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Invited Review Article

A comprehensive review on bovine tropical theileriosis under Indian scenario

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Abstract

Vector borne haemoprotozoan diseases are responsible for remarkable production losses due to mortality, the costs of treatment and the decrease in milk yield and average weight gain in animals especially in the cross bred and exotic cattle in the tropical and subtropical regions of the globe. Theileriosis is a group of tickborne diseases caused by protozoan parasites of the *Theileria* genus adversely affecting the livestock industry, but in bovines the two economically important pathogenic species are *Theileria annulata* and *T. parva* which are responsible for bovine tropical theileriosis and East Coast fever, respectively. The endemic distribution for *T. annulata* and *T. parva* is comparatively restricted. In India, *T. parva* has not been reported due to the absence of vector tick *Rhipicephalus appendiculatus*, however abundance data is available on the *T. annulata* transmitted by *Hyalomma anatolicum* ticks. It is a serious constraint to Indian dairy industry with more fatal infections in exotic cattle and substantial losses to cross-bred and indigenous zebu cattle. The clinical course of the bovine tropical theileriosis varies from subclinical to acute or per acute form. Disease is characterized by high fever, enlarged lymph nodes, weakness, weight loss, inappropriate appetite, conjunctival petechiation, and anaemia. The pathological changes attributed to cytokine production by T lymphocyte proliferation resulting into pathognomonic lesions in the form of punched out necrotic ulcers. The intrinsic factors associated with epidemiology of *T. annulata* infection are species of animal, age, breed and sex. The decreared levels of haematological parameters viz. red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration are indicator of anaemia. The biochemical alterations provide a supportive diagnostic aid in the clinical cases. Molecular tools are a better option for diagnosis of the disease than the conventional and serological techniques. Among several control methods the most practical and widely used method is treatment with buparavaquone and the chemical control of ticks with acaricides. A sustainable approach for controlling *Theileria* infection in developing world is focused on integrated measures comprising of chemotherapy, vaccination and vector management.

Key words: Bovines, Control, Diagnosis, India, *Theileria annulata*, Tropical bovine theileriosis

Introduction

Vector borne haematozoan diseases cause a major threat for the health and management of domesticated bovines in tropical and sub-tropical countries including India. Among these diseases theileriosis, babesiosis, trypanosomosis and anaplasmosis are of principal economic importance (Ashuma *et al.*, 2014) Theileriosis in animals is caused by several species tick borne obligate intracellular haemoprotozoa belonging to genus *Theileria* (phylum Apicomplexa, order Piroplasmida). The most pathogenic economically important species in bovines are *Theileria annulata* and *T. parva* causing bovine tropical theileriosis (BTT) and East Coast fever (ECF), respectively. *Theileria annulata* is transmitted by *Hyalomma anatolicum* and *T. parva* by *Rhipicephalus appendiculatus*. The endemic regions of *T. annulata* and *T. parva* do not overlap. *Theileria parva* is the most pathogenic and economically significant *Theileria* spp. in eastern, central, and southern Africa, where it causes East Coast Fever, Corridor disease, and January disease in cattle. *Theileria annulata* is endemic in southern Eastern Europe, southern Europe (Portugal, Spain, Italy, Greece, Bulgaria and Turkey), East and North Africa (Mauritania, Morocco, Algeria, Tunisia, Egypt, Sudan, South Sudan and Ethiopia) as well as in Asia (India, China, Middle East and Central Asia) and there is no report of East Coast fever from India (George *et al.*, 2015) due to the absence of its vector. Although, in India tick and tick borne diseases have great impact on the livestock production system as depicted from an estimated loss of USD 498.7 million per annum comprising tropical theileriosis that alone contributes to the tune of USD 57.2 million (Minjaw and

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McLeod, 2003).The disease threatens about 250 million cattle worldwide, having a very important negative impact on animal production, especially in developing countries. The impact of the disease is more in the cattle especially exotic and crossbred that showed the mortality rates upto 40-60% (Brown, 1997) and may reach up to 80% as was reported for the first times in 1970s' when cross breeding programme started in India to increase the productivity potential of dairy animals (Tuli *et al.*, 2015). Young calves less than three months of age are more prone to BTT as compared to adult animals (Aulakh and Singla 2006). The affected animals reveal fever, anemia, enlargement of lymph node, anorexia, and progressive loss of body weight, along with alteration in various haematolgical and biochemical parameters (Dua *et al.*, 2012). Most of the times typical punched out necrotic ulcers are observed on post mortem (Mahajan *et al.*, 2013). In rare cases theileriosis with cutaneous involvement suggesting metastasis and development of schizonts in the cutaneous lymphoproliferative nodules, leading to moderate parasitaemia and clinical disease has been reported (Narang *et al.*, 2019). We for the first time reported a molecular based confirmed case of cerebral theileriosis caused by *T. annulata* in a cross bred cattle calf (Agrawal *et al.*, 2023). The disease, as well as the carrier state induced by *T. annulata*, causes noteworthy production losses, due to mortality, the costs of treatment and the decrease in milk yield and average weight gain. Brown (1997) reported the economic losses endured by tropical theileriosis in India by stating 10 million cattle in India at risk for tropical theileriosis with an annual economic loss of US $800 million. The water buffaloes (*Bubalus bubalis*) are notably more resistant to theileriosis than indigenous cattle (Dhar *et al.*, 1988). A number of diagnostic assays encompassing conventional microscopy, immunological and molecular tools are in use for the detection of *Theileria* infection in livestock animals owing merits and demerits of individual test. The current review focused on biology, the recent updates on the prevalence, diagnosis and control measures adopted under Indian perspectives which will augment the profits to dairy farmers.

Life cycle

Theileria annulata has a complex life cycle involving the host animal and genus of *Hyalomma* ticks (Fig 1; Valente *et al.*, 2022). Sporogony and merogony take place in the bovine host while zygote and kinete are formed in ticks. In the course of tick feeding, the infective sporozoites inoculated through the saliva into the animal's body. They are rapidly captured by mononuclear leukocytes and multiply by merogony into schizonts. Microschizonts fairly thrive into macroschizonts, therefore inducing the host cells to become large lymphoblastoid cells which diverge in synchrony with the macroschizont. As a result of the parasite-induced lymphoproliferation, a large population of parasitised cells develops in the infected animal. Merozoites are then released from macroschizonts, which invade red blood cells thereby establishing the piroplasm stage to complete the life cycle within the bovine host (Mans *et al.*, 2015).

The development in the ticks include two stages namely gametogony and sporogony that occurs during the feeding cycle. Piroplasm differentiates to macro- and micro-gametes within lumen of tick's gut by gametogenesis. Gametes are morphologically similar and undergo syngamy to form a spherical diploid zygote. Subsequently, the zygote undergoes meiotic division, differentiates in epithelial cells of tick gut and ultimately forms motile uninucleate kinetes that lie free in cytoplasm. Kinetes cross the basal membrane as well as the lamina of gut cells to specifically enter salivary gland and are not found in any other tick organ. The salivary gland of ixodid ticks can be differentiated into type I, II and III. Type IV is present in male ticks only. Probably, kinete invades E-cells of Type III acinus due to its carbohydrate composition. Sporogony occurs in salivary gland and almost 30,000 to 50,000 sporozoites are produced in each infected acinar cell. The number of sporozoites in female tick is found to be higher than the male tick. Nymphal or adult ticks transmit non-motile sporozoites along with saliva into the bovine host during feeding.

Prevalence

The first *Theileria* species, *T. parva*, was discovered in 1898 by Robert Koch (Norval *et al.*, 1992). Another species was discovered in 1902 by Arnald Theiler and named as *Piroplasma annulatum* in 1904 (Dschunkowsky and Luhs 1904, Dobbelaere and McKeever 2002). Later on was reclassified as *T. annulata* following the identification of schizont stage in its life-cycle (Bettencourt *et al.*, 1907). Sen and Srinivasan (1937) published the first report of *T. annulata* in India. Thereafter a number of reports on natural outbreaks of theileriosis were reported (Gautam and Sharma, 1972; Gill, 1994). A number of reviews to give insight into the scenario of

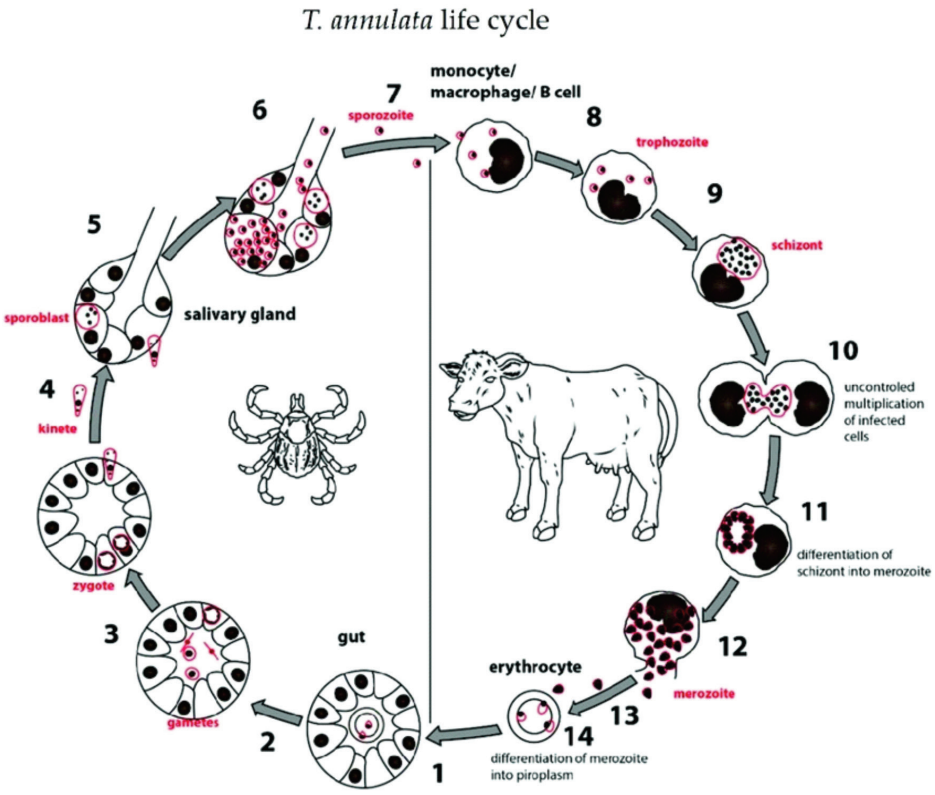

Fig 1: Life cycle of *Theileria annulata* (source Valente et al., 2022)

bovine tropical theileriosis in different states of India published time to time. Muraleedharan (2015) reviewed the *Theileria* infection among livestock and recorded the presence of *T. annulata* and *T. orientalis* in bovines of the Karnataka state. Kumar *et al.* (2018) reviewed the occurrence, control and the economic importance of *Theileria* spp. in Western Himalayan region of Uttar Pradesh. The prevalence estimates of BTT using a meta-analysis in India for the first time by Krishnamoorthy *et al.* (2021) revealed a higher prevalence in Puducherry and Assam. The method-wise breakdown revealed a higher prevalence by serology for India (39%) in comparison to other methods. Host species-wise scrutiny indicated a higher prevalence in cattle (22%) than buffaloes (14%). The occurrence of *T. annulata* in cattle and buffaloes and tick vectors from the different states based on the conventional and molecular diagnostic assays employed is summarized in Table 1

Risk factors

Various published reports from time to time on prevalence of BTT across the India attributed to various risk factors (Gul *et al.*, 2015; Tuli *et al.*, 2015) such as management practices, age, sex, season, health status, nutritional deficits, breed, herd size, agro-climate (humidity, temperature) affecting directly to the livestock health and predispose them to acquire the disease (Ghosh and Nagar, 2014; Ayadi *et al.*, 2016). A review using the meta analysis on the published data for the period 2015-2020 on the intrinsic risk factors for BTT in India revealed that age, sex and breed of cattle are significant risk factors to get infected with *Theileria* sp. (Bhangale *et al.*, 2021)

Pathogenesis

The species of *Theileria* grouped into schizont "transforming" and "non-transforming" species. Transforming parasites have uncontrolled proliferation of schizonts results in the pathologies associated with *T. parva* (East Coast fever, Corridor disease) and *T. annulata* (tropical theileriosis) in cattle and *T. lestoquardi* (malignant theileriosis) in goats and sheep. Non-transforming *Theileria* are regarded as being benign and but able to cause anaemia because of high piroplasm stage (Sivakumar *et al.*, 2014). Pathogenesis of theileriosis depends upon the production of schizonts

Table 1: Prevalence of *T. annulata* in host and vectors in India

State	Animal	Microscopy	PCR assay	Reference
Kashmir Valley	Cattle	1.80	-	Shaw,1989
Himachal Pradesh	Bovines	29.5	-	Jithendran,1997
Himachal Pradesh	Cattle	3.06	-	Sharma et al., 1999
West Bengal	Ticks	31.0	42.8	Das and Ray, 2003
Bangalore	Cattle	71.93	-	Ananda et al., 2009
Rajasthan	Cattle	12.5	-	Godara et al., 2010
Maharashtra	Cattle	5.70	-	Ugalmugle et al., 2010
Tamil Nadu	Cattle	13.63	18.18	Parthiban et al., 2010
	Ticks	-	13.33	
Northern Kerala	Cattle	16.0	-	Nair et al., 2011
Tamil Nadu	Cattle	55.27	-	Reetha et al., 2012
Punjab	Cattle	8.3	20.0	Tiwari et al., 2013
Gujarat	Cattle	82.94	-	Vahora et al., 2012
	Buffalo	84.29	-	
Eastern Haryana	Cattle	22.88	-	Chaudhri et al., 2013
Tamil Nadu	Cattle	13.0	-	Velusamy et al., 2014
Uttarakhand	Cattle	27.2	32.5	Kohli et al., 2014
Madhya Pradesh	Cattle	17.82	-	Agarwal and Das, 2014
Punjab	Cattle	9.23	29.26	Tuli et al., 2015
Karnataka	Cattle	0.75-58.91	-	
	Buffaloes	8.91-36.22	-	Muraleedharan 2015
Southern Rajasthan	Cattle	42.48	-	Bhatnagar et al., 2015
Telengana	Cattle	-	32.4	George et al., 2015
Punjab	Cattle	9.35	-	Kumar et al., 2015
South-West Gujarat	Cattle	7.08	-	Maharana et al., 2016
	Buffalo	5.30		
Madhya Pradesh	Cattle	7.2	20.8	Agarwal et al., 2016
Mizorum	Cattle	9.09	-	Sarma et al., 2016
Odisha	Bovine	4.56	68.0	Acharya et al., 2017
Odisha	Bovine	89	-	Singh et al., 2017
Haryana	Cattle	33.32	-	Ganguly et al., 2017
Kerala	Buffaloes	46.4	57.6	Priya et al., 2017
Madhya Pradesh	Cow	22.66	-	Dadrich et al., 2017
Telangana	Cattle	4.54	-	Kumar et al., 2018
	Buffalo	0.28	-	
Tamil Nadu	Cattle		13.95	Edith et al., 2018
Chhattisgarh	Cattle	23.33	-	Naik et al., 2016
Rajasthan	Cattle	-	41.0	Goyal, 2018
Bihar	Cattle	31.05	-	Kala et al., 2018
Maharashtra	Cattle	22.38	-	Khawale et al., 2019
Kashmir	Cattle	-	4.34%	Farooq et al., 2019
Tamil Nadu	Cattle	11.98	-	Gopalakrishnan et al., 2020
Tamil Nadu	Cattle	11.69	19.48	Anbu et al., 2020
West Bengal	Cattle	22.9	-	Debbarma et al., 2020
Odisha	Cattle	-	58.46	Selim et al., 2020
Jammu	Cattle	-	1.07	Kaur et al., 2021
Rajasthan	Cattle	67.22	-	Khatoon et al., 2021
South Bihar	Cattle	33.20	-	Sinha et al., 2021
Chhattisgarh	Cattle	37.1	56.6	Baghel et al., 2021
Bihar	Cattle	-	38.50	Kumar et al., 2022
Mizorum	Cattle	-	37.03	Joane et al., 2022
Odisha	Ticks	-	14.6%	Dehuri et al., 2022
Rajasthan	Cattle	24.0	-	Choudhary et al., 2022
	Buffaloes	8.0	-	
Rajasthan	Cattle	12.5	-	Bharti et al., 2022
Maharashtra	Cattle	6.79	80.58	Suryawanshi et al., 2022
Rajasthan	Cattle	15.38	-	Damor et al., 2023

in lymphocytes and piroplasm in RBCs. Besides the load of parasitaemia pathogenesis also depends on the strain of the parasite, age and health status of the host. The pathological impairment starts with the numerous schizont production(s) in lymphocytes that result into the massive and uncontrolled proliferation of both specific and nonspecific T lymphocyte resulting in enlarged lymph nodes in *T. annulata* and *T. parva* infection (Schneider *et al.*, 2007). The affected lymph nodes show reactive follicular hyperplasia, reticulo-endothelial hyperplasia, enlarged germinal centers and slight increase of interfollicular lymphoid tissue within the paracortical and cortical regions (Hassan *et al.*, 2000). Frothy nasal and ocular discharge in tropical theileriosis characterized by interlobular emphysema and severe pulmonary edema in acute cases is observed from the manifestations of proteinacious fluid in alveolar spaces, enlargement of pulmonary blood vessels with erythrocytes, and infiltration of inflammatory cells within the lung's interstitial tissue (Hassan *et al.*, 2000). These pathological changes are ascribed to T lymphocyte proliferation responsible for the production of pro-inflammatory cytokines TNF-α, IL-1α, IL-1β and IL-6 and IFNγ (Omer *et al.*, 2002). Lymphoid cellular infiltrations appear in the liver and kidney and hemorrhages and ulceration may be seen throughout the gastrointestinal tract due to the infiltration of renal interstitial fluid with mononuclear inflammatory cells (Hassan *et al.*, 2000). Tropical theileriosis is characterized by hemolytic anemia (Omer *et al.*, 2002) as a result of activation of the macrophages that releases the cytokines (TNFα), and may be the oxidative burst causing damage to RBCs (Reza and Dalir Naghadeh, 2006). The infected erythrocytes show morphological disorders which may be attributed to the presence of *Theileria* schizonts, immune-mediated processes and intravascular thrombi (Singh *et al.*, 2001). The excessive cytokines production is responsible for pathologies associated with tropical theileriosis (Forsyth *et al.*, 1999). The comparison of lymphoid tissues of *T. annulata* infected cattle showed non transformed T cells while *T. parva* infected cattle revealed extensive population of lymphoid cells. Agina *et al.* (2020) provided an extensive insight in the review about the clinicopathological and immunopathological profiles of *Theileria* infected cattle.

Clinical Symptoms

The clinical signs vary with the parasite strain, the host's susceptibility; severity of the disease is correlated with quantum of sporozoites inoculated by tick vector. Tropical theileriosis is generally characterized by high grade pyrexia, weakness, weight loss, inappropriate appetite, conjunctival petechiae, enlarged lymph nodes, unilateral or bilateral exophthalmia, nasal and ocular discharge and mild to moderate anemia. Lateral recumbency, diarrhea and dysentery are also associated with later stages of infection (Radostits *et al.*, 2007) and abortion in some cases (Kohli *et al.*, 2014) Dermatological involvement in cattle and buffaloes from distinct geographical locations include skin nodules, haemorrhagic lesions or necrotic lesions and cutaneous gangrene reported (Gharbi *et al.*, 2017; Narang *et al.*, 2019)**.**

Haematobiochemical alterations

Marked changes in complete blood cell count (CBC) are observed in naturally or experimentally *T. annulata* infected cattle (Singh *et al.*, 2001; Aulakh & Singla 2006; Ugalmugle *et al.*, 2010; Tuli *et al.*, 2015; Ganguly *et al.*, 2017; Acharya *et al.*, 2017; Kumari *et al.*, 2022; Prajapati *et al.*, 2022). *Theileria annulata* caused a regenerative anaemia with marked decreases in the RBC count, PCV, Hb concentration, mean corpuscular haemoglobin concentration (MCHC) with increased mean corpuscular volume (MCV), and marked reticulocytosis (Tuli *et al.*, 2015; Acharya *et al.*, 2017; Naik and Maiti, 2018). Alterations in biochemical parameters in *T. annulata* affected cattle and buffaloes are reported by several workers which include marked decrease in the level of total protein (TP), albumin and glucose (Tuli *et al.*, 2015). Parameters like blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and bilirubin showed marked increase in the affected cattle (Aulakh and Singla 2006; Tuli *et al.*, 2015; Acharya *et al.*, 2017; Ganguly *et al.*, 2017; Goyal, 2018; Prajapati *et al.*, 2022). The clinical pathology analysis may help to the clinicians as the supportive aid in the diagnosis, and therapy especially of the acute cases of theileriosis.

Diagnostic techniques

Tentative diagnosis of cases suspected for theileriosis is associated on the basis of clinical signs, knowledge of disease and vector distribution. The diagnosis of *T. annulata* based on clinical signs is challenging, because of overlapping of the symptoms especially with the blood haemoparasitic and other

infections of bovines (Forsyth *et al.*, 1999).

Conventional methods

Microscopic examination of Romanowsky stained blood smears for detection of piroplasms and lymph node aspirate smears for detection of schizont (Koch's blue bodies) of *T. annulata* is considered as gold standard test for routine use. However being robust, ease in use and is only suitable for acute clinical cases with high parasitaemia. Contrarily, it is unsuitable method for detecting the carrier or chronic phases of theileriosis and impossible to differentiate between piroplasms of other *Theileria* species which are morphologically homogeneous (Hoghooghi-Rad *et al.*, 2011). For detection of *T. annulata* infection in tick vectors, salivary glands stained with methyl green pyronin (MGP) is a traditional method (Walker *et al.*, 1979). In comparison to the Giemsa-stained thin blood film technique, acridine orange stains is more sensitive and a better option depending upon the availability of the florescent microscope (Omer *et al.*, 2011; Salih *et al.*, 2015).

Serological techniques

To overcome the deficits of routine microscopic examination, several immunological assays like complement fixation test (CFT), indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assays (ELISA) are employed worldwide for the epidemiological surveys of the theileriosis. These tests detect the circulating antibodies by using either piroplasms or cultured macroschizonts as the antigen. As per OIE, IFAT is the gold standard test, but ELISA is immensely accepted globally, because it is easy to perform, and having high through output. However IFAT have several drawbacks, cross reactivity, operator-dependent interpretation of results and low throughput. These tests are impractical because of the limitations as unable to process a large number of samples, cross reactivity with antibodies directed against other *Theileria* species, incompetent to differentiate the present and past infection, weakened immune response and insufficient antibody level during extended carrier phase. *Theileria* piroplasm may occasionally be present in the erythrocytes of long-term carriers whereas antibodies have a tendency to disappear. The animals may still be infected despite of negative serological test (Ali and Radwan, 2012). The identification of carrier animals is critical and serve a major reservoir source of transmitting infection to non-endemic areas.

Molecular techniques

The progress in the biotechnology field showed an outburst of diagnostic assays that targets specific genes and species of *Theileria* and proved to more sensitive and specific than conventional microscopic examination and serological assays (Criado-Fornelio, 2007). The molecular assays developed and employed in the mounted order include conventional polymerase chain reaction, nested PCR, reverse line blotting hybridisation using radio-isotope labelled probes (PCR RLB) and non-radio-active reverse line blot method that used chemiluminescence, probe based real-time PCR methods, SYBR green and Taqman real-time PCR assays, loop-mediated isothermal amplification (LAMP) assays, pan-FRET based assays and high-resolution melt analysis (Mans *et al.*, 2015; Tuli *et al.*, 2015, Ntesang *et al.*, 2022). The threshold of detection of the PCR-assay is an erythrocytic parasitaemia of 0.00008% corresponding to 16 infected bovine erythrocytes. The theoretical detection limit of real time PCR assay for *T. annulata* is of 23–54 million parasites in cattle weighing 500 kg while of PCR-RLB assay based on the cytochrome b gene is 100,000 parasites per liter of blood (Bilgic *et al.*, 2010). Mostly concurrent infections of tick borne haemoprotozoans occur in animals. Multiplex PCR technique that owes the capability to detect simultaneous amplification of more than one locus in the same reaction with less cost and time efficiency is of immense help in field condition (Tuli *et al.*, 2013; Kundave *et al.*, 2018).

Control measures

The present control measures against tropical theileriosis depend on chemotherapy, tick control using acaricides and improving cattle barns, as well as vaccination with attenuated cell line vaccines (Mhadhbi *et al.*, 2015). Each of these measures has its own merits and demerits. So the integrated approaches targeting the vaccination, tick vector control and mangemental strategies are useful to meet the specific requirements of livestock holders under different situations. The dissemination of the new technologies to the livestock owners, veterinarians must be like that they can develop appropriate strategies for the control of theileriosis as per their own circumstances (Minjauw and McLeod, 2003).

Chemotherapy

The chemotherapeutic agents recommended by OIE for theileriosis in bovines are tetracyclines,

parvaquone, buparvaquone and halofuginone (Mehlhorn, 2008). Tetracycline antibiotic was probably the first chemotherapeutic compound used against *T. parva* in early 1953. Long acting Oxytetracycline @ 20 mg/kg, intramuscular, three doses 4 days apart found effective only at the early phase of the infection. Thereafter in 1970, naphthoquinone compound (parvaquone and buparvaquone), with a wide therapeutic index are thought to be the drug of choice (Gachohi *et al.*, 2012) and recommended as curative prophylactic measure for the theileriosis (Qayyum *et al.*, 2010). The dose rate of buparvaquone is 2.5 mg/kg is effective for schizonts and piroplasm stage of the parasites. The parvaquone @ 20 mg/kg is effective only against schizonts. These drugs are not used by the farmers because of high cost and residual effects in body of the animals. Halofuginone was introduced 1986 in Kenya and is no longer approved for use against theileriosis caused by pathogen *Theileria* spp. in cattle (Mehlhorn, 2008). Drug resistance to buparvaquone reported in Tunisia and in southern Iran due to the failure of the treatment of the cattle affected with acute tropical theileriosis (Mhadhbi *et al.*, 2010). Resistance against antitheilerial drugs is due to point mutation in the parasite's cytochrome b gene (Sharifiyazdi *et al.*, 2012; Chatanga *et al.*, 2019).

Immunization

The first attempt of immunization in cattle against tropical theileriosis was made in Algeria in 1930s by inoculating the low virulent stains of *Theileria* pathogens into the healthy cattle results into no development of merozoites and protection for one year. Later same mechanism of immunization applied in Israel by inoculating cattle with low virulence Tunisian strain and boosting with local strain after two months to reinforce immunity (Pipano and Shkap, 2000). Infection and treatment is still in use where live sporozoites inoculated in cattle and then treatment with oxytetracycline leads to the cytotoxic T cell response against parasitic schizonts of *T. annulata.* Beside the broad endemic region of the *T. annulata* the impact of the attenuated *Theileria* vaccines is only limited to Israel, Iran, Morocco, Tunisia, India, China and Uzbekistan. In India Commercial vaccination for tropical theileriosis in cattle and buffaloes is Rakshavac-T containing attenuated schizont-infected cell lines given 3 ml, subcutaneously to the calves of >2 month age group. However being live vaccine is not widely accepted because it causes reversion to disease in calves and may be ineffective due to no cold-chain maintenance and due to short shelf life. *T. annulata* sporozoite antigen SPAG1 found to be vaccine candidates as it is able to induce the CD8+ T cell response. *Theileria parva* sporozoite p67 and *T. annulata* SPAG1 antigen confer a cross protection against each other, as anti-p67 serum recognizes SPAG1 and neutralizes *T. annulata* sporozoites, and vice versa (Nene and Morrison, 2016). The scope of the DNA vaccine consisting of plasmid DNA with genes of interest, molecular adjuvants, and chitosan may be the most promising next-generation vaccine for the control of bovine theileriosis (Agina *et al.*, 2020).

Tick control

Under Indian circumstances, acaricide application is the key method to control ticks. That results into multi drug resistance development beside the residual effect of these drugs in tissue and milk and the negative environmental impact. Management of the shed include individual tick proof houses without cracks and crevices, regular removal of stacks of bricks and dung cakes from the breeding place of the ticks (Vahora *et al.*, 2012). Quarantine and treatment of the newly purchased animals, manual removal of the ticks with forceps in case low frequency and then kill them by the smoldering on dung cake (Vahora *et al.*, 2012) and treatment of the heavily infested animals with the rotation of the acarcides application by dipping, pour-on, spraying and injectable methods. Hand application of the acaricides properly at the tick attachment sites inner pinna of ear, under part of tail and legs and udder is best approach for the effective acaricide application in addition to spray and dipping methods.

To overcome the acaricide resistance, emphasis on the search of alternative, environmental eco-friendly tick control strategies is the use of phytoacaricides derived from more than 200 plants that possess anti-tick or tick repellent properties. *Azadirachta indica* (Neem), *Lavendula augustifolia* (Lavender), *Pelargonium roseum* (Rose geranium) and *Cymbopogon* spp. (Lemongrass) showed acaricidal and larvicidal effects with 90–100% efficacy, comparable to those of currently used acaricides (Adenubi *et al.*, 2016). Bio-control of ticks by rearing of backyard poultry that eats the ticks off the body, spray of fungus like *Metarhizium anisopliae* and *Beauveria bassiana*, entomopathogenic nematodes of family Steinernematidae and Heterorhabditidae, predatory mites and parasitoids also found beneficial.

Exploitation of breed resistant to the tick and tick borne pathogens

A national policy was planned in India to reduce the population of exotic breeds of the cattle that led to reduction in prevalence of theileriosis (Omer *et al.*, 2002). Selection of cattle breeds with enhanced tick resistance (*Bos indicus,* Sahiwal) was proposed as a sustainable approach for controlling infection of tick and tick borne in developing world (Naik *et al.*, 2010). Breed wise low prevalence of parasite is reported in Sahiwal cattle than European breeds (Sajid *et al.*, 2009) and cross breed of cattle (Annand and Ross, 2001; Malmquist *et al.*, 2003). Holistic future sustainable approach for control of Tick Borne Haemoparastic Diseases is genetic manipulation either by the cross breeding programme or biotechnological tool to develop the cross bred cattle resistant to the ticks especially *H. anatolicum* (Gharbi *et al.*, 2020).

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Research Articles

Antibiogram of *Staphylococcus aureus* isolated from clinical cases of bovine mastitis

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Abstract

The present study was conducted to determine the antibiogram of *Staphyloccus aureus* isolates obtained from clinical cases of bovine mastitis. A total of 102 *S. aureus* isolated from mastitic milk samples were subjected to *In vitro* antibiotic sensitivity testing against 14 different antibiotics belonging to 9 different classes. The antibiotics comprised of mixture of conventionally and few non-conventionally used antibiotics. Diameters of zone of inhibitions were recorded and interpreted according to the breakpoints given by CLSI. On the basis of sensitivity pattern, isolates were categorized into multidrug-resistant (MDR), extreme drug-resistant (XDR), and pandrug-resistant (PDR). Variable sensitivity with a wide range was observed against antibiotics. Antibiotic sensitivity pattern revealed maximum sensitivity towards chloramphenicol (88.24%) and maximum resistance towards vancomycin (91.18%). Out of 102 isolates, 73 (71.56%) isolates were found to be multidrug-resistant. None of the multidrug isolates were found to be extreme drug-resistant and pan drug-resistant. No relation was observed between sensitivity pattern with conventional and non conventional antibiotics.

Keywords: Chloramphenicol, Mastitis, Multidrug resistant, *Staphylococcus aureus*

Mastitis is a major threat to dairy sector in India posing huge economic losses to the tune of Rs. 7165.51 crores per annum (Bansal and Gupta, 2009). Disease is multifactorial involving microorganisms, environment and host. More than 250 microorganisms have been reported to be associated with mastitis (Bhuvana and Shome, 2013). Bacteria are the most common etiological agent and among them *Staphylococcus aureus* has been reported as most prevalent by many researchers from India.

Staphylococus aureus expresses various factors which help its sustainability inside the tissue and environment. Treatment of mastitic animal has been the mainstay of control of disease. It is imperative to determine the antibiogram before the start of therapy but in case of empirical treatment practioners must know which antibiotic can be effective. Antimicrobial sensitivity prior to treatment will help in selecting suitable and cost effective antibiotic to treat an animal properly. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in a particular area; therefore, antimicrobial sensitivity is suggested before institution of treatment. Therefore, the present study was planned to determine the antibiogram against *Staphylococccus aureus* using a mixture of conventionally used antibiotics and fewer rarely used antibiotics.

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Materials and Methods

Isolation and identification of S. aureus

Cultural examination and phenotypic identification of S. aureus

Immediately after receiving, the milk samples were inoculated in 0.01 ml volume on 5% sheep blood agar (BA) and MLA plates with the help of a 4 mm diameter platinum loop. The plates were incubated aerobically at 37°C for 24-48 hrs. Catalase test was done to differentiate between staphylococci and streptococci. Catalase positive colonies were subjected to oxidase test to rule out the possibility of micrococci. Sub-cultures of the tested colonies were made on nutrient agar for purification of isolates and identified on the basis of Grams reaction and HiStaph™ latex test kit up to species level.

Species confirmation of S. aureus by PCR

Confirmation of pure culture of *S. aureus* was done by PCR assay. Single pure colonies obtained by quadrant streak method were subjected for DNA isolation.

Antibiotic sensitivity testing of S. aureus isolates

Antibiotic sensitivity testing was done according to Clinical and Laboratory Standards Institute (CLSI), 2013 guidelines by Kirby-Bauer disk diffusion method.

Preparation of inoculum for disk diffusion testing

With the help of an inoculation loop, 3-5 well isolated colonies were inoculated in 5 ml of BHI broth in a test tube. Culture was incubated for 2-6 hrs at 37°C, until it achieves or exceeds the turbidity standard of 0.5 McF. Turbidity of the actively growing broth culture was adjusted to 0.5 McF by diluting the culture in fresh BHI broth. This resulted in the suspension containing approximately $1x10^8$ cfu/ml. The adjusted suspension was used within 15 min of preparation.

Inoculation of bacterial culture on MHA plates

A sterile cotton swab was rotated several times into the inoculum and squeezed well at side above the suspension level. MHA plates were inoculated by streaking the swab on surface of plates by rotating the plate to ensure uniform inoculation. Rim of the plate was also swabbed.

Dispensing of antibiotic discs on inoculated plates

The antimicrobial discs were dispensed on the agar surface by light pressing, at least at a distance of 24 mm. Only 4 discs were placed on a 90 mm agar plate. The plates were inverted and incubated at 35°C for 16-20 hrs. The list of different antibiotics used for susceptibility testing is mentioned in table 1 along with the concentration.

Interpretation of results

The diameters of zone of inhibitions were measured with the help of a ruler. The results were recorded and interpreted according to breakpoints given by CLSI.

Determination of multidrug-resistant (MDR) bacteria

On the basis of sensitivity pattern, isolates were categorized into Multidrug resistant (MDR), Extreme drug resistant (XDR), and Pan drug resistant (PDR). Isolates resistant to three or more antibiotics belonging to different groups were classified as MDR. Among MDR isolates, isolates susceptible to only two antibiotics belonging to two different groups were considered XDR, while isolates resistant to all the antibiotics were considered as pandrug-resistant.

Results and Discussion

Multidrug resistant staphylococci strains associated with mastitis in dairy cows are being reported globally as a potential zoonotic agent possing threats to human health. In present study, a total of 102 S. aureus characterized isolates obtained from clinical cases of mastitis were subjected to determination of antibiogram. Variable sensitivity with a wide range was observed against antibiotics and have been depicted in the Figure 1. Maximum sensitivity was reported towards chloramphenicol (88.24%) followed by doxycyline and linezolid (74.51%), gentamicin (69.61%), cefoxitin (62.70%), clindamycin (59.80%),

Table 1. List of antibiotic discs used for sensitivity testing with concentration

Sr. no.	Group of Antibiotics	Antibiotics	Concentration
1	Cephalosporin	Cefoxitin	30 µg
		Cefoperazone	75 µg
		Cetriaxone	30 µg
2	Glycopeptide	Vancomycin	30 µg
3	Quinolone	Enrofloxacin	10 µg
		Ciprofloxacin	10 µg
4	Oxazolidinones	Linezolid	30 µg
5	Suphonamide	Sulfisoxazole	300 µg
6	Tetracycline	Doxycycline	30 µg
		Oxytetracycline	30 µg
7	Lincosamide	Clindamycin	02 µg
8	Chloramphenicol	Chloramphenicol	30 µg
9	Aminoglycoside	Neomycin	30 µg
		Gentamicin	30 µg

sulfisoxazole and oxytetracycline (58.82%), cefoperazone (56.86%), ceftriaxone (53.92%), enrofloxacin (46.08%), ciprofloxacin (39.21%), neomycin (29.41%) and least sensitive towards vancomycin (8.82%). No relation was observed between sensitivity pattern with conventional and non conventional antibiotics. This indicates a probable chances of transmission of resistance gene from human to animals.

Among different isolates, 73 (71.56%) isolates were found to be MDR and neither isolate was found to be XDR nor PDR. As compared to present study, higher percentage of isolates were reported as MDR by Li *et al.* (2017) and Bissong *et al.* (2020) whereas lower percentage of isolates were reported to be MDR by Mbindyo *et al.* (2021) (22.9%), Eid *et al.* (2022) (16%), Brahma *et al.* (2022) (57.5%) as compared to our study. Indiscriminate use of antibiotics, irregular doses of antibiotics or under dosing of antibiotics may lead to resistant mutants which can vary from area to area and region to region depending upon the prevailing practices. None of the isolates was found to be extreme drug resistant and pan drug resistant which might be due to the antibiotics (mixture of conventionally and non conventionally) used in present study for testing of sensitivity making isolates sensitive to one or other drugs.

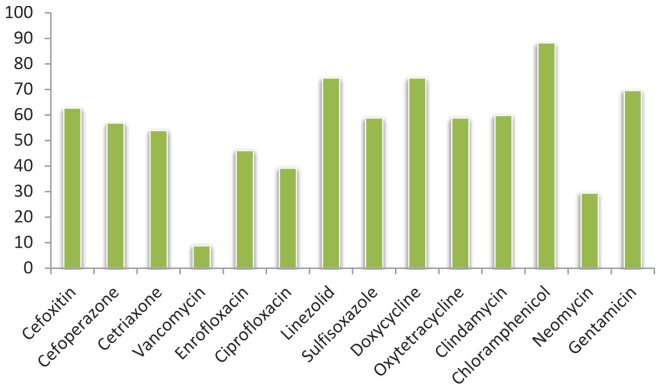

Fig 1. Bar diagram showing sensitivity pattern of Staphylococcus aureus against 14 different antibiotics belonging to 9 different classes.

In present study, Chloramphenicol to be the most sensitive among all the antibiotics tested with 88.24% isolates sensitive which is similar to Lemma *et al.* (2020) ,comparable to the sensitivity results of 84.28% isolates reported by Charaya *et al.* (2014) and lower than Gebremedhin *et al.* (2022). This result can be attributed to the fact that chloramphenicol is not much used in large animal clinical practices in our area. A range of sensitivity (62.70%-53.92%) of *Staphylococcus aureus* was observed for cephalosporins which was less as compared to Gebremedhin *et al.* (2022) and Lemma *et al.* (2020) reporting higher number of isolates to be sensitive for the drugs. In present study, *S. aureus* was found to be least sensitive towards vancomycin, which is lower than as reported by Gebremedhin *et al.* (2022) and Bissong *et al.* (2020). In conclusion, large number of *Staphylococcus aureus* isolates of milk origin have been found to be multidrug resistant indicating a huge threat to animal as well as human health. Therefore, it is imperative to conduct such studies to generate data which can be used to counteract these potential zoonotic dangerous bacteria.

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Clinical rhinoscopic and computed tomographic studies and therapeutic evaluation in twelve dogs with chronic inflammatory rhinitis

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Abstract

Inflammatory rhinitis is the second common chronic disease of nasal cavity in dogs. Clinical signs are often insufficient for reliable differentiation between other canine chronic nasal cavity diseases. Definitive diagnosis requires diagnostic imaging modalities like radiography, computed tomography and rhinoscopy along with biopsy to rule out other primary nasal lesions. The treatment strategies followed for chronic inflammatory rhinitis includes various antibiotics, antihistamines, steroids and non-steroidal anti-inflammatory drugs. The present study evaluates the clinical, rhinoscopic and computed tomographic changes and therapeutic evaluation of twelve dogs with chronic inflammatory rhinitis. Dogs brought to Madras Veterinary College Teaching Hospital, Chennai with clinical signs suggestive of nasal cavity diseases were screened. The dogs were subjected to radiography and computed tomography of skull and rhinoscopic assessment of the nasal cavity with a biopsy in case of mucosal lesions for ruling out other primary chronic nasal diseases like nasal tumour, foreign body rhinitis, fungal rhinitis or dental disease. Ten apparently healthy dogs brought for general check-up were utilized for obtaining reference values of parameters under study. Twelve dogs with chronic inflammatory rhinitis were grouped into the two treatment groups. Group I and II comprised of six dogs each administered with prednisolone alone and meloxicam followed by prednisolone respectively. Clinical signs, haemato-biochemical, radiographic, computed tomographic and rhinoscopic changes and therapeutic evaluation in dogs with chronic inflammatory rhinitis confirmed through histopathological studies are presented. The present work will be complementary to diagnostic imaging studies and treatment of canine chronic inflammatory rhinitis.

Key words: Dogs, Inflammatory rhinitis, Rhinoscopy, Computed tomography, Meloxicam, Prednisolone

Nasal diseases of chronic nature are a common clinical complain in clinical practice (Lefebvre *et al.*, 2005). Clinical manifestations tend to be similar, consisting of epistaxis, sneezing, reverse sneezing, halitosis, nasal discharge, stridor and facial pain (Auler *et al.,* 2015). Definitive diagnosis is essential for initiating appropriate medical or surgical treatment in chronic nasal diseases. A detailed history and clinical examination, followed by a stepwise diagnostic evaluation, often aids a specific diagnosis and development of an optimum treatment plan. Definitive diagnosis often requires additional imaging diagnostic modalities along with a biopsy (Lobetti, 2009). Diagnosis of inflammatory rhinitis is based on exclusion as it is also seen associated with nasal neoplasia, fungal rhinitis, dental disease, oronasal fistula or foreign body rhinitis (Moores and Walker, 2013). The reports on combined study of radiography, computed tomography and rhinoscopy with guided biopsy in dogs with chronic inflammatory rhinitis and the use of non-steroidal anti-inflammatory drugs for its management is limited, hence the present study was undertaken.

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Materials and Methods

Dogs brought to the Small Animal Out-Patient unit of Madras Veterinary College Teaching Hospital, Chennai were utilized for the study. Dogs with history and clinical signs suggestive of nasal cavity diseases like nasal discharge, stridor, foul-smelling breath, epistaxis, sneezing, respiratory distress or facial deformities were screened. These dogs were subjected to detailed anamnesis, clinical examination (Radostits *et al.,* 2000) and haemato-biochemical analysis (Abrams-Ogg and Schneider, 2010; Kaneko *et al.,* 2008). Special examinations including radiography, computed tomography of the skull and rhinoscopy were done on these animals. Ten apparently healthy dogs brought for routine check-up and deworming to the Small Animal Out-Patient unit of Madras Veterinary College Teaching Hospital were utilized for obtaining reference values of parameters under study. Twelve dogs with chronic inflammatory rhinitis were grouped into two treatment groups. Dogs under Group I were administered

with prednisolone (@ 1mg/kg orally for three weeks followed by 0.5 mg/kg orally for one week) and Group II were treated with meloxicam (@ 0.1mg/kg orally for three weeks) followed by prednisolone (@ 0.5mg/kg orally for one week).

Dogs were fasted for 12 hours before anaesthetic induction. An indwelling intravenous catheter and a cuffed endotracheal tube were placed. Anaesthetic protocol as described by Seymour and Gleed (1999) was followed. The animals were administered with glycopyrrolate (@ 0.01-0.02 mg/kg SC). Premedication with xylazine (@ 0.2 – 1.1 mg/kg IM) and induction of anaesthesia by propofol (@ 1-2 mg/kg IV) was done. Maintenance of anaethesia was carried out with either propofol or ketamine (@ 5 mg/kg IV) and diazepam (@ 0.25 mg/kg IV) combination or halothane. The drugs were chosen based on the age and clinical presentation of the animal. Modified infra orbital approach for maxillary nerve block as described by Fizzano *et al*. (2017) was followed for endoscopic procedures in dogs. Monitoring of electrocardiogram, blood pressure and oxygen saturation were done as per standard techniques.

The radiographic examination including a dorsoventral (DV) and a lateral projection of the entire skull was undertaken as described by Thrall *et al.* (2017) using Konica Minolta AeroRAD 32 Digital X-Ray system. Computed tomography of skull was performed as described by Schwarz and Saunders (2011) using third generation Toshiba Alexion 16 Multi–Slice scanner. Tomographic images were acquired with 120 kV, 160 mA and 1-second acquisition time and contiguous images were obtained from the caudal limit of the frontal sinuses to the nares with the animal on sternal recumbency under general anaesthesia.

Rhinoscopy was performed as per the procedure described by McCarthy (2005) using anterograde and retrograde methods under general anaesthesia. A flexible bronchoscope (bronchoscope 3.5 mm diameter with two-way deflection- Olympus type BF 1T150, Japan) was used for posterior rhinoscopy. An arthroscope with cystoscopy sheath (2.7mm 30-degree Karl Storz, Germany) was used for anterior rhinoscopy. Irrigation for anterior rhinoscopy was provided from bags of 0.9 per cent saline fitted with standard delivery sets connecting to one of the stopcocks of the cannula or working channel of the flexible endoscope. Nasal swabs obtained from both nasal cavities as described by Tress *et al.* (2017) were subjected for cytological analysis. Tissue sample obtained using endoscopic forceps were fixed in 10 per cent formalin and used for histopathological studies (Bancroft and Gamble, 2008). The data obtained in the study were subjected to statistical analysis as described by Snedecor and Cochran (1994).

Results and Discussion

In the present study, chronic inflammatory rhinitis was identified in 12 dogs. The sample in this study included mostly mesaticephalic breeds (66.7 per cent) followed by brachycephalic (16.7 per cent) and dolichocephalic (8.3 percent) dog breeds comprising of Labrador retriever (3), Spitz, Golden retriever and mixed breed (two each) and Great Dane, Rottweiler and non-descript (one each). Large breed dogs had a higher risk for the development of idiopathic inflammatory rhinitis (Pietra *et al*., 2010) similar to the findings in the present study. Mesaticephalic breeds were over-represented in this study (75.0 per cent) similar to the previous studies (Widsor and Johnson, 2006). In the present study, dogs were aged between 4 months and 11 years with a mean age of 4.42 years) similar to the findings of Windsor *et al* (2004). In contrast Lefbvre *et al.* (2005) and Lobetti (2014) reported that the disease was most common in older dogs with a mean age of 8.5 years and 9 years respectively.

Clinical findings of dogs with chronic inflammatory rhinitis in the present study are indicated in the in Table 1. The clinical findings in dogs with chronic inflammatory rhinitis were nasal discharge (11/12; 91.7 per cent), sneezing (9/12; 75.0 per cent), reverse sneezing (1/12; 8.3 per cent) and stertor (1/12; 8.3 per cent). The most common type of nasal discharge was epistaxis followed by mucopurulent, mucoid, serous, mucopurulent and purulent nasal discharge similar with the studies by Plickert *et al.* (2014). The nasal discharge (mucoid, mucopurulent and haemorrhagic) was mostly bilateral and no facial deformity was present in dogs with chronic inflammatory rhinitis according to Windsor *et al*. (2004) and Lobetti (2014). Similarly, nasal discharge was bilateral in 72.7 per cent of the dogs in the present study.

The haematobiochemical changes in dogs with chronic inflammatory rhinitis from apparently healthy dogs was analysed using independent t test (Table 2). The mean haemoglobin, haematocrit, RBC, platelet, WBC, neutrophils, lymphocytes, monocytes, eosinophils, total protein, albumin, creatinine and alanine transaminase in apparently healthy dogs were 15.09 ± 0.32 g/dL, 44.73

Table 1. Signalment and Clinical signs in dogs with nasal tumour

Case	Breed	Age (years)	Sex	Clinical signs
01	Spitz	9.0	F	B-Ep
02	Labrador retriever	4.0	M	B-Mu-ND, Sn
03	Great Dane	3.0	M	U-Ep, Sn
04	Spitz	11.0	F	B-MPu-ND, Sn
05	Golden retreiver	6.0	M	U-Ep, Sn
06	Mixed breed	8.0	F	B-Mu-ND, Sn
07	Mixed breed	3.0	M	B-Se-ND, Sn
08	Labrador retriever	3.0	M	B-Se-ND, Sn, St
09	Non-descript	0.7	M	R-Sn
10	Golden retriever	0.5	F	B-Mu-ND, Sn
11	Rottweiler	0.3	M	U-Ep, Sn
12	Labrador retriever	4.5	M	B-Pu-ND, Sn

U- unilateral; B - bilateral
M - Mucous; Pu – purulent; Se- Serous; Ep; Epistaxis
ND - nasal discharge; Sn - Sneezing; St - stertor

± 0.92 per cent, 6.85 ± 0.15 10^6 /cumm , 2.5 ± 0.25 10^5/ cumm, 12.53 ± 1.11 10^3/cumm, 9.14 ± 0.79 10^3/cumm, 2.85 ± 0.27 10^3/cumm, 0.52 ± 0.06 10^3/cumm, 0.1 ± 0.04 10^3/cumm, 7.34 ± 0.12 g/dL, 2.89 ± 0.14 g/dL, 1.05 ± 0.05 mg/dL and 51.3 ± 6.33 IU/L respectively. These values were within the normal range as reported by Rizzi *et al*. (2010).

Dogs with chronic inflammatory rhinitis in the present study were found to have reduced mean haemoglobin, haematocrit and RBC compared to the respective mean of the healthy dogs and the mean serum biochemistry values did not show any significant changes. The haematological and serum biochemical values in dogs with chronic inflammatory rhinitis were unremarkable in

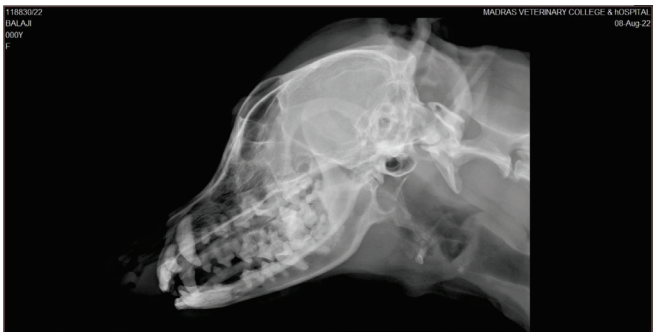
Table 2. Haemogram and serum biochemistry in apparently healthy dogs and dogs with nasal tumour (Mean ± S.E.)

Parameter	Apparently healthy dogs (n=10)	Dogs with chronic inflammatory rhinitis (n=12)	"t" test
Hb g/dL	15.09 ± 0.32	13.01 ± 0.67	2.616*
Haematocrit %	44.73 ± 0.92	36.63 ± 2.32	3.02**
RBC 10^6 /cmm	6.85 ± 0.15	5.83 ± 0.32	2.68*
PLT 10^5/cmm	2.5 ± 0.25	2.10 ± 0.38	0.828^{NS}
WBC 10^3/cumm	12.53 ± 1.11	14.99 ± 1.85	1.086^{NS}
Neutrophils 10^3/cumm	9.14 ± 0.79	11.53 ± 1.54	1.3^{NS}
Lymphocytes 10^3/cumm	2.85 ±0.27	2.37 ± 0.36	1.013^{NS}
Monocytes 10^3/cumm	0.52 ± 0.06	0.82 ± 0.14	1.796^{NS}
Eosinophils 10^3/cumm	0.1 ± 0.04	0.11 ± 0.04	0.226^{NS}
Total protein g/dL	7.34 ± 0.12	7.40 ± 0.22	0.223^{NS}
Albumin g/dL	2.89 ± 0.14	2.73 ± 0.13	0.825^{NS}
Creatinine mg/dL	1.05 ± 0.05	0.95 ± 0.11	0.782^{NS}
ALT IU/L	51.3 ± 6.33	43.25 ± 5.13	0.999^{NS}

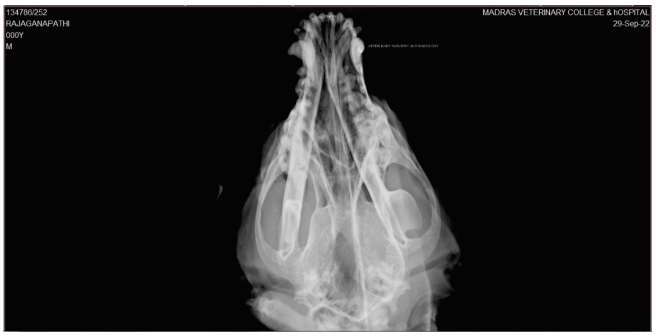
*Significant (p< 0.05), ** Highly significant (P < 0.01) and NS : Non significant (p > 0.05)

accordance with Windsor and Johnson (2006). Reduced mean haemoglobin, haematocrit and RBC in dogs with chronic inflammatory rhinitis in the present study might be due to chronic blood loss.

Radiography of the skull in 58.33 per cent of the dogs with chronic inflammatory rhinitis in the present study were devoid of any changes; the only significant change was increased radiopacity of nasal cavity (Plate 1). The typical radiographic change in dogs with chronic



Lateral radiograph of skull: Increased opacity of nasal cavity obscuring the normal turbinate pattern

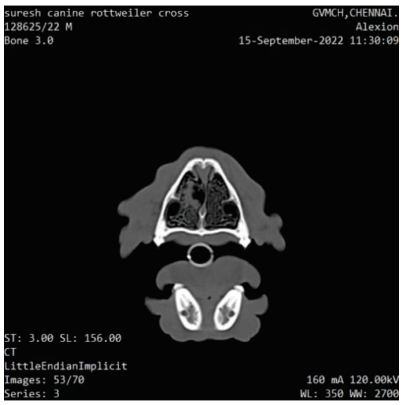


Dorso-ventral radiograph of skull: Increased opacity of nasal cavity obscuring the normal turbinate pattern

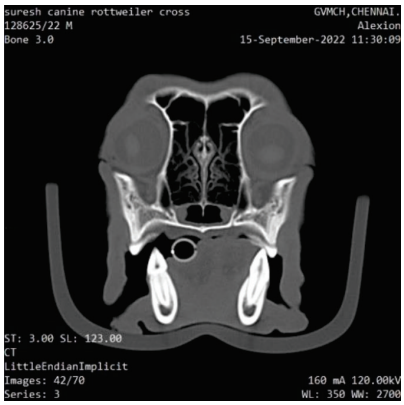
Plate 1: Radiography of skull of dogs with chronic inflammatory rhinitis

A. at the level of incisors

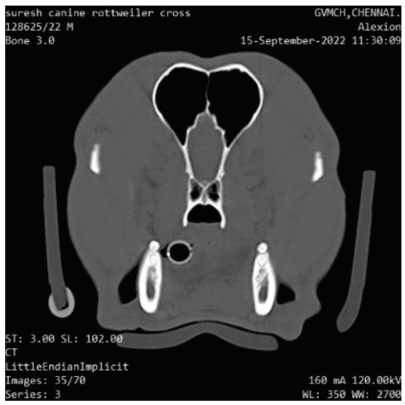
B. at the level of canine



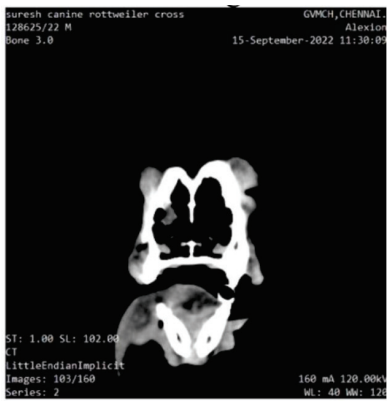
C. cranial to the orbital region



D. at the level of nose through orbital region



E. through frontal sinus



F. cranial to the orbital region (contrast)

Plate 2. Sequential computed tomography images through the nose of a dog with chronic inflammatory rhinitis (rostral to caudal)

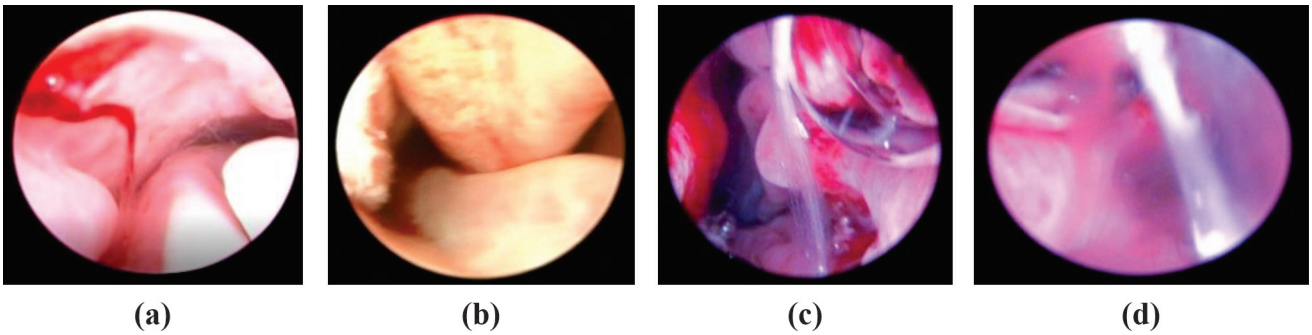


Plate 3: Rhinoscopy

Rhinoscopy in dogs with chronic inflammatory rhinitis: a. Increased fragility of the mucosa; b. Thickening of the turbinates; c. Turbinate destruction and severe bleeding; d. Nasal discharge with mild bleeding

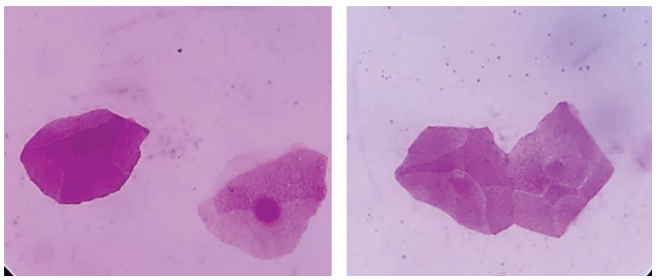
inflammatory rhinitis is opacification of the nasal passage Lobetti (2014) whereas the nasal radiographs were unremarkable in chronic inflammatory rhinitis according to Kaczamer (2018). Radiography had poor sensitivity in differentiating inflammatory rhinitis from fungal rhinitis and nasal neoplasia (Windsor *et al*., 2004 and Meler *et al.,* 2008).

Computed tomography of skull revealed either normal turbinate anatomy or mild turbinate destruction in dogs with chronic inflammatory rhinitis under the present study (Plate 2). Nasal computed tomography in dogs with idiopathic inflammatory diseases observed either normal turbinate structure without the presence of soft tissue densities or with mild to moderate turbinate destruction and scattered areas of soft tissue densities (Lefebvre *et al.,* 2005). Turbinate destruction was less severe in dogs with lymphoplasmacytic rhinitis compared with dogs with fungal rhinitis or nasal neoplasia (Windsor and Johnson, 2006).

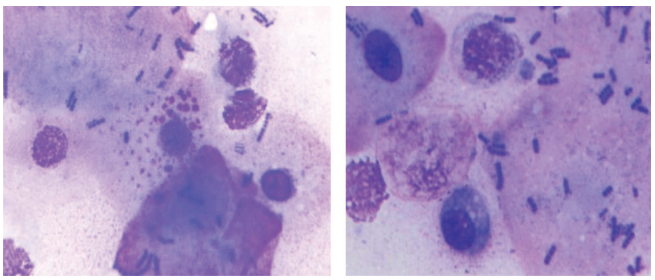
The rhinoscopic findings in dogs with chronic inflammatory rhinitis were hyperaemia, accumulation of nasal discharge and excessive fragility of nasal mucosa (Plate 3) similar to the findings by Sapierzyński and Żmudzka (2009). A combined approach with computed tomography and rhinoscopy ruled out other primary nasal lesion and confirmative diagnosis by histopathological examination was made by multiple biopsies from nasal mucosa.

Neutrophils, lymphocytes and plasma cells along with epithelial cells, bacterial rods and cocci were observed in the cytology of nasal swabs in dogs with chronic inflammatory rhinitis (Plate 4). Similar cytological features were described by Burton (2018) in dogs with chronic inflammatory rhinitis.

Chronic inflammatory rhinitis was characterized histopathologically by the appearance of nasal mucosal inflammation dominated predominantly by lymphocytes and plasma cells and to some extent by neutrophils (Plate 5) in accordance with the findings of Furtado and Constantino-Casas (2013). Histopathological changes in chronic inflammatory rhinitis were assessed based on the proportion of infiltrating inflammatory cell type;

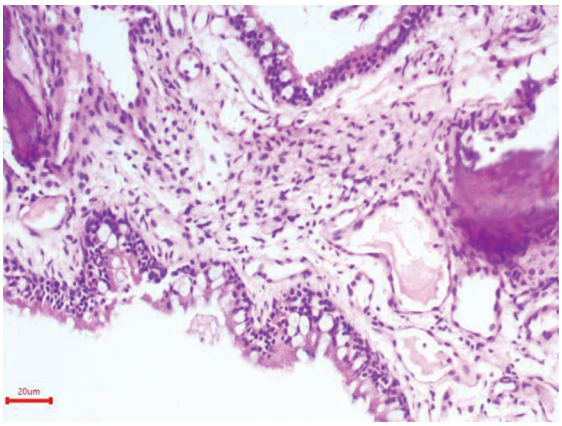


Normal nasal cytology: squamous epithelial cells with abundant eosinophilic cytoplasm 10X

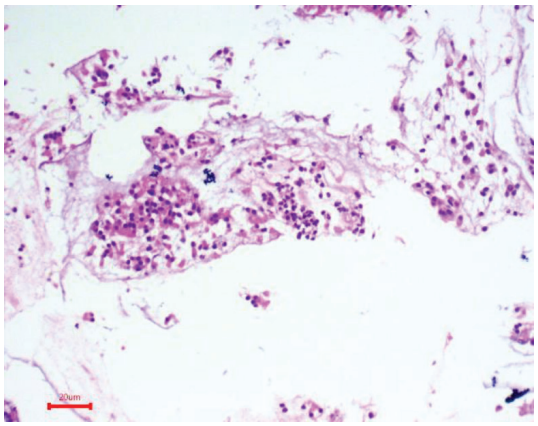


Nasal cytology of dog with chronic inflammatory rhinitis: Lymphocytes and plasma cells along with epithelial cells and bacteria 100X

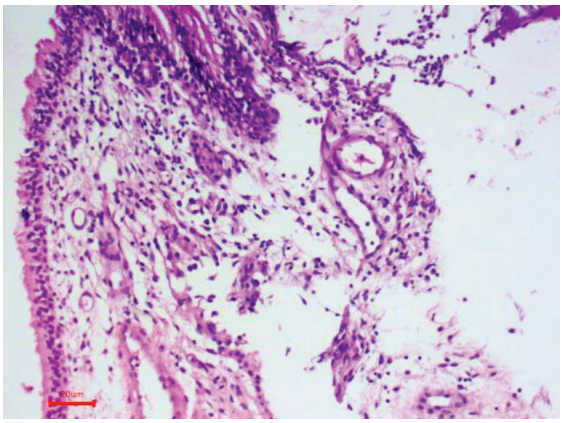
Plate 4: Nasal Cytology



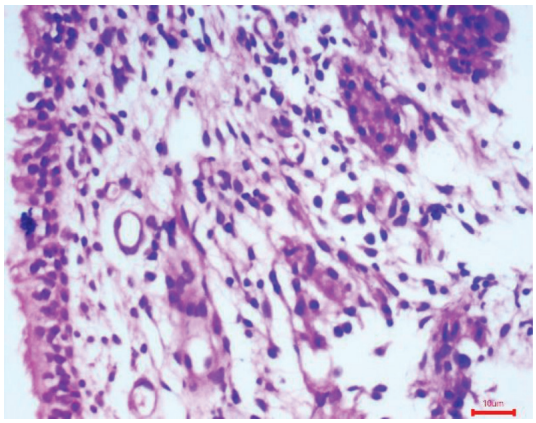
a. Mild degeneration of nasal epithelium with mononuclear cells infiltration, edema and fibrosis H&E- Bar=20µm



b. Neutrophil and mononuclear cells infiltration H&E- Bar=20µm



c. Lymphocyte and plasma cell infiltration -H&E- Bar=20µm



d. Lymphocyte and plasma cell infiltration -H&E- Bar=10µm

Plate 5 : Histopathology of nasal mucosa in dogs with chronic inflammatory rhinitis

lymphocytes, neutrophils, plasma cells and eosinophils, severity of inflammation, mucosal oedema and hyperplasia of epithelial and goblet cells (Kaczamer *et al*., 2018).

In dogs with chronic inflammatory rhinitis administered with prednisolone (@ 1mg/kg orally for three weeks followed by 0.5 mg/kg orally for one-week, gradual improvement in clinical signs was observed after 2 weeks followed by complete clinical resolution after 4 weeks. However, relapse of clinical signs was reported once the medication was stopped. No significant difference was observed in the mean haematology and serum biochemistry parameters before and after therapy. Windsor *et al.* (2004) and Lobetti (2014) reported that treatment with glucocorticoids lead to relapse of clinical signs in dogs with chronic inflammatory rhinitis whereas Burgener (1987) reported that glucocorticoids were effective in resolving clinical signs in 80 per cent of the dogs with chronic inflammatory rhinitis.

In dogs with chronic inflammatory rhinitis treated with meloxicam (@ 0.1mg/kg orally for three weeks) followed by prednisolone (@ 0.5mg/kg orally for one week), gradual improvement in clinical signs was observed after 2 weeks followed by complete clinical resolution after 4 weeks followed by no relapse. No significant difference was observed in the mean haematology and serum biochemistry parameters before and after therapy. The combination therapy with meloxicam and prednisolone was found to be most effective treatment for chronic inflammatory rhinitis according to the studies by Kaczamer (2018).

Based on the present study, it is concluded that, computed tomography of skull combined with rhinoscopy-

guided biopsy and histopathologic analysis in dogs with chronic inflammatory rhinitis provided confirmative diagnosis along with details on tissues of nasal cavity and extent of involvement of adjacent structures. Treatment with meloxicam (@ 0.1mg/kg orally for three weeks) followed by prednisolone (@ 0.5mg/kg orally for one week) produced complete remission and no recurrence in dogs with chronic inflammatory rhinitis when compared to dogs treated with prednisolone (@ 1mg/kg orally for three weeks followed by 0.5 mg/kg orally for one-week.

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Comparative therapeutic approach for anaemia in goats

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Abstract

The present study was conducted at Veterinary Clinical Complex, Veterinary College, Bidar, to know the therapeutic efficacy of various haematinics including blood transfusion in 24 anaemic goats. Selected goats were treated for endecto-parasites prior to therapeutic protocol. Detailed signalment, history, faecal, blood smear and clinical examination with recording of rectal temperature, respiration rate and heart rate were done. Moderate anaemic goats of Group IA were supplemented with ferric ammonium citrate complex orally, Group IB were treated with Inj iron dextran intramuscularly, severe anaemic goats of Group IIA were treated with Inj iron dextran intravenously and Group IIB with blood transfusion at the recommended dosage. Haemato bio-chemical parameters increased significantly ($p \leq 0.05$) post therapy in all the four group of goats. Post therapy improvement was earlier and significant ($p \leq 0.05$) with oral haematinic (Group IA) than parenteral iron (Group IB) in moderate anaemic goats and with blood transfusion (Group IIB) than parenteral iron (Group IIA) in severe anaemic goats. On comparative evaluation, it was found that oral haematinic was more efficacious than parenteral iron in moderate anaemia, whereas blood transfusion was superior to intravenous iron in treatment of severe anaemia in goats.

Key words: Moderate anaemia, Severe anaemia, Haematinics, Blood transfusion

Gastro-intestinal parasitism is the fore most cause of anaemia in goats and its prevalence is higher in young ones in comparison to adults (Singh *et al*., 2015). Severe infestation of acarids also linked to anaemia in goats. Nutritional deficiency is also incremented as major underlying cause of anaemia in flock and is further aggravated by non-supplementation of concentrates and mineral mixture in grazing goat results in deficiency of minerals and vitamins (Bhagure, 2002). Gastrointestinal parasites apart from causing loss of production, body weight and meat production, also make them more susceptible for diseases (Asif *et al*., 2014). Anaemia is characterized by decrease in hematocrit, erythrocyte mass and/or haemoglobin concentration leading to tissue hypoxia. It is the most common and important clinical condition in goats found to be associated with external, internal parasitic infestation, haemoprotozoan diseases and nutritional deficiency in goats (Shinde, 2007, Shalini, 2011 and Rajendra *et al*., 2021). Therapeutic protocol recommended for anaemia includes oral and parenteral haematinics and even blood transfusion in severe anaemic goats as lifesaving procedure (Sharma *et al*., 2009).

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Materials and Methods

Selection of goats

Twenty-four clinically anaemic goats were selected for the evaluation of therapeutic efficacy of various hematinics including blood transfusion as a part of therapeutic protocol. All the selected goats were treated for cypermethrin @ 2 mL per liter of water externally for the control of ectoparasites (Satale, 2001) and were dewormed using albendazole @ 10 mg/Kg body weight (Gondachar, 2002) for the control of endoparasites prior to therapeutic trail.

Clinical examination

Detailed signalment of age, breed, sex and body weight were recorded from the goats. A brief history of grazing, deworming, dipping, vaccination, feed and water intake, rumination, pregnancy status, kidding status, fever, pain and duration of illness, were recorded. Detailed clinical examination of anaemic goats was done by recording the parameters such as conjunctival mucus membrane, oral mucosa, vaginal mucosa, body condition, hair coat, presence of external parasites and their severity, soiled hind quarters, respiratory distress, exercise intolerance and recumbency. Further faecal samples were collected and subjected to microscopic

Table 1. Therapeutic protocol for anaemic goats

Groups	Moderate anaemia (n=12 goats)		Severe anaemia (n=12 goats)	
	IA (n=6 goats)	IB (n=6 goats)	IIA (n=6 goats)	IIB (n=6 goats)
Therapy	Ferric ammonium citrate complex 10 mL per goat per day per oral route for 15 days regularly	4 mg x Δ Hb x BW = Total dosage in milligram administered in divided doses on alternate day by deep intra muscular route	4 mg x Δ Hb x BW = Total dosage in milligram administered in divided doses on alternate day by intravenous route in 100 mL normal saline	Blood transfusion @ 10 mL of blood per kg body weight slow intravenous route using transfusion set

Hb = Haemoglobin Δ Haemoglobin = Desired haemoglobin – actual haemoglobin Desired Haemoglobin = 8 g/dL.

examination by parasitological techniques (Soulsby, 1984). Rectal temperature (°F), heart rate (beats per minute) and respiratory rate (breaths per minute) was recorded as per the standard procedures (Kelly, 1984).

Therapeutic protocol for anaemic goats

Among selected 24 anaemic goats selected for therapeutic trail, were divided into group I consist of 12 anaemic goats with haemoglobin value between 5-8 g/dL diagnosed as moderate anaemic goats, which were further sub divided into 6 goats in each group as IA and IB. Moderate anaemic goats in group IA were supplemented with ferric ammonium citrate complex (3-D RED®) at the rate of 10 mL per goat per day by oral route for 15 days (Sarkar, 1989 and Shinde, 2007) as a therapeutic protocol. Group IB moderate anaemic goats were supplemented with injection iron dextran (Imferon®), total dosage administered in divided doses on alternate day by deep intra muscular route as a therapeutic protocol (Ghadge, 2008). Twelve anaemic goats with haemoglobin value below 5 g/dL were diagnosed as severe anaemic (Santiago *et al*., 1975) with group II, which were further sub divided into 6 goats in each group as IIA and IIB. Group IIA were supplemented with injection iron dextran (Imferon®) in divided doses on alternate day by intravenous route in normal saline as a therapeutic protocol (Shalini, 2011). Group IIB severe anaemic goats were administered with blood transfusion at the rate 10 mL per Kg body weight using CPDA blood transfusion bag and blood transfusion set by slow intravenous route (Dey, 2017).

Haematological analysis

After proper restraining of goat, 2 mL blood was collected aseptically from the jugular vein in EDTA (CML BIO-TECH (P) LTD INDIA, *Safelab*®K2 EDTA) coated vial (1mg/mL) for the estimation of RBC count ($x10^6/\mu L$)), Haemoglobin (g/dL), Packed cell volume (PCV %), Mean corpuscular volume (MCV fL), Mean haemoglobin concentration (MCH pg), Mean corpuscular haemoglobin concentration (MCHC g/dL) and Platelet count (PLT $x10^3/\mu L$) using fully automatic haematology analyser (ERMA PCE 210® and results were recorded

Table 2. Changes in Mean ± SE values of clinical parameters in goats with moderate and severe anaemia

Clinical parameters	Severity	Moderate anaemia (n=12)		Severe anaemia (n=12)	
	Period	IA (n=6)	IB (n=6)	IIA (n=6)	IIB (n=6)
Rectal temperature (˚F)	0 day	$103.11 \pm 0.1^{1a}x$	103.25 ± 0.27^{ax}	103.17 ± 0.27^{ax}	101.50 ± 0.44^{by}
	10th day	103.28 ± 0.09^{ax}	103.30 ± 0.08^{ax}	102.70 ± 0.23^{ay}	101.90 ± 0.25^{by}
	15th day	103.40 ± 0.29^{ax}	103.46 ± 0.06^{ax}	102.70 ± 0.16^{ay}	101.80 ± 0.29^{by}
Respiratory Rate (breaths per minute)	0 day	26.33 ± 0.67^{ax}	26.67 ± 0.49^{ax}	33.17 ± 0.31^{ax}	34.50 ± 1.02^{ax}
	10th day	21.17 ± 0.40^{bx}	22.50 ± 1.02^{by}	25.50 ± 0.62^{bx}	23.67 ± 0.88^{by}
	15th day	19.67 ± 0.56^{bx}	20.17 ± 0.31^{cz}	20.17 ± 0.54^{cx}	19.50 ± 0.72^{cx}
Heart Rate (beats per minute)	0 day	82.67 ± 0.99^{ax}	81.83 ± 0.91^{ax}	93.67 ± 1.20^{ax}	94.50 ± 1.38^{ax}
	10th day	74.33 ± 1.09^{bx}	75.50 ± 0.34^{by}	82.17 ± 1.37^{bx}	79.33 ± 0.99^{by}
	15th day	71.50 ± 0.50^{cx}	72.17 ± 0.75^{cy}	72.33 ± 0.88^{cx}	74.34 ± 0.84^{cy}

Note: Mean ± SE with different superscripts (a, b and c) within groups and (x, y) between groups differ significantly at ($p < 0.05$).

on 0 day, 10th day and 15th day of the therapeutic trial.

Bio-chemical analysis

4 mL blood was aseptically collected from the jugular vein in serum collection vial, centrifuged at 5,000 rpm for 5 minutes to separate the serum. Serum collected from anaemic goats was used for the determination of total protein, albumin and glucose using semi-automated biochemical analyser (MICROLAB-300®, Eli Tech Group) using commercially available ERBA® kits as per standard procedure on 0 day and 15th day of therapeutic protocol. Serum globulins were calculated by subtracting the serum albumin from serum total protein and the results were expressed in g/dL. Albumin: Globulin ratio was calculated by dividing Albumin values with Globulin values and results were expressed as A: G Ratio.

Statistical analysis

Data obtained in the present study analysed by Statistical methods described by Snedecor and Cochran (1994).

Results and Discussion

Diagnosis of anaemia in goats

Goats with pale pink conjunctival mucus membrane and haemoglobin value more than 5 and less than 8 g/dL were diagnosed as moderately anaemic and goats with pale or paper white conjunctival mucus membrane and haemoglobin value less than 5 g/dL were diagnosed as severely anaemic. These findings are in accordance with Santigo *et al*., (1975) and Mohanmbal *et al*., (2018). Parasitic infestation has been reported to be the cause of moderate and severe anaemia in goats (Sarkar, 1989). Erythrocyte indices confirmed normocytic normochromic anaemia in moderate anaemic group of goats and normocytic hypochromic in severe anaemic goats in the present study. These findings are in accordance with Shinde (2007) and Shalini (2011).

Clinical signs in anaemic goats

Pale mucus membrane, lethargy, rough hair coat, variable appetite, loose faeces, mild dehydration moderate anaemic goats and paper white mucosae, cachexia, exercise intolerance and sternal to lateral recumbency in severely anaemic goats were important clinical observations in present study. These findings were in accordance with Sarkar (1989), Shalini (2011), Dey (2017), Bhatane (2018) and Tufani *et al*., (2018). Loss of blood due to ectoparasites and endoparasites, disturbances in digestion and absorption of essential elements in endoparasitic infection (Sarkar, 1989), inability to cope up with normal activity due to lack of oxygen carrying capacity in anaemic goats (Shinde, 2007 and Ghadge, 2008) have been attributed for observations of these signs in anaemic goats which were more pronounced in severely anaemic goats. Goats became active, appetite return to normal, regained its mucus membrane colour to pinkish, glistening hair coat, passing pelleted faeces and all clinical signs were returned to normal post therapy. These findings were in accordance with Sarkar (1989),

Table 3. Changes in Mean ± SE values of haematological parameters in goats with moderate and severe anaemia

Haematological parameters	Severity	Moderate anaemia (N=12)		Severe anaemia (N=12)	
	Period	IA (n=6)	IB (n=6)	IIA (n=6)	IIB (n=6)
RBC Count (x10^6/µL)	0 day	7.20 ± 0.29^{ax}	7.28 ± 0.12^{ax}	4.17 ± 0.15^{ax}	3.98 ± 0.59^{ax}
	10th day	9.57 ± 0.27^{bx}	8.70 ± 0.15^{by}	6.33 ± 0.21^{bx}	7.80 ± 0.89^{by}
	15th day	11.93 ± 0.26^{cx}	10.47 ± 0.14^{cy}	8.73 ± 0.22^{cx}	11.68 ± 0.51^{cy}
Haemoglobin (g/dL)	0 day	7.07 ± 0.22^{ax}	6.82 ± 0.14^{ax}	3.88 ± 0.18^{ax}	3.03 ± 0.64^{ax}
	10th day	9.10 ± 0.11^{bx}	8.05 ± 0.16^{by}	5.72 ± 0.23^{bx}	6.24 ± 0.64^{by}
	15th day	11.02 ± 0.26^{cx}	9.70 ± 0.12^{cy}	7.70 ± 0.11^{cx}	8.59 ± 0.37^{cy}
Packed cell volume (%)	0 day	21.72 ± 0.70^{ax}	21.20 ± 0.40^{ax}	12.75 ± 0.41^{ax}	11.07 ± 1.80^{ax}
	10th day	27.67 ± 0.42^{bx}	25.27 ± 0.49^{by}	20.33 ± 0.81^{bx}	21.39 ± 0.91^{bx}
	15th day	33.78 ± 0.84^{cx}	29.5 ± 0.37^{cy}	24.93 ± 0.41^{cx}	29.10 ± 1.15^{cy}
Platelet Count (x10^3/µL)	0 day	146.54 ± 3.12^{ax}	144.02 ± 3.89^{ax}	109.63 ± 3.31^{ax}	118 ± 5.72^{ay}
	10th day	177.46 ± 3.36^{bx}	185.47 ± 3.40^{by}	132.83 ± 5.03^{bx}	168.70 ± 6.96^{by}
	15th day	209.90 ± 2.00^{cx}	206.40 ± 2.90^{cx}	187.17 ± 7.36^{cx}	216.50 ± 5.78^{cy}

Note: Mean ± SE with different superscripts (a, b and c) within groups and (x, y) between groups differ significantly at ($p < 0.05$).

Table 4. Changes in Mean ± SE values of erythrocyte indices in goats with moderate and severe anaemia

Erythrocyte indices	Severity	Moderate anaemia (N=12)		Severe anaemia (N=12)	
	Period	IA (n=6)	IB (n=6)	IIA (n=6)	IIB (n=6)
MCV (fL)	0 day	30.25 ± 0.57^{ax}	29.12 ± 0.23^{ax}	30.65 ± 0.35^{ax}	27.03 ± 1.07^{ay}
	10th day	29.50 ± 1.17^{ax}	29.03 ± 0.23^{ax}	32.17 ± 0.24^{bx}	26.48 ± 0.95^{ay}
	15th day	28.33 ± 0.60^{ax}	28.20 ± 0.18^{ax}	28.58 ± 0.53^{cx}	26.93 ± 0.13^{by}
MCH (pg)	0 day	9.82 ± 0.18^{ax}	9.40 ± 0.08^{ax}	8.92 ± 0.11^{ax}	8.80 ± 0.20^{ay}
	10th day	9.70 ± 0.33^{ax}	9.27 ± 0.07^{ax}	9.03 ± 0.11^{ax}	8.45 ± 0.53^{ax}
	15th day	9.23 ± 0.18^{ax}	9.23 ± 0.03^{ax}	8.62 ± 0.20^{ax}	8.63 ± 0.08^{ax}
MCHC (g/dL)	0 day	32.53 ± 0.11^{ax}	32.17 ± 0.25^{ax}	30.42 ± 0.11^{ax}	29.13 ± 0.14^{ax}
	10th day	32.82 ± 0.24^{ax}	31.85 ± 0.08^{ax}	28.12 ± 0.21^{bx}	30.92 ± 0.90^{ay}
	15th day	32.62 ± 0.13^{ax}	32.90 ± 0.15^{ax}	31.17 ± 0.18^{cx}	30.63 ± 0.70^{ax}

Note: Mean ± SE with different superscripts (a, b and c) within groups and (x, y) between groups differ significantly at ($p < 0.05$).

Shalini (2011), Kaltungo *et al., (*2016) and Tufani *et al*., (2018). However, improvement in clinical signs was fast and near to normal in oral haematinic group (Group IA) than parenteral iron (Group IB) in moderate anaemic goats and in blood transfusion (Group IIB) than parenteral iron (Group IIA) in severe anaemic goats.

Clinical parameters in anaemic goats

Significant increase ($p \leq 0.05$) in respiratory and heart rate in goats with moderate and severe anaemia were recorded in present investigation which was more pronounced in severe anaemic goats. The present observation is in accordance with earlier reports of Sarkar (1989), Shalini (2011) and Bhatane (2018).

However, no significant variation ($p \leq 0.05$) was recorded in rectal temperature in goats with moderate anaemia and severe anaemia pre and post therapy, these findings are in accordance with earlier reports of Shalini (2011) and Dey (2017).

Increase in respiratory and heart rate in anaemia were important observations which had been attributed to compensatory mechanism due to reduced oxygen perfusion to tissues and increased cardiac output and lung perfusion and compensatory mechanism to decrease the circulation time of RBC as reported by Shinde (2007) and Shalini (2011).

Post therapy, respiratory and heart rate regained to normal physiological values in both moderate and severe anaemic group of goats. However, improvement was earlier and significant ($p \leq 0.05$) in oral haematinic group (Group IA) than parenteral iron (Group IB) in moderate anaemia and in blood transfusion (Group IIB) than parenteral iron (Group IIA).

Significant increase ($p \leq 0.05$) in respiratory and heart rate in goats with moderate and severe anaemia were recorded in present investigation which was more pronounced in severe anaemic goats. The present observation is in accordance with earlier reports of Sarkar (1989), Shalini (2011), Goklaney (2011) and Bhatane (2018). However, no significant variation ($p \leq 0.05$) was recorded in rectal temperature in goats with moderate anaemia and severe anaemia pre and post therapy, these findings are in accordance with earlier reports of Shalini (2011) and Dey (2017). Post therapy, respiratory and heart rate regained to normal physiological values in both moderate and severe anaemic group of goats. However, improvement was earlier and significant ($p \leq 0.05$) in oral haematinic group (Group IA) than parenteral iron (Group IB) in moderate anaemia and in blood transfusion (Group IIB) than parenteral iron (Group IIA).

Haemogram changes in anaemic goats

Significant decrease ($p \leq 0.05$) in RBC Count, haemoglobin, packed cell volume and platelet count was recorded in present investigation on goats with moderate and severe anaemia. These changes were more predominant in severe anaemia than moderate anaemia which are in agreement with earlier reports of Sarkar (1989), Shalini (2011) and Dey (2017). Ecto and endo parasitism has been attributed as most important cause of anaemia owing to loss of blood from the host. Chronic blood loss also compounds iron deficiency in such animal which has been attributed to inadequate RBC regeneration as well as incorporation of haemoglobin (Naigamwalla *et al*., 2012). The lowered changes in Haemogram were reverted to near normal after therapeutic intervention.

Table 05. Changes in Mean ± SE values of bio-chemical parameters in goats with moderate and severe anaemia

Bio-chemical parameters	Severity	Moderate anaemia (N=12)		Severe anaemia (N=12)	
	Period	IA (n=6)	IB (n=6)	IIA (n=6)	IIB (n=6)
Blood glucose (mg/dL)	0 day	44.83 ± 1.64^{ax}	39.17 ± 1.9^{ax}	29 ± 1.12^{ax}	28 ± 3.26^{ax}
	15th day	65.67 ± 1.11^{bx}	56.67 ± 1.54^{by}	60.34 ± 1.52^{bx}	68.16 ± 1.4^{by}
Total protein (g/dL)	0 day	4.2 ± 0.08^{ax}	4.35 ± 0.12^{ax}	3.94 ± 0.29^{ax}	3.48 ± 0.40^{ax}
	15th day	7.12 ± 0.06^{bx}	6.2 ± 0.14^{by}	6.03 ± 0.09^{bx}	7.48 ± 0.32^{by}
Albumin (g/dL)	0 day	1.74 ± 0.04^{ax}	1.8 ± 0.04^{ax}	1.69 ± 0.13^{ax}	1.48 ± 0.17^{ax}
	15th day	3.58 ± 0.05^{bx}	3.18 ± 0.07^{by}	2.91 ± 0.03^{bx}	3.73 ± 0.14^{by}
Globulin (g/dL)	0 day	2.47 ± 0.04^{ax}	2.52 ± 0.07^{ax}	2.14 ± 0.15^{ax}	2.01 ± 0.22^{ax}
	15th day	3.67 ± 0.06^{bx}	3.18 ± 0.07^{by}	3.11 ± 0.03^{bx}	3.75 ± 0.18^{by}
Albumin: Globulin Ratio	0 day	0.70 ± 0.01^{ax}	0.72 ± 0.01^{ax}	0.75 ± 0.01^{ax}	0.74 ± 0.01^{ax}
	15th day	1.01 ± 0.02^{bx}	0.94 ± 0.01^{by}	0.96 ± 0.01^{bx}	1 ± 0.02^{by}

Note: Mean ± SE with different superscripts (a, b and c) within groups and (x, y) between groups differ significantly at ($p < 0.05$)

However, improvement was earlier and significant ($p \leq 0.05$) in oral haematinic group (Group IA) than parenteral iron (Group IB) in moderate anaemia and in blood transfusion (Group IIB) compared to parenteral iron (Group IIA).

Changes in erythrocytic indices in anaemic goats

Non-significant ($p \leq 0.05$) decrease in MCV, MCH and MCHC was recorded in present investigation in goats with moderate and severe anaemia. These changes were more predominant in severe anaemic goats. Altered erythrocyte indices were suggestive of normocytic and normochromic anaemia in moderate anaemic group of goats and normocytic and hypochromic anaemia in severe anaemic goats. These findings are in accordance with Shinde (2007) and Shalini (2011).

Bio-chemical changes in anaemic goats

Hypoglycaemia, hypoproteinaemia, hypoalbuminaemia, hypoglobulinemia and altered Albumin: Globulin Ratio were significant ($p \leq 0.05$) in goats with moderate and severe anaemia on the day of presentation. These findings were in accordance with earlier reports of Satale (2001) and Goklaney (2011). Prolonged inappetence and defective glucose metabolism (Goklaney, 2011), anoxia and alteration in carbohydrate metabolism due to reduction in red blood cells and haemoglobin concentration (Ramesh,1998), reduced absorption of nutrients from gut, rapid absorption, and utilization of nutrients by helminth in the gastro-intestinal tract (Pophale, 2002) have been attributed as major reasons for hypoglycaemia in goats. Similarly, inappetence, absence of concentrate supplementation in extensive type of rearing (Pophale, 2002), catabolism of protein by parasitism and loss of protein from damaged intestinal mucosa (Bhagure, 2002), poor absorption of dietary proteins, enteritis, loss of plasma proteins in blood loss (Gondachar, 2002) and anorexia and disturbances in liver function have been proposed as causes of hypoproteinaemia, hypoalbuminaemia and hypoglobuminemia in anaemic goats. Decreased level of Albumin caused decreased A: G Ratio in anaemic goats (Haq *et al*., 2017). These lowered values of biochemical parameters viz., blood glucose, total proteins, Albumin, Globulin and Albumin: Globulin Ratio increased significantly ($p \leq 0.05$) on day 15 post treatment in all the four group of goats. These findings are in accordance with earlier reports of Satale (2001) and Goklaney (2011). However, improvement was earlier and significant ($p \leq 0.05$) in oral haematinic (Group IA) than parenteral iron (Group IB) in moderate anaemia and in blood transfusion (Group IIB) compared to parenteral iron (Group IIA).

Conclusion

Supportive haematinics therapy is essential to regain the normal physiological status from anaemia in goats. Oral haematinic therapy will be ideal for moderate anaemic goats whereas blood transfusion will be more suitable supportive therapy for severe anaemic goats to regain normal haemato-biochemical parameters in general and overall health.

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Evaluation of a polyherbal preparation applied topically in curing intramammary infections and subsiding udder inflammation in dairy cows

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Abstract

Excessive use of antibiotics may result in establishment of antibiotic resistance, human sensitivity disorders and adverse effects on the dairy industry. So, an antibiotic alternate strategy for successfully treating the disease is preferred. In present study, a polyherbal preparation "Sepal" developed by the Post-graduate Institute of Medical Research, Chandigarh, was evaluated in the treatment of specific subclinical mastitis in 13 HF × Sahiwal crossbred cows in their early lactation. The health status of the quarters was defined considering culture and somatic cell count (SCC) results of quarter foremilk (QFM) for three consecutive days, following the IDF guidelines. The QFM and udder composite milk samples were collected pre-treatment (0d), and 7 and 21 d post-treatment. The samples were analyzed for bacteriology, CMT, SCC, electrical conductivity (EC), and biochemical composition to analyze the effect of therapy on intramammary infections (IMI), udder inflammation and milk quality. The udder of cow was cleaned properly, dried, and stimulated for letdown of milk. The, drug was then applied morning and evening for 07 consecutive days by massaging on the quarters/ udder portions affected with specific or non-specific mastitis. The therapy could cure 12/22 (54.54%) quarters with specific or non-specific mastitis, i.e., IMI eliminated, and milk SCC reduced to $<400\times10^3$ cells/ml at 21d post-last treatment. The therapy could eliminate 08/13 (61.54%) of specific IMI and 05/07 (71.43%) latent infections. No new IMI was established in the treated quarters, while 09/23 (39.13%) of untreated quarters developed IMI. The milk SCC, EC, and CMT scores decreased significantly ($p<0.05$) post-treatment. At the cow level, a significant improvement in the biochemical composition of milk occurred; while milk SCC and CMT score decreased, the SNF, density, protein, and lactose increased. Thus, the herbal topical therapy may be recommended to treat early cases of mastitis in dairy cows.

Keywords: Cows, Mastitis, Topical herbal therapy, Cure, Milk quality

Despite adopting various disease control strategies at the farm, mastitis cases continue. Although many cases of mastitis may be successfully treated with antibacterial therapy, the use of antibiotics has its side effects. Intramammary infusion of antibiotics has been observed to be immune suppressive, and excessive use of antibiotics results in antibiotic ineffectiveness with the establishment of antibiotic resistance and resistance genes. The impact of antibiotic usage on the production of dairy products and the emergence of human sensitivity disorders are other harmful side effects. To avoid the side effects of the antibiotics used to treat both clinical and sub-clinical mastitis, a different strategy is required in successfully treating and preventing the condition without exhibiting any adverse side effects. For example, early mild cases of mastitis may be treated with antibiotic alternatives that are more cost-efficient, environmentally friendly, secure, and effective in treating the disease. A significant amount of study on various plant species and their medicinal properties is currently being done to revalue traditional medicine worldwide. The World Health Organization has acknowledged its effectiveness against multiple diseases. Many medicinal herbs have been found to serve as antibiotic alternatives that efficiently control microorganisms without causing undesirable side effects. Likewise, the Post-graduate Institute of Medical Research, Chandigarh, developed a polyherbal preparation called "Sepal" (drug components not revealed). The preparation is claimed to possess antibacterial and anti-inflammatory characteristics. Assuming that this polyherbal preparation may prove beneficial in treating early cases of mastitis, a study was planned to assess its therapeutic potential in specific subclinical mastitis of dairy cows.

Materials and Methods

Animals and Collection of milk samples

In this study, 13 HF × Sahiwal crossbred cows in their early lactation having at least one quarter affected with specific subclinical mastitis were involved. The

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trial was conducted at an organized dairy farm where the animals were maintained under a semi-loose housing system with sandy and brick or concrete floors.

The quarter foremilk samples from the enrolled cows were collected, taking all possible aseptic precautions thrice before the start of therapy (0 d), at 07 days, and 21 days post-last treatment. Additionally, on completion of the milking, about 30 ml of udder/ cow composite milk sample was collected in a clean plastic vial. Every time, the milk samples from each quarter/cow were collected and analyzed for bacteriology, electrical conductivity (EC), SCC, and biochemical composition for 03 consecutive days. The health status of the quarters was defined considering the 03 days QFM SCC and culture results as per IDF guidelines as follows. Also, the SCC and other biochemical parameters of cow composite milk represented the average of 03 days values to enhance data accuracy.

Quarter health status	**Status of bacteriology and SCC analysis of QFM of 03 consecutive days**	
	Bacteriology	**Somatic cell count (cells/ml milk)**
Healthy	≥ two times negative	≥ 2 times < 400,000
Latent mastitis	≥ two times positive	≥ 2 times < 400, 000
Nonspecific mastitis	≥ two times negative	≥ 2 times ≥ 400, 000
Specific mastitis	≥ two times positive	≥ 2 times ≥ 400, 000

The cows included in the trial represented 13 specific, 09 non-specific, 07 latent infections, and 23 healthy quarters.

*Analytical Procedures Used***:**

(i) Bacteriology of milk samples

Culturing milk samples for bacterial isolation and identification was done per the standard microbiological procedures (Brown *et al.*, 1969). The 5% sheep blood agar was used as the primary medium for culturing milk samples. Each blood agar plate was marked to have four parts specific to individual quarters of the animal. The 0.05 ml of QFM sample was inoculated on the respective part of the agar plate with a platinum loop following all sterilization precautions. The plates were kept overnight in the incubator, having the temperature set at 37°C. The plates were examined for the appearance of any bacterial growth after18-24 hours and a re-incubated maximum of up to 36 hours to find the development of any slow-growing bacterium such as Corynebacterium. The presence of any bacterial growth was further processed for identification of the organism through MALDI-TOF MS (Matrix-assisted laser desorption ionization-time of flight mass spectrometry). During the MALDI-TOF MS process, microbes are identified using either intact cells or cell extracts. The process is rapid, sensitive, and economical in terms of both the labor and costs involved. The working principle of MALDI-TOF MS is based on the analysis of chemical compounds and measuring the mass-to-charge (m/z) ratio for identifying the microbe. For microbiological applications, mainly TOF mass analyzers are used (Singhal *et al*., 2015)

(ii) Milk SCC

The somatic cell count of milk was analyzed by SomaScope Smart from DELTA Instruments, The Netherlands. The machine worked on the principle of Flow Cell Cytometry. Results were expressed in $\times 10^3$ cells/ ml of milk.

(iii) Modified California Mastitis Test

Sodium lauryl sulphate was used as the test reagent. Otherwise, the standard procedure and interpretation for CMT were used.

(iv) Electrical conductivity

The electrical conductivity was recorded in milli Siemens per cm (mS/cm) with the help of a Digital Conductivity Meter (Eutech Instruments, CON 700).

(v) Biochemical composition of the milk

To determine the milk's biochemical composition, i.e., fat, SNF, protein, lactose, and density, milk samples were brought to room temperature before analysis. It was analyzed using the Milk analyzer "Lactoscan LA from Milkotronic LTD, Bulgaria," where results were expressed in % (W/V).

Drug application

The farm was visited during the routine milking times. After thorough cleaning, dryness, and stimulation of the udder for a milk letdown, the herbal preparation was applied and massaged one by one on the quarters (udder portions) affected with specific or non-specific mastitis. The drug was used morning and evening for 07 consecutive days.

Table 1. Elimination of existing intramammary infections in treated and untreated quarters

Organism causing IMI	Elimination of IMI					
	Treated Quarters (n=22) (13 Specific Mastitis and 09 non-specific mastitis)			Not treated quarters (n = 30) (23 Healthy Quarters and 07 Latent infections)		
	IMI present Pre-treatment	IMI eliminated post last treatment at		IMI present pre-treatment	IMI eliminated post-last treatment at	
	0d	7d	21d	0d	7d	21d
S. aureus	6	5	4 (66.67)	03	01	01 (33.33)
S. chromogenes	3	2	2 (66.67)	02	02	02 (100)
S. hemolytic	3	0	1 (33.33)	01	01	01 (100)
S. hominis	1	1	1 (100)	-	-	-
Actinobacteria ursingii	-	-	-	01	01	01 (100)
Total	13	08	08 (61.54)	07	05	05 (71.43)

Elimination of infections: $\chi^2 = 0.1956$. P = 0.6582 Not significant at p<.05
Occurrence of new IMI in eligible quarters: Treated group 0/9; untreated group 09/23 (39.13%); $\chi^2 = 3.234$ (P = 0.07)
Overall cure rate of specific and non-specific mastitis quarters, i.e., IMI eliminated, and milk SCC reduced to $<400\times10^3$ cells/ml at 21d post last treatment = 12/22 quarters = 54.54%

Evaluation of the effectiveness of therapy

The effectiveness of the therapy was determined in terms of the elimination of existing intramammary infections, the establishment of new infections in eligible quarters (i.e., quarters free from infection), subsiding of udder inflammation as evident from milk SCC/ CMT score and the effect of biochemical milk composition (Fat, Total Protein, Lactose and SNF, and electrical conductivity) 7 and 21 d post last application of therapy. The significance of results concerning the effect of treatment on SCC, EC, and the biochemical composition of milk in the post-treatment phase was analszed by applying a t-test. The elimination/ development of new intramammary infections was analysed using the Chi-square test.

Results and Discussion

Twenty-two mastitis quarters (13 specific and 09 non-specific) in HF×Sahiwal crossbred cows identified based on milk high SCC and cultures were treated with topical application of a polyherbal gel. The pathogens of specific subclinical mastitis recorded were *S. aureus (6), S. chromogenes* (3), *S. hemolytic* (3)*, and S. hominis* (1). Overall, therapy could cure 12/22 (54.54%) quarters with specific or non-specific mastitis, i.e., IMI eliminated, and milk SCC reduced to $<400\times10^3$ cells/ml at 21d post-last treatment. The therapy could eliminate 08/13 (61.54%) of specific intramammary infections in treated quarters and 05/07 (71.43%) latent infections in untreated quarters (Table 1), a statistically non-significant difference (p>0.05). These results could be seen in three possible ways (i) Application of the drug proved productive as it could eliminate tough specific mastitis infections (ii) On application, absorption of the drug to adjacent quarters was a natural process, and hence elimination of latent infections present in those quarters might occur (iii) the therapy is not efficacious enough to cure intramammary

Table 2. Effect of therapy on the inflammatory reaction of quarters

Period	Treated Quarters Mean ± SE			Not treated Quarters Mean ± SE		
	SCC ($\times10^3$ cells/ml)	EC (mS/cm)	CMT (score)	SCC ($\times10^3$ cells/ml)	EC (mS/cm)	CMT score
Pre-treatment	1850 ± 281^1	5.80 ± 0.17^1	2.18 ± 0.18^1	142 ± 21^1	4.84 ± 0.10^1	0.27 ± 0.08^1
7d post-treatment	891 ± 234^2	4.85 ± 0.12^2	1.23 ± 0.23^2	366 ± 125^{12}	4.70 ± 0.10^1	0.53 ± 0.16^{12}
21d post-treatment	644 ± 162^3	4.85 ± 0.11^2	1.02 ± 0.20^3	530 ± 165^2	4.83 ± 0.10^1	0.67 ± 0.17^2

Within columns, figures possessing at least one similar superscript do not reveal significant differences (p>0.05)

Table 3. Effect of therapy on SCC and biochemical composition of milk at cow level (Mean ± SE)

Period	SCC (×10³ cells/ml)	CMT Score	Fat %	SNF %	Density	Protein %	Lactose %
Pre-treatment	873±204[1]	1.65±0.22[1]	2.18±0.16[1]	8.81±0.34[1]	32.66±0.55[1]	3.28±0.06[1]	4.93±0.09[1]
7d post-treatment	585±184[2]	0.95±0.31[2]	2.11±0.14[1]	9.49±0.13[2]	34.86±0.44[2]	3.47±0.05[2]	5.20±0.07[2]
21d post-treatment	505± 149[2]	1.18±0.26[12]	2.09±0.18[1]	9.43±0.14[2]	34.49±0.50[2]	3.44±0.05[2]	5.18±0.08[2]

Within columns, figures possessing at least one similar superscript do not reveal significant differences (p>0.05)

infections. No new intramammary infection was established in the treated quarters, while 09/23 (39.13%) of quarters not subjected to drug application showed the development of intramammary infections over 21 days post-treatment. As such, therapy application seems to prevent new intramammary infections, although statistically, results seem to be non-significant, probably due to a limited number of observations. In cows treated for subclinical mastitis with Mastilep gel, a 60% bacteriological cure was noted on the 5th day. This cure rate was 70% following treatment with AV/AMS/15 herbal formulation (Hase *et al*., 2013). Shafi *et al*. (2020), while evaluating *W. somnifera* root powder in the therapy of specific subclinical mastitis in cows, observed 64.28% elimination of intramammary infections ($\chi 2 = 4.14$; 01 df; $p<0.05$). Patel and Gupta, 2020 observed that treating specific subclinical mastitis cases in crossbred cows with non-antibiotic preparation "Magic-3" could cure 70.73% of intramammary infections at 15d and 80.49% of infections at 30d post-treatment. Whereas a therapy with *M. koenigii* (G3) could not demonstrate any considerable fall in the total bacterial count of milk from 0d (34.50 ± 2.73) to 7d (29.00 ± 1.51), but at 14d (22.63 ± 3.73) and 28d (10.88 ± 2.89), post-treatment.

The milk SCC, EC, and CMT scores in treated quarters decreased significantly ($p<0.05$) from pre-treatment to 7 and 21 days post-treatment (Table 2). In untreated quarters, an increase in the milk SCC and CMT score was observed. This could be justified by the observations of Owens et al. (1988), who reported that quarters where infections have not been cured, supported more growth of *Staph aureus* during treatment than those healed. At the cow level, a significant improvement in the biochemical composition of milk was also observed; while milk SCC and CMT score decreased, the SNF, density, protein, and lactose increased post-treatment (Table 3). The role of herbal therapy in lowering inflammatory reactions of the udder and improving milk composition has been reported previously. For example, Hase *et al.* (2013) showed that on the fifth day of therapy, SCC in the AV/AMS/15 sprayed group and Mastilep gel treated group dramatically decreased. Milk output increased in cows with subclinical mastitis, although milk fat percentage remained the same. Vala *et al*. (2013) evaluated the efficacy of herbal teat dip Mastidip liquid in subclinical mastitis in cows. They observed that the pH and SCC returned to normal following treatment, and milk output increased. Correspondingly, a considerable recovery in milk SCC and milk yield was noted in mastitis udders treated with topical application of AV/AMP/34 gel, applied twice daily × 5 days following milking by Ranaut et al. (2015). Tawheed *et al*. (2018) observed that therapy of mastitis udders with "Masticure®," a non-antibiotic preparation, resulted in a significant decrease of udder inflammation as judged by CMT score and SCC, pH, and EC, and an increase in lactose, protein, and SNF. Likewise, oral therapy of specific subclinical mastitis in cows with *O. sanctum* leaf powder proved very beneficial in lowering milk SCC, CMT score, pH, and electrical conductivity of milk (Shafi *et al*., 2018). Thus, polyherbal topical therapy may be recommended to treat mild/ early cases of mastitis in dairy cows.

Conclusions

The topical application of herbal preparation seems to possess good therapeutic potential in curing the intramammary infections and subsiding udder inflammation in specific subclinical of mastitis in dairy cows. The therapy also showed promising results in restoration of compositional milk quality. Thus, mastitis, at least early milk cases of may be best managed by use of effective medicinal herbs.

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Therapeutic and prognostic studies in canine parvoviral gastroenteritis

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Abstract

Canine parvoviral enteritis, a highly contagious and often fatal disease, is characterized by vomiting, foul-smelling haemorrhagic enteritis and severe dehydration. In this study conducted at multispecialty Veterinary Hospital of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, 24 dogs confirmed with CPV Immuno-chromatographic assay(Ubio quick VET rapid Antigen test kit, Cochin, India) were divided into 3 treatment groups (8 dogs each) viz. conventional therapy (Group I), Conventional and amino acid therapy (Group II) and conventional and plasma therapy (Group III). All these dogs were selected based on selection criteria of hypoproteinemia (total protein and albumin <4.5g/dL and <2.0g/dL, respectively). The levels of total protein increased in all the treatment groups within 7-days, however there was maximum rise in level of total protein from 3.9±**0**.11 to 5.93± **0.**14 in group III followed by group II and least rise was seen in group I. Similar improvement was seen in albumin values in group III followed by group II and group I. Out of 24 dogs, 19 survived, while 5 pups died despite treatment. TLC, absolute neutrophils, absolute lymphocyte values were significantly lower in non-survivors as compared to survived dogs. Total protein and albumin levels were almost similar on day of first presentation between survivors and non-survivors groups. However subsequently, these levels increased markedly in survivor groups but showed no improvement in non-survivors' group. The present study concludes that plasma therapy increases total protein and albumin levels in CPV affected pups; and these along with TLC, absolute neutrophils and absolute lymphocyte count can be used as prognostic indicators of CPV.

Key words: CPV, Immunochromatographic assay, Plasma therapy, Parvovirus

Canine parvovirus (CPV) is a well-known disease in dogs, accounting for around 27% of diarrhoeic cases (Sakulwira *et al*., 2001). Canine parvoviral enteritis is highly contagious, often fatal disease, characterized by vomiting, foul-smelling haemorrhagic enteritis, severe dehydration and myocarditis in dogs. CPV is an endemic disease in India, causing high morbidity and frequent mortality in pups (Kumar & Nandi, 2010) . Pups between 6 weeks to 6 months of age are highly susceptible (Hueffer et al., 2003).

Various diagnostic tests include demonstration of CPV in the faeces of affected dogs by immunochromatography (Oh *et al*., 2006) or PCR (Mohyedini *et al*., 2013). If treatment is delayed, CPV causes severe morbidity and mortality in puppies and occasionally adult dogs. The survival rate depends on early diagnosis and aggressive treatment with intravenous fluids (crystalloids and colloids) for correction of hypoglycemia and electrolyte disturbances, antiemetics, antacids, and combination of antibiotics (Bargujar *et al*., 2011).

Hypoalbuminaemia is a common finding in CPV-2 cases and can be due to protein-losing enteropathy, caused by the CPV-2 induced intestinal damage. Albumin facilitates normal platelet function, coagulability and scavenges free radicals; and amino acid drip infusions has shown promising results along with conventional therapies (Kumar *et al*., 2020).Plasma, a natural colloid, could be used in CPV-2 cases as it provides antibodies, crucial serum proteins as well as treat hypovolaemia.

Materials and Methods

The study was performed in Department of Veterinary Medicine at the multispecialty Veterinary Hospital of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab. The criteria of selection of cases was CPV confirmed case with hypoproteinemia (total protein and albumin <4.5g/dL and <2.0g/dL, respectively).

Epidemiological data such as age, breed, gender etc. was recorded and a detailed physical examination was carried out. Two mL blood was aseptically collected from the cephalic or the lateral saphenous venepuncture in sodium EDTA (Ethylenediaminetetraacetic acid) vacutainer vials for the haematological analysis. Five mL of blood was drawn and centrifuged to extract serum for biochemical examination. Blood samples were collected

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on four occasions for CPV i.e. at the time of presentation and before initiation of the treatment (day 0), day 3, day 5 and day 7.

Diagnosis of CPV by Immuno-chromatographic assay

Using a gloved finger, faeces samples were obtained per-rectum from the suspected dogs. Quick VET Qualitative Immuno-chromatographic assay kit (Plate 1a) (Ubio Biotechnology systems Pvt. Ltd, Cochin, India) was used for rapid detection of Canine Parvovirus antigen in canine faeces. 10-15 drops of assay diluent were put into the eppendorf tube and then swab was used to collect faecal sample either directly from rectum or sample provided in the faecal container. Faecal loaded swab was inserted into assay diluent and agitated, then 3 drops of sample were added into sample well provided in the rapid kit and result was checked within 10 minutes (Plate 1b) as per details (Plate 1c). The result was considered invalid after 15 minutes.

Therapeutic studies

24 dogs confirmed with Immuno-chromatographic assay for canine parvoviral enteritis were divided into 3 treatment groups (n=8 each). First group was administered conventional therapy consisted of fluid therapy viz. Ringer's Lactate, Normal Saline Solution or Dextrose Normal Saline (DNS) on the basis of dehydration status along with Ampicillin @10-20 mg/kg b.wt. i.m.b.i.d, amikacin @15-30 mg/kg b.wt. i.m. b.i.d. and metronidazole @15 mg/kg b.wt. i.v. b.i.d. The antioxidant therapy (Vitamin C @20 mg/kg b.wt. i.v.o.d), antacids (rantidine @2-4 mg/kg i.v,s.c), antiemetic (ondansetron @0.2-0.4 mg/kg i.m), antipyretic (analgin @25 mg/kg b.wt. i.m.), vitamin B complex i.m,antifibrinolytic agents (Tranexamic acid @10 mg/kg b.wt.) was administered as per the clinical condition of the animal.

In second group, amino acid infusion (Astymin-3, Allianz Biosciences (P) Ltd.) was used for 3-days in addition to conventional therpay @ 1.2–2 ml/kg i/v containing different amino acids, such as L-Arginine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine HCL, L-Methionine, L-Phenylalanine, L-Threonine (5.4 mg), L-Tryptophan (1.8 mg), L-Valine (6.1 mg), Glycine (10 mg), Sorbitol (50 mg). Third treatment group comprised of plasma therapy for 3-days at a dose rate of 10– 15mL/kg Body weightalong with conventional treatment. After cross-matching and screening of donor for infectious pathogens, whole blood collected from donor was separated into packed RBCs and plasma. Whole blood collected in double bag was centrifuged in cryofuge for 5 minutes (4°C) at 5000x g to separate plasma. The plasma was then transferred into a satellite bag by plasma extractor. Plasma was transfused slowly at initial rate of <5mL/kg/hour for first 15–30 minutes, and the recipient dog were closely monitored for transfusion reactions. Then an administration rate of 5– 10mL/kg/hour was maintained. The efficacy of different treatments was accessed on the basis of clinical improvement and haemato-biochemical improvements (CBC, total protein, albumin etc.).

Prognostic studies

The survivor (n=19) and non-survivors (n=5) suffering from CPV were separated, and hemato-

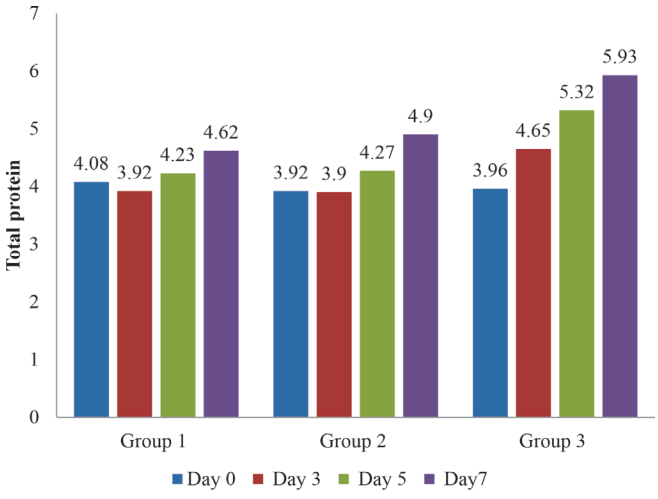

Figure 1a: Effect of treatment regimen on total protein value in CPV enteritis.

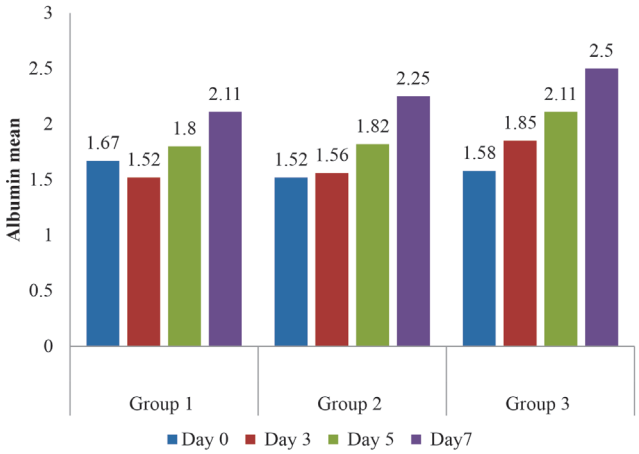

Figure 1b: Effect of treatment regimen on albumin value in CPV enteritis

biochemical parameters on day of first presentation, and follow ups were compared.

Statistical analysis

Results were analyzed using computer based SPSS statistical software. The values were found to be statistically significant if p value is <0.05.

Results and Discussion

Mean age (Days) of CPV affected dogs was 94.83±7.97. Similarly, more cases were recorded in pups below 6 months age by (Bhargavi *et al*., 2017). The quick and high epithelial cell turnover rate of the intestines, along with the increased mitotic index of the intestinal crypts' enterocytes, may contribute to the high CPV infection in young puppies. In present study higher number was observed in male (18; 75%) than in female population (6; 25%), that might be due to over presentation of males in clinics. Previous study in GADVASU clinics (Sen et al., 2016)found similar results. In present study CPV was more common in unvaccinated (18; 75%) than vaccinated (6; 25%) pups.

Faecal samples of dogs showing signs of CPV enteritis were collected and were tested with Ubio quick VET rapid Antigen test kit, Cochin (India).24 dogs positive for the infection were included in the study for clinical therapy. Immunochromatography based test were rapid, reproductive, simple, sensitive diagnostic test (Bhargavi *et al*., 2017).

Effect of treatment regimen on clinical signs and physical parameters

Anorexia, vomiting and haemorrhagic diarrhoea, were the predominant clinical signs encountered in all the animals. Similar findings were recorded in previous studies (Ogbu *et al*., 2017). Hematemesis was observed in 11 out of 24 CPV affected dogs on day of presentation.

On day 3, out of 8 animals only 2 (25%) dogs were left with anorexia, 3 (37.5%) with vomition and 2 (25%) with haemorrhagic diarrhoea in group III. Group III showed significant improvement on day 3 itself followed by group II, while group I showed least improvement. Similarly better results were observed in group III on day 5 and day 7 as compared to group I and group II. All the CPV affected dogs were either lethargic or recumbent as they were severely dehydrated or in the state of shock.

Effect of treatment regimen on haemato-biochemical values

On day 0 (day first of presentation), mean ± SE of Hb, PCV, TEC values in group I was 10.6± 0.7, 30.81± 1.8, 4.8± 0.3 respectively, while in group II the value of Hb, PCV, TEC was 9.9± 0.8, 29.9± 1.8, 4.5± 0.3 and in group III values were 10.1± 0.5, 30.6± 1.7, 4.5± 0.3, respectively. There was no significant difference between these groups on day 0. Similarly, no significant difference was between treatment groups on day 3 , day 5 and day 7.

Overall, In CPV affected dogs, Hb, PCV, TEC values were lower than the normal range of healthy dogs. That was due to haemorrhagic diarrhoea noticed in all the affected dogs that occurs due to loss of capillary bed of intestine (Bhat *et al*., 2013).

TLC, absolute neutrophils, absolute lymphocyte values were within reference range in most of animals. Some dogs showed marked leukopenia, neutropenia and lymphopenia that is due to bone marrow suppression in CPV infection. Similar results have been reported in previous studies (Salem, 2014)

Overall, in all the groups with treatment, count of TLC, absolute neutrophils, absolute lymphocyte showed increase in values from day 0 to day 7.

As depicted in (Figure 1a and 1b), the levels of total protein were increased in all the treatment groups, However there was maximum rise in level of total protein from 3.9±0.11 to 5.93± 0.14 in group III involving conventional as well as plasma therapy followed by group II (Conventional plus amino acid drip) and least rise was seen in group I (Conventional alone).

It has been reported that plasma is a rich source proteins, immunoglobulins and serum protease inhibitors, which can assist to neutralize circulating virus and reduce the inflammatory response (Prittie, 2004). So, plasma therapy results in significant increase of total protein. Also in group II infusion of amino acid drips resulted in increase in total protein value indicating the usefulness of amino acid drips in CPV cases (Kumar *et al*., 2020).

Similar to total protein values, albumin levels were low in all the CPV affected dogs and maximum improvement was seen in dogs treated with combination of conventional and plasma therapy followed by group II (Conventional plus amino acid drip) and least rise was seen in group I (Conventional alone) as depicted in (Figure 1).

Table 11. Prognosis in parvoviral enteritis based on haemato-biochemical parameters

Parameters	Days	Survivors (n=19)	Non-survivors(n=5) (#)
Hb (g/dL)	0	10.2 (10.4±0.4, 6.6-14.2)	9.5 (9.4±0.4, 7.8-10.6)
	3	9.7 (9.6±0.2, 7.5-12.1)	9.2 (9.3±1.1, 6.5-12.2)
	5	10.2 (10±0.2, 7.6-11.5)	9.4 (9.4±1.1, 7.5-11.5)
	7	11.1 (10.9±0.2, 8.8-12.6)	-
PCV (%)	0	30.5 (31.2±1.1, 24.1-40.6)	28.1 (27.6±1.05, 24.1-30.6)
	3	28.9 (29.3±0.5, 26.2-35.2)	28.9 (28.8±2.6, 20.3-35.3)
	5	30.2 (30.1±0.5, 26.2-34)	27.3 (26.3±3.3, 20.2-31.6)
	7	32.3 (33±0.7, 27.1-38.3)	-
TEC ($\times10^6$/ µl)	0	4.9 (4.7±0.2, 2.7-6.1)	3.9 (4±0.1, 3.8-4.4)
	3	4.2 (4.4±0.1, 3.2-5.5)	4.1 (3.8±0.6, 2.2-5.3)
	5	4.7 (4.6±0.1, 2.9-5.7)	4.5 (4.3±0.3, 3.5-4.9)
	7	5.1 (5±0.1, 3.9-5.7)	-
Platelets ($x10^3$/µl)	0	321 (349.4±48.7, 60.5-852)	254 (339±127.1, 160-838)
	3	284 (374.5±55.8, 80.7-888)	254.5 (267.4±56.7, 166.7-482)
	5	279.5 (341.9±43.9, 90-736)	155 (157±47.9, 75-241)
	7	253 (362.4±49.7, 132-929)	-
TLC ($\times10^3$/µl)	0	8500 (13316±1930, 6500-29000)*	1800 (1900±321, 1100-3000)*
	3	11000 (13002±2083, 8000-33400) *	900 (1100±290, 700-2300) *
	5	13400 (14674±1215, 8300-25100) *	500 (700±252, 400-1200) *
	7	15670 (15510±875, 10400-22400)	-
Absolute Neutrophils (cells/µl)	0	7254 (11450±1823, 3660-26100) *	1440 (1661±395, 605-2940) *
	3	10000 (13021±1952, 4590-30060) *	1380 (1460±310, 420-2860) *
	5	9916 (11954±1211, 5780-24598)	-
	7	11322 (12658±999, 7350-20832)	-
Absolute Lymphocytes (cells/µl)	0	1440 (1824±331, 800-5400) *	60 (239±114, 42-540) *
	3	1936 (1953±296, 650-5740) *	45 (190±98, 30-490) *
	5	2736 (2618±294, 900-5152)	-
	7	2530 (2746±258, 1140-4701)	-
Total Protein (g/dL)	0	4 (4±0.06, 3.50-4.40)	4 (3.9±0.1, 3.50-4.20)
	3	4.20 (4.20±0.1, 3.50-5.20) *	4.10 (3.82±0.2, 2.80-4.40) *
	5	4.70 (4.71±0.1, 3.7-5.7) *	4.10 (3.83±0.7, 2.5-4.90) *
	7	5.10 (5.12±0.1, 4.1-6.3)	-
Albumin (g/dL)	0	1.70 (1.60±0.06, 0.70-1.90)	1.50 (1.52±0.10, 1.30-1.80)
	3	1.90 (1.67±0.05, 1.2-2.10)	1.50 (1.52±0.09, 1.2-1.80)
	5	2.0 (1.90±0.07, 1.50-2.50) *	1.60 (1.46±0.13, 1.20-1.60) *
	7	2.30 (2.27±0.09, 1.60-3.10)	-

Superscript * depict significance between the groups
At day 5, n=3 in non-survivors as 2 animals died on day 5.

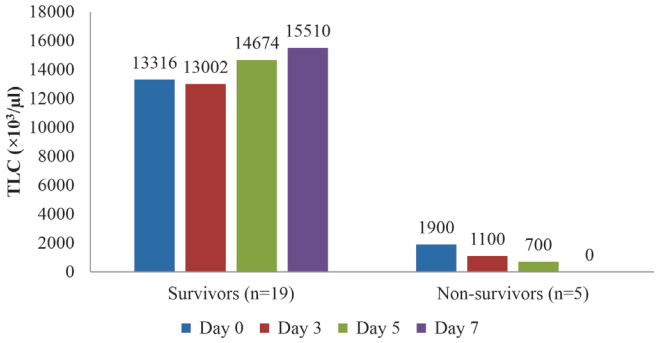

Figure 2a: Prognosis in CPV enteritis on the basis of TLC count.

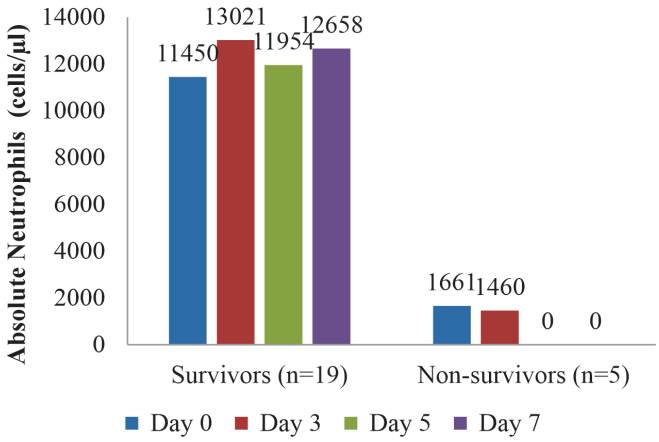

Figure 2b: Prognosis in CPV enteritis on the basis of absolute neutrophils count.

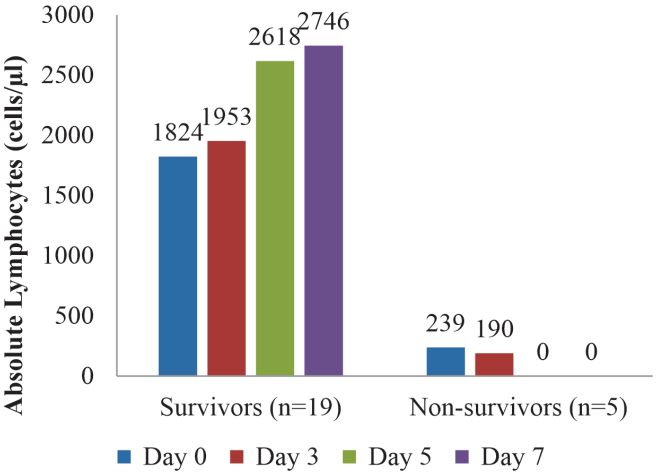

Figure 2c: Prognosis in CPV enteritis on the basis of absolute Lymphocyte count.

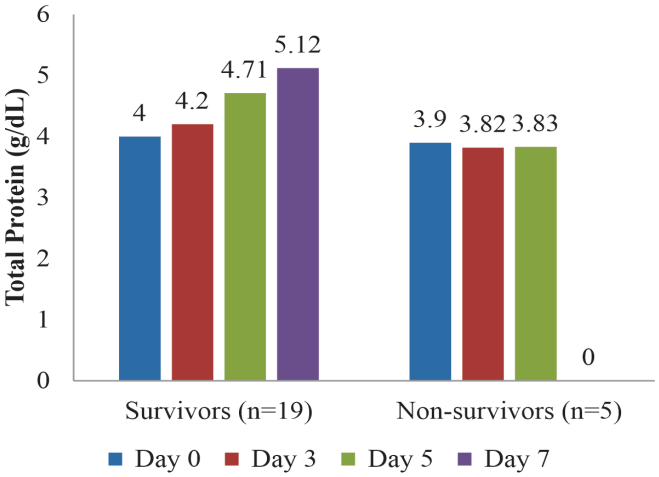

Figure 2d: Prognosis in CPV enteritis on the basis of total protein values.

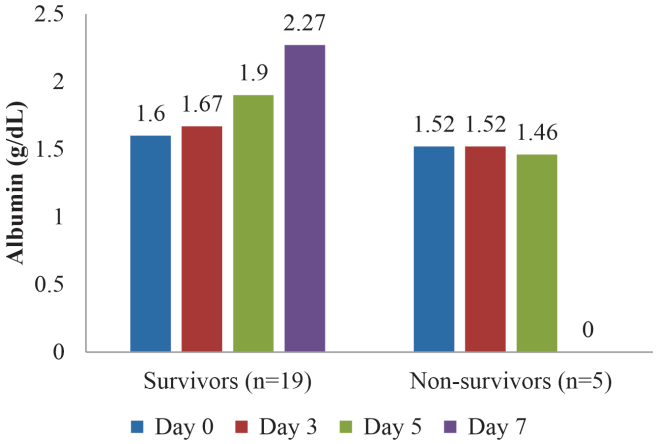

Figure 2e: Prognosis in CPV enteritis on the basis of albumin values.

The values of total protein and albumin were lower than the normal values in all the three groups. Our study is in accordance with previous study (Shah *et al*., 2013) that found decreased total protein and albumin levels in 80 % and 66 % of cases respectively. Similar results were also recorded by Bhargavi *et al*. (2017). Hypoproteinaemia and Hypoalbuminemia seen in CPV may be caused by serum protein leakage through intestinal villi capillaries, as well as by decreased protein absorption through the injured villi.

Prognostic indicators in parvoviral enteritis dogs

Out of 24 dogs treated with different approaches, 19 survived out of 24, while 5 pups died despite treatment. Hematobiochemical values in survivors and non-survivors were compared on first day of presentation and follow ups as shown in Table 1.

There was significant difference in survivors and non-survivors' group with respect to TLC (Figure 2a), absolute neutrophils (figure 2b), absolute lymphocytes (Figure 2c). Similar results were observed in earlier studies (Salem, 2014).

Regarding total protein (figure 2d) and albumin (Figure 2e), the levels between survivors and non-survivors CPV groups were almost similar on day of first prestation. However subsequently, these levels increased markedly in survivor groups but showed no improvement in non-survivors' group.

Our study was in accordance with a previous study (Geetha and Selvaraju, 2021) that revealed leukopenia, hypoalbuminemia (Caddy and Bexfield, 2010) and mild anaemia were the prognostic indicators for CPV infected dogs. Goddard *et al*., (2008) also recorded lower leukocyte count in the non-survivors as compared to survivors and concluded that accurate prognosis can be obtained by evaluating the change in total leukocyte, neutrophil, lymphocyte counts between 24-48 hours. Hypoproteinaemia and Hypoalbuminemia indicate poor prognosis in CPV patients, as they are linked to a lower effective circulating blood volume, poor tissue perfusion, and a worse outcome (Judge, 2015). Therefore, it is advised that in addition to CPV infection confirmation, haemato-biochemical analysis be performed to advice the prognosis to owner. In CPV, destruction of haematopoietic progenitor cells of the various leukocyte types in the bone marrow leads to leukopenia and in turn high susceptibility to secondary bacterial infections may be the cause of death in non-survivors (Goddard *et al*., 2008).

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Evaluation of a neem (*Azadirachta indica*) herbal formulation for management of sarcoptic mange in dromedary camels

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ABSTRACT

In the present study, a neem herbal formulation was prepared using fresh neem leaves, mustard oil, turmeric powder, camphor oil and distilled water for treatment of sarcoptic mange in adult dromedary camels. Total seven applications of this formulation was applied in six mange affected camels at every alternate day. The efficacy of this formulation was compared with six camels receiving two doses of ivermectin injection (dose rate 0.2mg/Kg body weight) at 7 days interval. To evaluate the therapeutic efficacy of the formulation, improvement in skin lesions of mange were recorded, and skin scraping and skin biopsy examination was performed before and after treatment. Macroscopically all the mange affected camels showed grey colored dry skin lesions over different body parts with intense pruritus, alopecia, thickening and corrugation. At the end of treatment trial, the neem formulation treated camels showed complete recovery without any remnants of scar or keratinization at affected sites, further the skin became smooth, shiny and glossy with appearance of new hair growth. In ivermectin treated camels although recovery from mange lesions was observed after 14th day, however the grey areas of the skin with some remnants of scar or keratinization and patches of alopecia were not completely cured in 50% of the treated camels. The result indicated that neem based herbal formulation have better efficacy than ivermectin against treatment of sarcoptic mange in dromedary camels and can provide a cheap, safe and eco-friendly alternative.

Key words: Acaricidal properties, Camel, Herbal, Mange, Neem

In dromedary camels sarcoptic mange caused by *Sarcoptes scabiei* var. *cameli* is a highly contagious zoonotic mite infection characterized by crusty, pruritic dermatitis and hair loss (Aziz *et al.,* 2020). Sarcoptes mite has wide host range and apart from camels it can infect cattle, dogs, sheep, goats, horses, swine, llamas, alpacas and humans (Bornstein *et al.,* 2001). Sarcoptes is a burrowing mite as it penetrates deeply through the skin surface of the infected camel and cause intense pruritus and exudative dermatitis (Aziz *et al.,* 2020). The skin lesions spread rapidly over the body surface, very difficult to manage and are responsible for causing weight loss, reduced growth rate and also predispose affected camels to other infections. Sarcoptic mange is also of zoonotic nature and camel owners are the main sufferers due to close association with camels (Parsani *et al.,* 2008). The topical application of different acaricide compounds and ivermectin injection are the most widely practiced for treatment of mange in camels. However, effective control of mange mites remains a big challenge due to frequent reoccurrence and resistance (Gopinath *et al.,* 2018). The continuous and indiscriminate use of chemicals also leads to chemical toxicity, emergence of resistance problem, residual effect in animal food products, and environmental pollution (Alemu *et al.,* 2022). Consequently, new alternative drugs are urgently needed. Locally available medicinal plants may provide an alternative means of mite control as they are rich source of bioactive chemicals, environmentally safer, cost effective, relatively non-toxic to humans and have broad spectrum of insecticidal and acaricidal activities, which help to prevent development of drug resistance (Pasipanodya *et al.,* 2021). Since neem (*Azadirachta indica*) is a widely available tree species in camel inhabited arid region of India and have varied insecticidal, larvicidal, and acaricidal effects, hence in the present study, we evaluated the efficacy of neem leaves in combination with other herbal ingredients for successful treatment of sarcoptic mange in dromedary camels.

Materials and Methods

Animals and clinical observations

The treatment trial was conducted in camels of an organized farm located in Bikaner district, Rajasthan, India. A total of 12 adult camels (irrespective of sex

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and breed) suffering from natural infection of Sarcoptic mange (diagnosed on the basis of history, type of lesions and skin scraping examinations) were divided into 2 groups of 6 camels each for drug trials. These camels were provided with identical feeding and management practice. All the mange affected camels of the present study were in category of moderate to severe lesions. Clinical examination and body weight of each camel was determined before and after treatment. Camels of Group 1 were subjected to topical application of neem based herbal formulation which was prepared by incorporating fresh neem leaves paste- 1 Kg, Mustard (*Brassica juncea*) oil- 1 liter, Turmeric (*Curcuma longa*) powder- 250gm, Camphor (*Eucalyptus globulus*) oil- 100ml and distilled water- 1 liter. This paste like preparation was applied on mange affected parts of the body covering entire lesions using gloved hands for every alternate day for total 14 days duration. Before application of this formulation, clipping of hairs, cleaning with water and drying the skin with cotton cloth was practiced. Camels of Group 2 were given Ivermectin injection (1% Ivermectin w/v) at the rate of 0.2mg/ kg body weight by subcutaneous route which was repeated at 7th day.

Skin scraping and histopathology examinations

Skin scrapings collected from mange infected camels before and after treatment were suspended in a 10% solution of potassium hydroxide and kept in water bath at 37°C for 2 hours and then centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded and one drop of glycerin was added to the sediment before it was microscopically examined for mites or their remnants. *Sarcoptes scabiei* var *cameli* mites were identified on the basis of their morphological characteristic features such as circular outline having four pairs of short and stumpy legs. For histopathology skin biopsy samples were collected from mange affected camels before and after treatment (14th day) from camels of both groups in 10% neutral-buffered formalin. The formalin fixed skin tissue samples were embedded in paraffin, cut into 4–5 micron sections using a semi automatic microtome and stained with hematoxylin and eosin (HE) stain.

Results and Discussion

Clinical manifestations

Out of 12 mange infected camels, 6 camels (3 in each group) were suffering from severe sarcoptic mange characterized by poor body condition with emaciation, lack of appetite, severe mange skin lesions covering large areas of the body surface and bloody skin injuries due to frequent rubbing and scratching. The affected areas showed hyper pigmentation, crusting, scaling and severe scratching with oozing of blood due to penetration of skin by the mites which provoked the animal to rub its body against rough objects like walls, trees, etc (Fig.1A). The remaining six camels (3 in each group) in the moderate lesion category had medium sized visible mange lesions in patches with no body skin injuries and occasional pruritus. In all the affected camels, mange lesions were characterized by grey coloured dry skin lesion having mild to severe itching, alopecia, thickening, crusting and corrugation causing overall deterioration of quality of skin. These lesions were observed more frequently on the face (66.66%) and neck (66.66%) region followed by axilla (58.33%), ventral and lateral part of abdomen (50%), brisket (50%), perineum (50%), base of tail (50%), flank (50%), shoulder (50%), inner and posterior surface of thighs (50%) and limbs (50%). Sarcoptic mange was also confirmed by skin scraping which were taken from

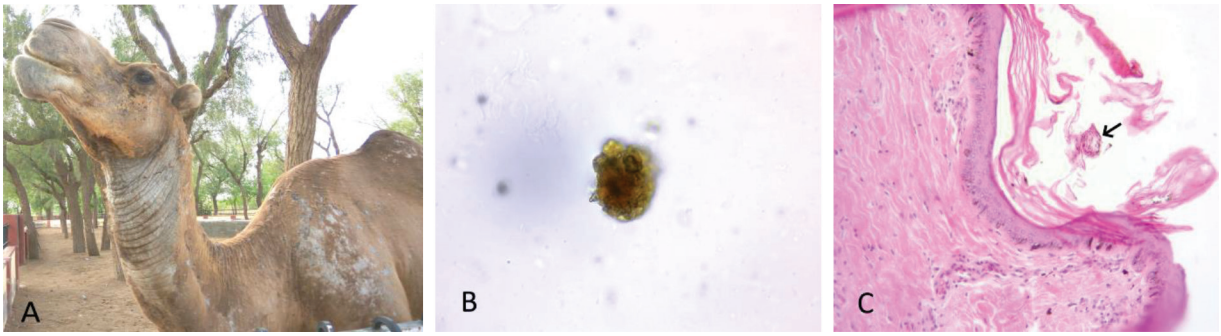

Fig. 1 A. Camel showing thick, grey, hairless, keratinized mange lesions on face, neck and shoulder region; **B.** Skin scraping examination showing *Sarcoptes scabiei* var. *cameli* in 40 X magnification; **C.** Histopathology of skin of mange infected camel showing hyperkeratosis and presence of a sarcoptic mite (arrow) in keratin layer. HE X 200.

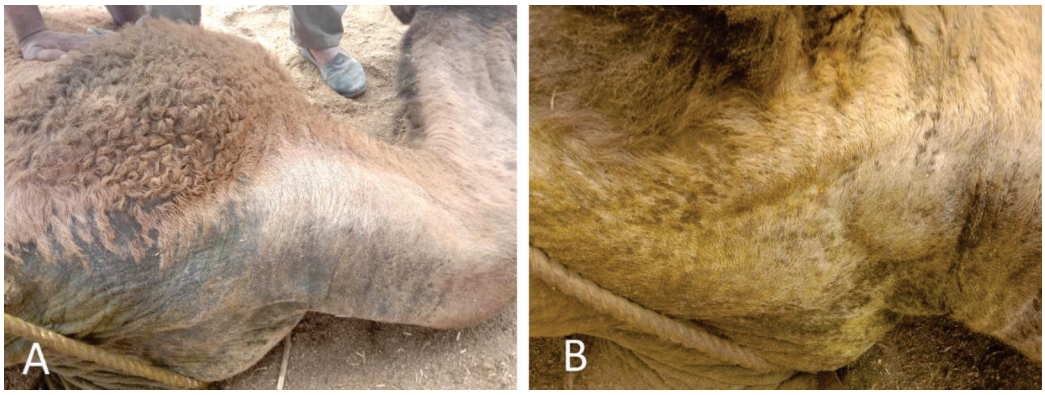

Fig 2 A. Group 1 camel showing alopecia, keratinization and wrinkled grey coloured skin at ventral side of neck and axillary region before treatment; **B.** Same camel after 7th application of neem herbal formulation showing recovery with absence of mange-like lesions and growth of new hairs.

various affected sites of the animals before treatment in which presence of mites, their remnants or eggs were observed (Fig. 1B). In histopathology hyperkeratosis, acanthosis, presence of mange mite in keratin layer and occasional eosinophil infiltration was observed (Fig. 1C).

Evaluation of the clinical recovery

The visible recovery from clinical signs of mange started to appear after 7 days of application of neem herbal formulation in Group 1 camels. This recovery was expressed through observation of absence of itching, scale formation and restlessness. The affected hairless, dry, scaly, grey coloured skin turned into smooth, dark coloured with appearance of new hair growth (Fig. 2 A and B). After 14 days of application of neem herbal formulation complete recovery was observed with disappearance of any visible mange-like clinical signs such as itching, scale formation, alopecia and restlessness. In addition the skin also became smooth, soft, glossy with appearance of new hair growth and there was no scar present over the skin, thereby, improving the overall appearance of animal due to the effect of treatment (Fig. 3 A and B). One week post treatment, skin scrapings from the neem formulation-treated group were found to contain remnants of dead mites and after two weeks of treatment, no observable mites were detected. In all the recovered camels, total seven applications of neem herbal formulation were required for complete recovery. Although there was no significant difference between the mean body weight of camels before treatment (396.83±25.01 Kg) as compared to mean body weight of camels after treatment (406±25.69 Kg), but it was found slightly increased with improvement of body condition.

Almost all parts of the neem tree reported to have varied insecticidal, larvicidal and acaricidal effects,

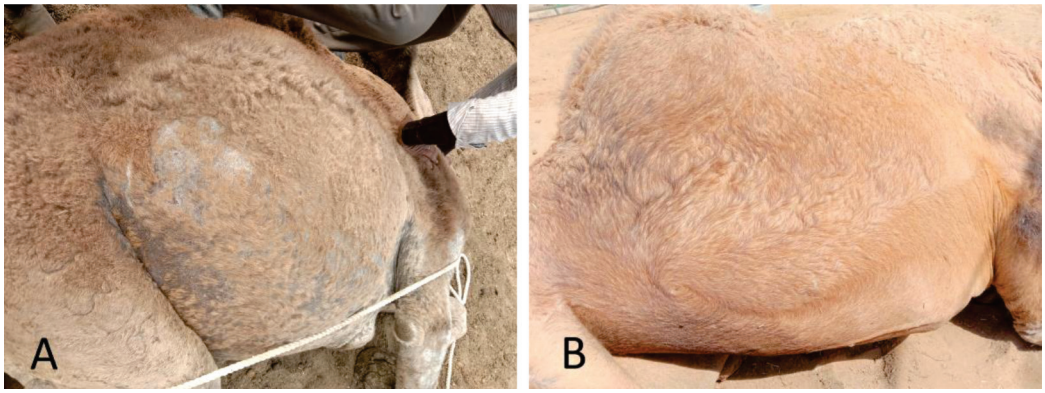

Fig 3 A. Group 1 camel showing grey coloured skin, alopecia and keratinization at abdominal region; **B.** Same camel showing complete recovery after 7th application of neem herbal formulation. Also note soft, smooth skin without keratinization and appearance of shiny and glossy new hair coat.

mediated by groups of phytochemical compounds such as cardenolide, triterpene, azadirachtins, salannin, nimbin, and 6-desacetylnimbin (Pasipanodya *et al.,* 2021). The camphor oil and mustard oil used in this neem formulation reported to have acaricidal effect and they help in reliving itching, skin dryness/ roughness and alopecia (Fang *et al.,* 2016). The mustard oil can cause mechanical blockage and penetrate the integument. This would affect the ability of the arthropods to breathe by blocking their spiracles which are the external openings that allow oxygen to enter the body of the arthropods (Suraj *et al.,* 2019). Mustard oil is rich in Vitamin E content and help to optimize hair growth, relieve dryness and itchiness of the skin and have cleansing effect making the skin more soft (Sharma and Joshi, 2004). The freshly prepared camphor oil also found effective in treating human facial demodicosis (Morsy *et al.,* 2003). Curcuma longa powder used in the present herbal formulation has been used in previous studies for successful treatment of sarcoptic mange in buffalo calves (Sharma and Joshi 2004; Naresh *et al.,* 2005). In another study, daily topical application of neem leaves and turmeric paste along with ivermectin injection showed significant clinical improvement by the third week of therapy in mange affected camels (Periasamy *et al.,* 2018). The synergistic effect of all the herbal components used in the present formulation may be thought to be responsible for drastic recovery of the camels within short span compared to previous studies using herbal preparations.

The neem formulation was also found safe and no specific adverse effect on skin of camels has been recorded. The composition was also found effective in healing of cuts, cracks, minor wounds and erythema observed on the skin of infected camels. As ascertained in previous studies, the neem has found to have high safety index and do not display any adverse effects when applied on skin or ingested by the animals (Pasipanodya *et al.,* 2021).

In ivermectin treated group recovery was observed on 7th day after first injection in three moderately infected camels, while in three severely infected camels recovery was delayed until the end of the trial. In moderately infected camels, the clinical symptoms started to disappear after first injection and the recoveries in these camels were manifested by subsided clinical symptoms like itching, alopecia, corrugation and thickening of skin, hyperemia, and disappearance of keratinization. In three severe cases, although recovery was observed after end of experiment; however some remnants of scar tissue and patches of alopecia remained. In earlier studies it was reported that the ivermectin may have low efficacy against mange mites in chronically infected camels and may take upto 21 days for complete cure of mange lesions (Darwish *et al,* 2020). Moreover there could be possible resistance of mites against Ivermectin since indiscriminate use of Ivermectin is very common in India. Camel farmers in India usually self treat the animals which may lead to underdosing or overdosing and may initiate the resistance among mites. In both the treatment groups, in skin scraping examination remnants of dead mites were found on the 7th day whereas they were absent on 14th day. The histopathology of skin of camels after 14th day showed absence of hyperkeratosis, acanthosis, any inflammatory cells and mites in both the groups.

Overall the neem herbal formulation of the present study was found to have comparable therapeutic modality with ivermectin, with superiority of healing of lesions and overall improvement of skin condition with short duration of treatment. The results of the present study also revealed that camels treated with neem formulation showed slight increase in feed intake, weight gain and improvement of body condition due to reduction in itching, irritation and restlessness. The preparation of this formulation does not require any sophisticated technique and farmers can easily prepare this formulation with locally available cheap ingredients. Hence, this can prove to be a farmer friendly and environment friendly solution for treating mange in camels.

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Epidemiological, Hemato-biochemical and Therapeutic study on Nutritional Hemoglobinuria in Cattle and Buffaloes

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Abstract

A total of 22 animals (16 buffaloes and 6 cattle) suspected for nutritional hemoglobinuria were enrolled under the study. Blood and urine samples for various investigations were collected for hemato-biochemical and urine analysis**.** The results of epidemiological analysis revealed higher incidence of nutritional hemoglobinuria during winters as compared to other seasons. Most of the cases were recorded during post-partum period in both buffaloes and cattle. The highest incidence was seen in buffaloes and cattle in ≥ 5th lactation group (62.5% and 66.67%). Most of the cases (64.28% in buffaloes and 80% in cattle) were recorded within 0-30 days post-partum. Berseem constituted a major component (90%) of fodder for most nutritional hemoglobinuria affected animals. Brown red to coffee-coloured urine was the most obvious clinical symptom. The most predominantly observed clinical signs were reduced milk production (100%), followed by tenesmus (92.45%), coffee coloured urination (86.36%), pale mucous membrane (77.27%), complete anorexia (72.72%), constipation (68.18%), reduced water intake (45.45%) and other signs of gastro-intestinal disturbances. Heart rate and respiration rate were increased in both cattle and buffaloes. Significantly decreased ($p<0.01$) mean hemoglobin level, total erythrocyte count, packed cell volume, serum phosphorus and significantly increased ($p<0.01$) serum AST, total bilirubin, BUN, creatinine, glucose were recorded in hemoglobinuric cattle and buffaloes as compared to healthy controls. Total leukocyte count and absolute neutrophils count were significantly increased ($p<0.05$) in both cattle and buffaloes. No significant difference was recorded in absolute lymphocyte count, serum calcium and ALKP between affected and healthy animals. Urinalysis revealed presence of blood and leukocytes as common findings whereas microscopic examination revealed presence of epithelial cells, urothelial cells, few RBCs, and waxy casts. USG of kidney evidenced loss of parenchyma in most of the affected animals. After the treatment with sodium acid phosphate in combination with ascorbic acid, hemoglobin, PCV, total erythrocyte count and serum phosphorus improved significantly ($p<0.01$) and absolute neutrophils count decreased significantly ($p<0.05$) whereas, serum AST, total bilirubin, BUN, creatinine, and glucose decreased significantly ($p<0.01$). The recovery rate for combination therapy along with supportive treatment was found to be 59.09% respectively.

Key words: Nutritional hemoglobinuria, Urinalysis, Phosphorus, Sodium acid phosphate

Adult cattle and buffaloes are susceptible to the non-infectious hemolytic condition known as nutritional hemoglobinuria, which affects numerous animals each year during pregnancy and the first few months of lactation. Severe anaemia, hemoglobinuria, intravascular hemolysis, and mortality from anoxia and anaemia are its hallmark symptoms (Resum *et al*., 2017). In the native parlance, nutritional hemoglobinuria is known as "Post Parturient Hemoglobinuria" "Lahu mutna" or "Rakth mutna."

Despite the fact that the aetiology of nutritional hemoglobinuria is unknown, several risk factors have been connected to it, including the consumption of cruciferous plants, berseem saponin, dietary phosphorus insufficiency, decreased serum copper and selenium, and excessive molybdenum (Soren *et al*., 2014). The low phosphorus content of berseem, lucerne, sugar cane tops, sugar beets, kale, mustard as well as the presence of inhibitory factors like metallic ions interfere with dietary phosphorus absorption and assimilation, all contribute to a precipitous phosphorus deficiency (Dhonde *et al*., 2007). As a result of hypophosphatemia, red blood cell glycolysis and ATP generation is reduced causing an increase in fragility and hemolysis, all of which can lead to hemoglobinuria and hemoglobinemia (Ogawa, 2018). Within 20±10 days before or after parturition, infected buffaloes generally show the first noticeable evidence of passing red to coffee-coloured urine (Purohit *et al.*, 2014). Faeces might be solid, dry, and bile-coloured

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(Macwilliams *et al*., 1982).

Illness is clinically defined by the appearance of pale mucous membrane, depression, tachypnoea, tachycardia and slight salivation (Madheswaran *et al*., 2017). The diagnosis of nutritional hemoglobinuria is made using the clinical history, along with clinical signs like pale mucous membrane, tenesmus, and coffee to red brown urine. Keeping in view the economic losses due to high mortality, marked decrease in milk yield and long convalescent period in few survivors due to poor response to treatment, makes this an important disease. Based on above facts the present study is designed to evaluate the epidemiological, hemato-biochemical, and therapeutic aspect of nutritional hemoglobinuria.

Materials and Methods

Clinical cases presented to referral animal hospital, (Medicine unit), College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab during the period of past one year from August 2021 to July 2022 were included in this study. Twenty-two animals (16 buffaloes and 6 cattle) showing signs (coffee to red brown coloured urination) of nutritional hemoglobinuria were screened by routine and microscopic examination of urine. Incidence of the disease was studied with respect to season, stage of lactation, lactation number and period of parturition.

Hematological and serum biochemical analysis

Five ml of whole blood was collected from affected (n=22, 16 buffaloes and 6 cattle) and control (n=16, 8 cattle and buffaloes each) animals. Two ml of EDTA added blood was used for estimation of hematological parameters using MYTHIC 18 Hematology system (Siemens Healthcare diagnostics Inc. Deerfield, IL, USA). Serum samples were collected by centrifuging 3 ml of whole blood at 3000 rpm for 10 minutes for estimation of serum biochemical parameters (AST, total bilirubin, phosphorus, calcium, ALKP, BUN, creatinine and glucose).

Urinalysis

Urine was collected from the affected animals and analysed for physical appearance, microscopic examination, and routine urinalysis using multi- diagnostic strips/ dipsticks. Confirmation of hemoglobinuria was done by centrifuging the urine sample at 3000 rpm for 5 minutes and subsequently observing for any sedimentation.

Ultrasonography of kidney and liver

The ultrasonography of liver and kidney was done with a portable ultrasound scanning machine (Sonosite M- Turbo) with a 5-10 MHz curvilinear transducer (L38, Sonosite, serial number: 03RQ5K).

Therapeutic efficacy of sodium acid phosphate in combination with ascorbic acid

All the affected animals were treated with intravenous administration of sodium acid phosphate (80 gm in 400 ml normal saline) for 3-5 days (depending on the recovery of the animal) and ascorbic acid (25 ml/100 kg) OD for 3 days along with supportive treatment (injection B-complex 10 ml I/M, injection multi-vitamin 10 ml I/M, Tablets VET-CUCO, mineral mixture 50 gm /day). The hematological and serum biochemical parameters of the survived animals were compared before and after treatment.

Statistical analysis

The data was statistically analysed with the SPSS software version 27 using the Mann- Whitney test, Wilcoxon Signed Rank test and unpaired T- test. The analysis was done to know about the efficacy of the treatment provided at various level of significance ($p<0.05$, $p<0.01$).

Results and Discussion

The study includes 22 clinical cases (16 buffaloes and 6 cattle) having history of recent parturition, pale mucous membrane, reddish-brown coloured urine, and hypophosphatemia on hemato-biochemical analysis were enrolled in this study. The most predominantly observed clinical signs were reduced milk production (100%), followed by tenesmus (92.45%), coffee coloured urination (86.36%), pale mucous membrane (77.27%), complete anorexia (72.72%), constipation (68.18%), reduced water intake (45.45%) and other signs of gastro-intestinal disturbances (Figure-1). Three animals (1 cow and 2 buffaloes) were anuric on the day of presentation due to which percentage of animals showing coffee coloured urine was decreased. In a study on 40 PPH affected buffaloes red to coffee coloured urine, constipation and pale mucous membrane was recorded in 100% of the cases (Soren *et al*., 2014). Contrary to reports of hemosiderin deposition in earlier investigations, it is suspected that gastrointestinal disorders such as ruminal stasis,

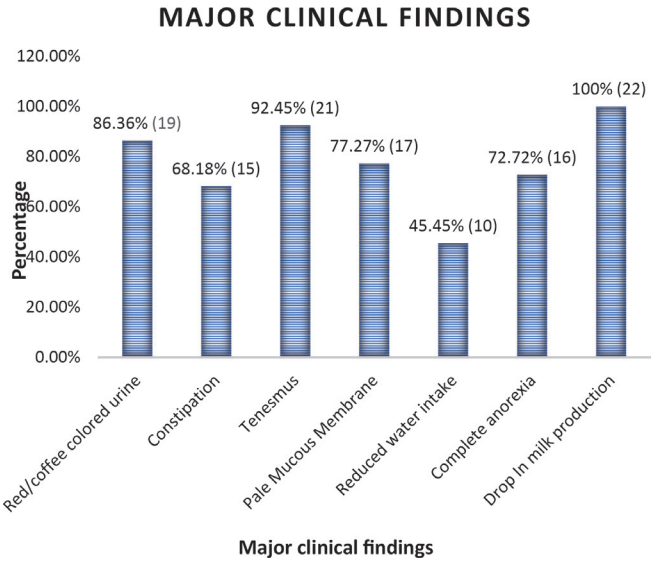

Fig. 1. Major clinical findings in nutritional hemoglobinuria affected cattle and buffaloes

constipation, straining, and dark colouration of faeces in the present study may be associated to nephrosis and reduced kidney function (Sharma *et al.*, 2014). Increase in heart rate and respiration rate was observed, whereas ruminal motility and milk yield were decreased. Similar findings were reported in previous studies (Soren *et al*., 2014; Zaghawa *et al*., 2019).

Higher incidence of nutritional hemoglobinuria was observed in winters during which 62.5% of buffaloes and 66.7% of cattle were affected (Table no. 1). Similar findings were also reported in previous studies (Macwilliams *et al*., 1982; Khan *et al*., 2006; Soren *et al*., 2014; Zaghawa *et al*., 2019). Khan *et al*. (2006) suggested that buffaloes experience stress due to mineral imbalance at their peak of production, which is exacerbated by pregnancy and the late stages of gestation which ultimately corresponds with the consumption of cruciferous plants in the winter, leading to the development of disease. Most of the cases of nutritional hemoglobinuria were recorded during post-partum period as compared to pre-partum period in both buffaloes and cattle (Table no. 1). Similar observations were made by previous researchers (Dalir-Naghadeh *et al*., 2006 and Gahlawat *et al*., 2007). More number of affected buffaloes and cattle (62.5% and 66.67%) were in 5th lactation or above. Similar findings were reported in previous studies (Mahmood *et al*., 2012) with highest prevalence of parturient hemoglobinuria at the 5th lactation and lowest at 1st lactation. With respect to stage of lactation, maximum incidence of the disease (9 buffaloes and 4 cattle) was observed within 30 days of parturition (Table no. 1). This finding in our study is in accordance with Zaghawa et al. (2019) who reported that majority (59.26%) of the buffaloes developed parturient hemoglobinuria in the post calving period between 1-23 days of calving followed by 29.63% and 11.11% cases in between 61-270 days and 24-60 days of calving, respectively. Additionally, high incidence in early lactation has also been reported previously (Sarma *et al*., 2014; Kumar *et al*., 2019). With respect to feeding, out of 22 animals, 20 (90.90%) cattle and buffaloes were fed berseem fodder and concentrate ration. As this is main fodder available in Punjab during the winter months so most of the cases were associated with berseem feeding. PPH is strongly associated with berseem feeding in the winter season as reported in previous studies (Akhtar *et al*., 2007). By virtue of its hemolytic saponin contents, this might predispose the animals to nutritional hemoglobinuria.

Affected animals had significantly lower hemoglobin, total erythrocyte count and packed cell volume ($p<0.01$) as compared to control groups (Table

Table 1. Epidemiological risk factors in animals suffering from nutritional hemoglobinuria

Parameters		No. of buffaloes affected (%)	No. of cattle affected (%)
Season	Winter	10 (62.50)	4 (66.67)
	Other seasons	6 (37.50)	2 (33.33)
Period of parturition	Pre-partum	2 (12.50)	1 (16.67)
	Post-partum	14 (87.50)	5 (83.33)
Lactation number	≤4th lactation	6 (37.50)	2 (33.33)
	≥5th lactation	10 (62.50)	4 (66.67)
Stage of lactation (2 buffaloes and 1 cattle were affected pre-partum)	0-30 days	9 (64.28)	4 (80)
	> 30 days	5 (35.72)	1 (20)

no. 2), whereas TLC and absolute neutrophils counts were significantly increased ($p<0.05$) in both cattle and buffaloes. A decrease in TEC, Hb, and PCV indicated severe anaemia in hypophosphatemic buffaloes and cattle that could be attributed to intravascular hemolysis (Pandey and Misra, 1987; Digraskar *et al*., 1991). Increasing and decreasing trends in neutrophils and lymphocyte count could be attributed to endogenous release of glucocorticoids (Montiel *et al*., 2007).

Serum phosphorus was reduced significantly ($p<0.01$) in cattle and buffaloes as compared to healthy control animals (Table no. 2). Similar findings have been consistently documented in previous studies (Chugh *et al.*, 1996; Kumar *et al*., 2014; Rashid *et al*., 2021).This could be attributed to utilization of phosphorus for foetal development and its drainage through milk in lactating buffaloes and deficiency of phosphorus in fodder and soil (Bhikane *et al*., 2004). It may also be associated with prolonged feeding on cruciferous or toxic plants (Khan *et al.*, 2006).

Other serum biochemical parameters like serum AST, total bilirubin, BUN, creatinine, and glucose ($p<0.01$) were significantly increased in affected animals. This rise of serum AST in affected animals indicates liver damage. Similar findings were also reported in previous studies (Mahmood *et al*., 2019; Kumar *et al*., 2019; Rashid *et al.*, 2021). Loss of hepatic functions results in decreased capacity for bilirubin uptake, conjugation or secretion

Table 2. Mean ± SE values of hemato- biochemical parameters in healthy and nutritional hemoglobinuria affected buffaloes and cattle

Parameters	Diseased buffaloes (n=16)	Control (n=8)	Diseased cattle (n=6)	Control (n=8)
Hb (g/dL)	4.10±0.22** (2.9-5.9)	11.25±0.34 (9.8-12.4)	4.05±0.41** (2.9-4.9)	9.39 ±0.19 (8.4-10.3)
PCV (%)	12.96±0.78** (7.80-18.80)	33.75±1.03 (29.4-37.2)	12.32±1.22** (8.70-17)	27.84 ±0.54 (25.3-30.3)
TEC ($\times10^6/\mu L$)	1.91±0.78** (1.1-4.0)	6.85±0.22 (5.8-7.6)	2.55±0.44** (1.3-3.9)	6.30 ± 0.21 (5.59-7.17)
TLC (/µL)	11177±803* (7600-15300)	8925±824.3 (5800-12500)	11333±1806* (7700-19900)	8262.50 ±638.34 (5900-11200)
Absolute lymphocytes count	3352±326 (1216-5507)	4153.88±390.05 (2784-6136)	3976±507.82 (3196-6368)	4293.25±323.11 (2832-5824)
Absolute neutrophils count	7374±677* (4925-11702)	4771.13±503.17 (3016-5664)	7357±703* (4466-10300)	3669.25 ±351.40 (2680-5376)
Ca (mg/dl)	9.56±0.18 (8.6-10.9)	10.36±0.25 (9.3-11.3)	8.90±0.23 (8.3-9.7)	9.70±0.51 (8.3-12)
P (mg/dl)	2.33±0.20** (0.7-3.2)	5.43±0.12 (4.9-6.0)	2.12±0.25** (1.2-2.8)	5.39±0.11 (4.9-5.9)
AST (U/L)	419.81±25.18** (264-635)	103.63±3.31 (85-115)	416.33±73.65** (280-767)	98.25±4.73 (74-110)
Total bilirubin (mg/dl)	3.95±0.25** (2.8-6.5)	0.54±0.06 (0.3-0.8)	3.18±0.21** (2.5-3.8)	0.40±0.11 (0.1-0.7)
ALKP (U/L)	119.25±6.01 (56-180)	116.00±10.92 (79-160)	141.83±10.98 (120-190)	110.50±16.86 (54-200)
BUN (mg/dl)	47.56±1.16** (40-54)	28.25±1.67 (20-32)	49.17±1.21** (43-54)	29.63±1.36 (23-34)
Creatinine (mg/dl)	2.5±0.11** (1.8-3.5)	1.29±0.11 (0.8-1.6)	2.65±0.19** (1.8-3.2)	1.16±0.09 (0.8-1.6)
Blood glucose (mg/dl)	99.92±2.12** (80-110)	60.25±1.49 (54-65)	100.17±1.98** (87-101)	67.00±3.14 (54-76)

*Significant level at $p<0.05$, **Significant level at $p<0.01$

(Latimer, 2003; Akhtar *et al*., 2008; Mahmood *et al*., 2013) leading to significant increase in total bilirubin. Endogenous release of corticosteroids, starvation and tubular epithelial necrosis (Khan and Akhtar, 2007) contributes to significant increase in blood urea nitrogen. Findings were similar to previous studies (Rashid *et al*., 2021). Reduced GFR in affected animals leads to decrease in creatinine clearance from the renal cells, eventually leading to increase in the creatinine concentrations (Latimer, 2003). Similar findings could be attributed to damage to the kidney resulting from anaemic hypoxia due to acute hemolysis (Resum *et al*., 2017). These observations were in agreement with findings reported in previous studies (Latimer, 2003; Zaghawa *et al*., 2019; Kumar *et al*., 2019). Significant difference in glucose level of hemoglobinuric and healthy buffaloes was contrary to the previous reports (Khan *et al*., 2006; Akhtar *et al*., 2008; Mahmood et al., 2019). Possible reason could be attributed to release of glucocorticoids due to stress, stimulating glycogenolysis and gluconeogenesis, resulting in hyperglycaemia (Latimer, 2003). Some biochemical parameters like serum calcium, ALKP were not significantly different as compared to healthy control animals.

The urine from the affected animals varied from light brown to deep coffee coloured, depending upon the severity and stage of the disease. The coffee-coloured urine was seen in 50 % of the buffaloes and red brown coloration of urine was seen in 37.5% of the buffaloes (Figure-3). Similarly, coffee-coloured urine was seen in 66.67 % of the cattle and red brown colouration was seen in 16.67% of the cattle. Similar findings were reported in previous studies (Khan and Akhtar, 2007). The affected cattle and buffaloes had mean specific gravity of 1.010±0.02, and mean pH of the urine sample was 8.41±0.13. Higher pH of urine in hemoglobinuric animals has also been reported in previous studies (Akhtar *et al*., 2008; Soren *et al*., 2014). Microscopic analysis of urine in affected buffaloes revealed waxy casts (21.42%), epithelial cells (57.14%), few RBCs in urine (92.85%) and urothelial cells (57.14 %). Similarly, microscopic analysis of urine in affected cattle revealed waxy casts (0%), epithelial cells (40%), few RBCs in urine (60%) and urothelial cells (40%). Presence of waxy casts and epithelial cells could be attributed to renal stasis due to decreased blood flow across the kidney tissue causing ischemia, necrosis and renal failure (Ringsrud, 2001). The presence of waxy cast indicates renal failure caused due to nephrotoxic effect of heme proteins (Ringsrud, 2001). Presence of urothelial cells indicates infection of the urinary bladder induced by descending infection in the kidneys (Ringsrud, 2001). The most common abnormalities on urinalysis were presence of blood, bilirubin, and leukocytes. The less common findings were glycosuria, proteinuria, presence of nitrates and ketones.

The dimensions of the right kidney were measured in affected buffaloes and cattle. The mean horizontal and vertical diameter of right kidney in affected buffaloes were 5.83 ± 0.20 cm and 8.76 ± 0.19 cm, respectively, and in affected cattle were 5.70 ± 0.49 cm and 8.88 ± 0.27 cm, respectively. Most of the affected animals (71.42% buffaloes and 60% cattle) evidenced parenchymal loss which might be due to nephrotoxicity induced by heme proteins (Figure-2). Present study concludes that there is

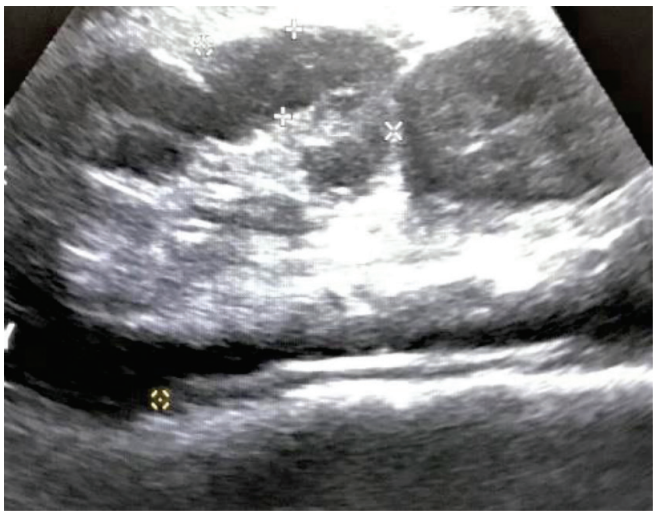

Fig 2 : Parenchymal loss in the right kidney of buffalo

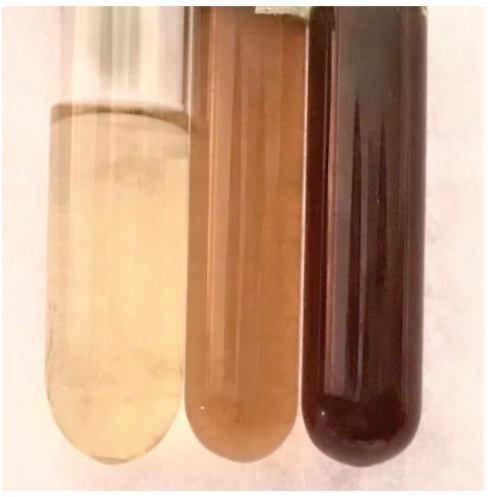

Fig 3: Different urine coloration in buffaloes and cattle suffering from nutritional hemoglobinuria

Table 3. Effect of treatment on hemato- biochemical parameters in cattle and buffaloes

Parameter	Before treatment (n=9)	After Treatment (n=9)
Hb (g/dL)	4.40±0.26 (3.4-5.60)	8.94 ± 0.31** (7.80-10.20)
PCV (%)	13.42±0.95 (8.6-17.70)	26.52± 1.03** (22.20-30.60)
TEC($\times10^6/\mu L$)	2.26±0.34 (1.22-3.98)	4.95 ± 0.26** (4.10-6.00)
TLC ($\times10^3/\mu L$)	11566±1056 (7700-17210)	9744± 237 (8700-11000)
Absolute Lymphocyte count ($\times10^3/\mu L$)	3766±363 (2336-5507)	3709± 213 (3060-4725)
Absolute neutrophil count ($\times10^3/\mu L$)	7799±824 (4928-11703)	6036±205* (5264-7140)
P (mg/dl)	2.36±0.25 (1.20-3.60)	6.31±0.11** (5.80-6.90)
AST (U/L)	385.4±21.2 (290-487)	108.67±1.80** (98-115)
ALKP (U/L)	141.83±10.98 (87-190)	120±0.76 (64-150)
Total bilirubin (mg/dl)	3.72±0.27 (2.50-5.50)	0.85±0.05** (0.70-1.20)
BUN (mg/dl)	46.67±1.15 (42-54)	22.00±0.03** (19-25)
Creatinine (mg/dl)	2.52±0.10 (1.80-2.70)	1.20±0.03** (1.10-1.30)
Blood Glucose (mg/dl)	98.4±2.05 (90-110)	68±1.60** (56-78)

*Significant level at $p<0.05$, **Significant level at $p<0.01$

no significant enlargement of the kidney, rather the size of the kidney is much variable in bovine species (Smith, 1990).Ultrasonography of liver was done in 14 buffaloes and 5 cattle. Fifty percent (7) of buffaloes were having normal liver echotexture whereas, four buffaloes were having hepatic congestion and rest three were having hyperechoic parenchyma. Normal hepatic echotexture and congested liver was evidenced in two cattle each and one cattle was having hyperechoic parenchyma. So, not much change in the liver parenchyma was observed in the present study.

Out of the 22 affected animals, 13 animals (9 buffaloes, 4 cattle) survived (Table no. 4). Clinical signs in all the affected animals subsided and milk yield improved in all the survived cases. However, only 9 animals (cattle and buffaloes) were presented at 7th day post-treatment and hemato-biochemical parameters of these nine animals were compared before and after treatment (Table no. 3). Hemoglobin, PCV and TEC were increased significantly ($p<0.01$) after the treatment (Table no. 3). No significant change in TLC and absolute lymphocyte count was found whereas absolute neutrophil count was significantly decreased ($p<0.05$) before and after the treatment.

As hypophosphatemia decreases glucose utilization rate and ATP production in erythrocytes, this leads to decreased synthesis as well as reduction of glutathione. This predisposes erythrocytes to harmful effects of lipid peroxidation. Sodium acid phosphate reduces lipid peroxidation by acting through antioxidant mechanism, thereby restoring plasma inorganic phosphorus level (Gahlawat *et al*., 2007). Serum phosphorus was increased significantly ($p<0.01$) after the treatment, whereas serum AST, total bilirubin, BUN, creatinine, and glucose were significantly decreased

Table 4. Comparison of survivors and non- survivor animal suffering from nutritional hemoglobinuria

Category	Cattle (n=6)	Buffalo (n=16)	Total (n=22)
Survivors	4(66.66%)	9(56.25%)	13 (59.09%)
Non survivors	2(33.33%)	7(43.75%)	9 (40.90%)

($p<0.01$) (Table no. 3) after the treatment. No significant difference was found in other biochemical parameters like ALKP before and after the treatment.

Conclusion

Present study concludes that hypophosphatemia and increased blood glucose level were the consistent findings in nutritional hemoglobinuria affected animals. Around 2/3rd of the cases were observed in winters followed by other seasons. Most of the cases were observed within 0-30 days post-partum in both cattle and buffaloes. Most of affected buffaloes and cattle were in 5th lactation or above. Kidney and liver function were invariably compromised in all the cases with increased BUN, creatinine, AST, and total bilirubin levels. Therapeutic regimen comprising of intravenous administration of ascorbic acid and sodium acid phosphate was effective in saving the life of hemoglobinuric animals with recovery rate of 59.09%. Blood transfusion along with the standard treatment may be the best treatment if given in proper time keeping in view the duration of sickness to prevent the fatal outcome of the disease.

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Conflict of interest

Authors declare there is no conflict of interest.

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Electrocardiographic reference parameters in healthy pug dogs

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Abstract

The present study was designed to establish electrocardiographic data in healthy Pug dogs with respect to body weight, age and gender. Forty clinically healthy pug dogs presented to Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University presented for routine deworming, vaccination and general health check-up were selected for the present study. Fifteen dogs were in the body weight range of 1-7 kg and 25 dogs in 7-15 kg range. To study the effect of age on various electrocardiographic measurements dogs were divided into 3 age groups (1-3, 3-6 and >6 years of age). Heart rate was found to be high (146.3±7.7 bpm) ranging from 100-200 bpm in 1-7 kg body weight as compared 7-15 kg body weight 128.3±4.0 bpm ranging from 90-170 bpm although, there was no significant effect on mean heart rate. All the amplitudes and durations of electrocardiographic waves and complexes did not show any significant variation with respect to body weight. The heart rate belonging to age group 1-3 years was 148.08±8.76 bpm ranging from 100-200 bpm significantly high ($p<0.05$) as compared to dogs of 3-6 years (124.23±5.81 bpm) ranging from 90-160 and > 6 years old (122.3±2.41 bpm) ranging from 105-135 bpm and there was no significant variation ($p >0.05$) in amplitude and durations of electrocardiographic data with respect to age. Heart rate was higher in females (140.74±6.25 bpm) ranging between 100 and 200 bpm than in males (129.90±4.99 bpm) ranging between 90 and 180 bpm but was found to be non-significant ($p > 0.05$). P-R interval, was found to be significantly ($p < 0.05$) higher in females as compared to males.

Key words: Dog, Electrocardiogram, Pug, Heart rate

Electrocardiography (ECG) is a non–invasive and relatively inexpensive technique for recording heart rate, heart rhythm, conduction disturbances, electrical axis, myocardium and pericardium affections along with detection of electrolyte imbalance, drug toxicities, and endocrine imbalances (Mattera *et al*., 2012). ECG is also used for routine health screening, cardiac monitoring during surgical intervention, trauma, routine pre-surgical examination, and preventive health screening on adult dogs. Although some had documented normal electrocardiographic readings for dogs and there is variation based on breeds, body size, and other factors. Breed differences are thought to have the greatest impact on electrocardiographic reference values. Electrocardiographic reference ranges derived from different breeds may be unreliable (Saini, 2014). As compared to different developed countries, India has limited literature on the electrocardiographic reference data in pets (Thirunavukkarasu, 2019). Saini (2014) and Gugjoo *et al*. (2014) had established electrocardiographic data in healthy Labrador Retrievers. Similarly, Sidhu (2015) had documented electrocardiographic data in healthy German shepherd dogs. No reference electrocardiographic data has yet been established in pug breed of dog.

Therefore, the present study was designed to establish electrocardiographic data in healthy Pug dogs with respect to body weight, age and gender.

Materials and Methods

Study place and animals

Forty clinically healthy pugs presented at Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, were selected for the study which were presented for routine deworming, vaccination and general health check-up. Dogs presented normal on physical examination were selected for electrocardiography to determine the reference electrocardiographic data in pugs. They were categorized into different groups on the basis of body weight (Table 1), age (Table 2) and gender (Table 3).

Table 1: Distribution of healthy pugs on the basis of body weight (Kg):

Body weight (Kg)	Healthy pugs (n=40)
1-7	15
7-15	25

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Table 2: Distribution of healthy pugs on the basis of age (years):

Age (years)	Healthy dogs (n=40)
1-3 Years	13
3-6 Years	13
>6 years	14

Table 3: Distribution of healthy pugs on the basis of Gender:

Gender	Healthy dogs (n=40)
Male	21
Female	19

Electrocardiography

Electrocardiogram was recorded in all dogs as described by Tilley (1992) by using BPL cardiart 8108 six channel ECG machine.

Patient positioning and placement of leads

The patient positioned in right lateral recumbancy with the limbs held perpendicular to the body. All limbs were held parallel to one another without allowing any contact. Limb electrodes were positioned over the stifle and either distal or proximal to the elbow's caudal surface.

Electrocardiographic recordings

Electrocardiographic recordings were made at a speed of 50 mm/s, 10mm= 1mV. The leads I, II, III, aVR, aVL, and aVF from the Bailey's hexaxial limb lead system were adopted. The rhythm strip from Lead II was used to measure each parameter. The amplitude was measured in millivolts, while the wave width was measured in seconds.

Following ECG parameters were recorded:

P wave: It represents depolarization of the atria that indicates time required for an impulse to pass from SA node to AV node that may be positive, notched, negative or biphasic. P wave amplitude is measured in (mV) and duration in seconds.

PR interval: It is the time required for an impulse to travel from sino-atrial node to the ventricles. PR interval is measured in seconds.

QRS complex: It is the representation of depolarization of the ventricles. QRS complex amplitude is measured in (mV) and duration in seconds.

ST segment: It is the time interval from the end of the QRS interval to the beginning of the T wave (early phase of ventricular depolarization). It can be elevated, depressed or coving etc. ST segment is measured in seconds.

T wave: It is the representation of depolarization of the ventricles. It can be positive, notched, negative or diphasic. P wave amplitude is measured in (mV) and duration in seconds.

QT interval: It is measured from onset of QRS complex to the end of T wave. QT interval is measured in seconds.

Mean electrical axis was recorded By Using Baileys Hexaxial system chart as described by (Tilley, 1992).

Results and Discussion

Electrocardiographic measurements

Mean± SEM of electrocardiographic (ECG) parameters of healthy pug dogs and the effect of body weight, age and gender on all ECG parameters are presented in Table 4.

Effect of body weight on electrocardiographic parameters

In the current study, heart rate was found to be high (146.3±7.7 bpm) ranging from 100-200 bpm in 1-7 kg body weight as compared 7-15 kg body weight (128.3±4.0 bpm) ranging from 90-170 bpm although, there was no significant effect ($p > 0.05$) on mean heart rate with respect to body weight in healthy pug dogs (Table 4) . All the amplitudes and durations of waves and complexes did not show any significant change ($p > 0.05$) with respect to body weight. Similarly, Lerdweeraphon *et al.* (2020) also did not find any significant effect of body weight on heart rate as well as amplitude and durations of various waves in electrocardiographic data in military working dogs.

Effect of age on electrocardiographic parameters

In the present study, the heart rate belonging to age group 1-3 years was significantly high ($p<0.05$) (148.08±8.76 bpm) ranging from 100-200 bpm as compared to dogs of 3-6 years (124.23±5.81 bpm) ranging from 90-160 and > 6 years old (122.3±2.41 bpm) ranging from 105-135 bpm (Table 4). In contrast to our study, Mukherjee *et al*. (2020) found lowest heart rate in the age group of 6 months to 2.5 years old and maximum in the age group of 10.5 -12.5 years of age. However, according to Varshney (2020), heart rate is affected with age as age increases heart rate decreases, young dogs have high heart

Table 4: Effect of body weight, age and sex on Electrocardiographic parameters (MEAN ±SEM) in healthy pugs (n=40)

	Heart rate (bpm)	P wave Amplitude (mV)	P wave Duration (sec)	PR interval (sec)	R wave Amplitude (mV)	QRS Duration (sec)	Q wave (mV)
Body weight (kg)							
1-7 (n=15)	146.3±7.7 (100-200)	0.170±0.015 (0.1-0.3)	0.032±0.002 (0.02-0.04)	0.080±0.008 (0.02-0.12)	1.26±0.092 (0.6-1.8)	0.034±0.003 (0.02-0.05)	0.18±0.031 (0.1-0.5)
7-15 (n=25)	128.3±4.0 (90-170)	0.168±0.014 (0.1-0.3)	0.0320±0.005 (0.02-0.15)	0.085±0.003 (0.06-0.12)	1.068±0.062 (0.9-2.2)	0.029±0.002 (0.02-0.05)	0.17±0.023 (0.1-0.5)
Age (years)							
1-3 (n=13)	148.08±8.76* (100-200)	0.188±0.018(-) (0.1-0.3)	0.040±0.009 (0.02-0.15)	0.077±0.009 (0.02-0.12)	1.29±0.104 (0.9-2.2)	0.035±0.003 (0.02-0.05)	0.18±0.035 (0.1-0.5)
3-6 (n=13)	124.23±5.81 (90-160)	0.180±0.019 (0.1-0.3)	0.029±0.002 (0.02-0.04)	0.090±0.003 (0.06-0.10)	1.076±0.087 (0.6-1.8)	0.028±0.002 (0.02-0.05)	0.19±0.036 (0.1-0.5)
>6 (n=14)	122.3±2.41 (105-135)	0.139±0.013 (0.1-0.2)	0.026±0.002 (0.02-0.04)	0.082±0.005 (0.06-0.12)	1.057±0.078 (0.5-1.5)	0.03±0.002 (0.02-0.04)	0.16±0.022 (0.1-0.3)
Gender							
Male (n=21)	129.90±4.99 (90-180)	0150±0.012 (0.1-0.3)	0.028±0.002 (0.02-0.04)	0.085±0.004 (0.05-0.12)	1.12±0.072 (0.6-1.9)	0.03±0.002 (0.02-0.05)	0.15±0.023 (0.1-0.5)
Female (n=19)	140.74±6.25 (100-200)	0.189±0.014 (0.1-0.3)	0.035±0.006 (0.02-0.15)	0.101±0.002* (0.07-0.12)	1.15±0.081 (0.5-2.2)	0.03±0.002 (0.02-0.05)	0.20±0.027 (0.1-0.5)

*P<0.05 shows significant difference

Table 4 continued….

	ST segment (sec)	T wave Amplitude (mV)	T wave Duration (sec)	QT interval (sec)	MEA (degree)
Body weight (kg)					
1-7 kg (n=15)	0.116±0.008 (0.1-0.2)	0.203±0.030 (0.1-0.5)	0.039±0.005 (0.02-0.08)	0.18±0.004 (0.15-0.22)	80.0±3.8 (60-90)
7-15 kg (n=25)	0.106±0.003 (0.1-0.15)	0.214±0.028 (0.1-0.8)	0.038±0.003 (0.02-0.08)	0.19±0.003 (0.16-0.22)	80.4±2.9 (60-90)
Age (years)					
1-3 (n=13)	0.119±0.009 (0.1-0.2)	0.173±0.023 (0.1-0.3)	0.037±0.005 (0.02-0.08)	0.180±0.005 (0.15-0.22)	83.08±3.65 (60-90)
3-6 (n=13)	0.107±0.005 (0.1-0.15)	0.192±0.017 (0.1-0.3)	0.031±0.002 (0.02-0.04)	0.187±0.005 (0.16-0.22)	78.46±4.21 (60-90)
>6 (n=14)	0.103±0.003 (0.1-0.15)	0.260±0.051 (0.1-0.8)	0.045±0.004 (0.02-0.08)	0.182±0.004 (0..16-0.2)	79.29±3.99 (60-90)
Gender					
Male (n=21)	0.102±0.002 (0.1—0.15)	0.202±0.017 (0.1-0.3)	0.043±0.003 (0.02-0.08)	0.180±0.003 (0.15-0.22)	80.0±3.16 (60-90)
Female(n=19)	0.118±0.006 (0.1—0.2)	0.218±0.039 (0.1-0.8)	0.033±0.004 (0.02-0.08)	0.187±0.003 (0.16-0.22)	80.53±3.29 (60-90)

rate in comparison to adult dogs. In the present study, we did not found any significant variation in amplitude and durations of various waves of electrocardiographic data with respect to age.

Effect of Gender on electrocardiographic parameters

In the present study, heart rate was higher in females (140.74±6.25 bpm) ranging between 100-200 bpm than in males (129.90±4.99 bpm) ranging between 90 and 180 bpm but was found to be non-significant ($p > 0.05$) (Table 4). Mukherjee *et al*. (2020) also observed no significant effect of heart rate on gender. In the present study, we did not find any major differences in electrocardiographic parameters ($p > 0.05$) except P-R interval, which was found to be significantly ($p < 0.05$). Similarly, Hanton and Rabemampianina, (2006) also did not found significant differences in electrocardiographic parameters in healthy beagles on the basis of gender.

Conclusion

The present study generates basic reference eletrocardiographic data in pugs which will help the clinicians to monitor cardiac abnormalties in pugs.

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Evaluation of fluralaner as a novel treatment for Scabies in naturally infested dogs

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Abstract

A single treatment of fluralaner orally once is sufficient and safe to kill *Sarcoptes* mites and improve the clinical condition of diseased dogs. *Sarcoptes* mite was detected in eight dogs having the presence of pinnal-pedal reflex and having sarcoptic mange-related skin lesions (erythema, alopecia, papule, crust etc.). Skin scrappings were taken from at least five different body areas judged to be most likely to have mean mite infestation on the days 0, 28, 56, and 84 and improvement in resolution of skin lesions was assessed on days 28, 56, and 84. On day 28th after treatment, all dogs were completely parasitological cured and most of the dermatological lesions were resolved on day 28th and completely resolved on days 56th and 84th. Marked alterations in the haemato-biochemical panels were observed as compared to healthy dogs. All studies of haemato-biochemical panels improved towards normalcy on day 56 post-treatment showing encouraging response to the therapy.

Key words: Canine, Efficacy, Mite, Isoxazoline, Sarcoptes

Canine scabies (sarcoptic mange) is a non-seasonal, intensely pruritic, and transmissible mite infestation of the skin of dogs caused by *Sarcoptes scabiei* var. *canis*. Mites preferentially inhabit less hairy areas of the host's body, and the severity of clinical signs and mite infestation on skin surface areas may vary from one host to the next and from one species to another (Scott *et al*., 2001). The clinical signs include intense constant pruritus, an erythematous rash, papules and yellowish crusts on the skin's surface, and alopecia (Arlian *et al.*, 1995). Sarcoptic mange is an emerging or re-emerging infectious ectoparasitic disease that threatens both human and animal health. Most conventional medications recommended for the treatment of mite-induced dermatitis are extremely hazardous to both animals and owners, To overcome this concern, there is a dire need to test efficacy of newly launched acaricidal drugs such as Fluralaner in the treatment of canine scabies. Fluralaner potent and long-lasting acaricidal and pharmacokinetic properties suggest that it could be useful in the treatment of sarcoptic mange in dogs. The purpose of the study reported here was to evaluate efficacy of fluralaner in the treatment of scabies caused by *Sarcoptes scabiei* var. *canis* in dogs.

Materials and Methods

The present study was carried out in canine population presented to Referral Veterinary Hospital, Small Animal Medicine OPD, Dr. G. C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur (H.P.) The study was conducted over a period of one year and two months w.e.f. February 2021 to April 2022. Dogs naturally infested with *Sarcoptes scabiei* var. *canis* further confirmed with the help of skin scrapings, pinnal pedal reflex (Plate:1), visible clinical signs linked with *Sarcoptes scabiei* var. *canis* infestation (Plate:2) e.g., erythema, alopecia, papule, crust and negative *Demodex* spp. on skin scrapings were selected. Haemato – biochemical panels were assessed between healthy and sarcoptic mange-affected dogs. Haematobiochemical panels were assessed after the 56th day post-treatment. The clinical signs were evaluated pre-treatment and at 28th, 56th and 84th day post-treatment. Clinical signs score assessment based on severity in this study include +++ for severely affected, ++ for moderately affected, + for mild affected, and – for not affected. Skin scrapings were taken from at least five different body areas that showed the most severe lesions at the enrolment visit (Day 0) and subsequent visits on days 28, 56, and 84, indicating the presence of local mite infestation. Each scraping was taken over an area of approximately 2.5cm^2 with a mineral oil-coated blade or spatula to an approximately constant depth, allowing capillary oozing to be visible. The scraping was examined under a microscope for the presence of mites, and the scraping results were evaluated based on mite counts. All dogs were included in the study and treated with oral fluralaner @25mg/kg body weight on day 0 (i.e., day of treatment). The total number of mites counted

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Plate 1: Pinna-pedal reflex in scabies dog

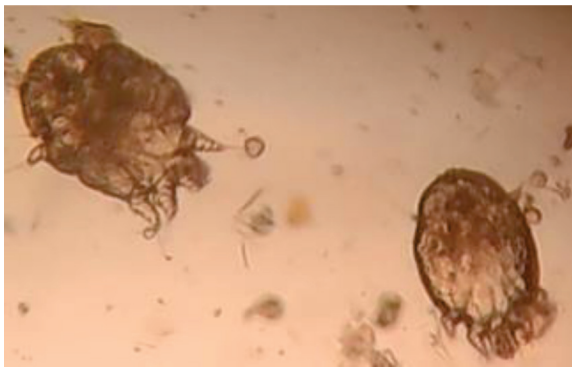

Plate 2: *Sarcoptes scabiei* var. *canis* mites 10X

in skin scrapings following treatment was the primary assessment variable in the study. Using geometric means and Abbott's formula, the percentage of efficacy against *Sarcoptes scabiei* var. *canis* mites was calculated as:

$$\text{Efficacy (\%)} = \frac{\text{Mpre - Mpost}}{\text{Mpre}} \times 100$$

Where, Mpre was mean number of pre-treatment mite counts and Mpost was mean number of post-treatment mite counts.

Results and Discussion

Based on skin scrapings and clinical signs, eight dogs were diagnosed with sarcoptic mange infestation. The mean value of Hb in sarcoptic mange-affected dogs was significantly ($p<0.01$) lower than in the healthy dogs. The finding of low TEC, HCT, and Hb observed in dogs with sarcoptic mange could be attributed to restlessness, hyporexia, and blood loss from scratching. The chronic and widespread nature of the disease may be too responsible for the decreased haemoglobin levels which is in accordnance with the earlier studies (Saleh *et al.,* 2011; Beigh *et al.,* 2013). The loss of epidermal protein and oxidative stress brought on by the infection may be responsible for the reduced haemoglobin concentrations. The mean value of TLC in sarcoptic mange was significantly ($p<0.01$) higher as compared to healthy dogs and is in parallel to the findings of Sakina and Mandial (2012) and Reddy *et al.* (2014). Leucocytosis may have been brought on by toxins generated because of tissue injury, necrosis brought on by inflammation, or secondary bacterial infection. The mean value of lymphocytes in sarcoptic mange dogs was significantly ($p<0.01$) lower than the healthy dogs which could be due to body's immune response and is in concurrence with observations of Chandy *et al.* (2000) and Pritam *et al.* (2008). Neutrophil mean value was non-significant higher in sarcoptic mange. The mean value of monocytes in sarcoptic mange dogs was significantly ($p<0.01$) lowered as compared to healthy dogs. According to Valenciano *et al.* (2014), due to the typically low levels of monocytes in healthy dogs, monocytopenia is not diagnostically significant. The mean value of eosinophil was significantly ($P< 0.05$) higher as compared to healthy dogs. Eosinophilia may be caused by a large number of mites with a significantly substantial antigen concentration and a resulting antigen-antibody reactivity. The comparison of haematological parameters between healthy and sarcoptic mange infested are presented in Table 1.

Table 1. Comparison of haematological parameters (Mean ± SE) between healthy and sarcoptic mange dogs

Parameters	Healthy	Sarcoptic mange
Hb (g/dL)	12.95±0.90	9.25±0.16**
HCT (%)	38.85 ±2.70	28.44±0.54**
TLC (10^9/L)	9.88±0.94	23.17±1.85**
TEC (10^{12}/L)	6.47 ±0.98	5.24±0.30
Lymphocytes (%)	23.73±1.07	16.93±1.04**
Neutrophil (%)	69.85±2.78	75.57±1.92
Monocytes (%)	3.84±0.53	1.46±0.16**
Eosinophil (%)	2.69±0.55	6.02±1.25*

* Significant at 5% (P< 0.05); ** Significant at 1% (P< 0.01)

The activity of serum enzyme alanine aminotransferase in sarcoptic mange significantly ($P<0.01$) higher as compared to healthy dogs. The increased liver specific enzymes were attributed to the hepatic damage caused by the toxic by-products of tissue breakdown and might be attributed to stress induces

hepatic impairment in diseased dogs. The mean value of AST was significantly (P<0.01) higher in sarcoptic mange as compared to healthy dogs. Markedly elevated AST activities could be attributed partly to generalized cellular damage in the skin of diseased dogs. Moreover, pruritus-induced increased muscular activities, while frequent scratching and chronic debility induced by the disease condition, might be attributed to the elevated activities of AST in dogs. The mean value of total protein in sarcoptic mange was significantly (P<0.01) higher as compared to healthy dogs. Increased inflammatory response mixed with subsequent bacterial infection may be the cause of the increased total protein values also reported by Shyama and Vijayakumar (2011). According to Aujla *et al*. (1999) value of total protein increased due to increased immunoglobulins and circulatory immune responses. Comparison of biochemical parameters between healthy and sarcoptic mange infested are presented in Table 2.

Table 2. Comparison of biochemical parameters (Mean±SE) in between healthy and sarcoptic mange dogs

Parameters	Healthy	Sarcoptic mange
ALT (U/L)	36.4± 4.23	64.91±7.34**
AST(U/L)	31.02±3.87	62.24±9.2**
Total Protein(g/dL)	6.61±0.27	7.61±0.16**

* Significant at 5% (P< 0.05); ** Significant at 1% (P< 0.01)

In comparison of mean value of pre and post-treatment with fluralaner the mean values of Hb, HCT, lymphocytes, and monocytes were significantly (P< 0.01) higher on day 56th post-treatment when compared to day 0th and the mean value of TEC was significantly (P< 0.05) higher on day 56th post-treatment when compared to day 0th and is in agreement to the findings reported by Sivakumar *et al*. (2017) and Habeeb (2015). The mean value of TLC and neutrophil was significantly (p<0.01) lower on day 56th post-treatment when compared to day 0th and the mean value of eosinophil was significantly (P< 0.05) lower on day 56th post-treatment when compared to day 0th. Comparable findings were reported by Behera *et al.* (2011), whereas the non-significant lower values of TLC, neutrophil, and eosinophil and non-significant higher values of Hb, HCT, lymphocytes, and monocytes were observed on day 21th post-treatment as compared to 0th day (Khushbhoo 2021). The results of haematological findings are presented in Table 3.

Table 3. Haematological alterations pre and post treatment in fluralaner treated dogs orally.

Parameters	0th day	56th day
Hb (g/dL)	9.25±0.16	12.6±0.2**
HCT (%)	28.44±0.54	39.5±0.39**
TLC (10^9/L)	23.17±1.85	9.45±0.1**
TEC (10^{12}/L)	5.24±0.30	6.17±0.1*
Lymphocytes (%)	16.93±1.04	27.25±0.12**
Neutrophil (%)	75.57±1.92	66.87±0.48**
Monocytes (%)	1.46±0.16	3.56±0.11**

* Significant at 5% (P< 0.05); ** Significant at 1% (P< 0.01)

The mean value of ALT, AST, and Total protein was significantly (p<0.01) lower on day 56th post-treatment when compared to day 0th. Whereas significant reduction of ALT and non-significant reduction of AST and total protein on 21st day post-treatment was reported by Khushbhoo (2021). The results of biochemical findings are presented in Table 4.

Table 4: Biochemical alterations pre and post treatment in fluralaner treated dogs orally.

Parameters	0th day	56th day
ALT (U/L)	64.91±7.34	35.11±2.19**
AST(U/L)	62.24±9.2	30.47±3.32**
Total Protein (g/dL)	7.61± 0.16	6.7±0.04**

** Significant at 1% (P< 0.01)

The clinical signs were evaluated in this study and on the 0th day, there was severe erythema, alopecia, and papules. After treatment of all dogs with oral fluralaner @25mg/kg b.wt once, there was reduction of clinical signs on 28th day. There was mild alopecia and crust seen on 28th day as compared to 0th day. Complete resolution of signs was seen on 56th and 84th day and was consistent with Khushboo (2021) who reported clinical recovery of

Table 5. Evaluation of the clinical signs of sarcoptic mange affected dogs Pre and post-fluralaner treatment

Lesions	Day 0	Day 28	Day 56	Day 84
	Fluralaner single treatment on day 0			
Erythema	+++	-	-	-
Alopecia	+++	+	-	-
Papule	+++	-	-	-
Crust	+++	+	-	-

+++ = severe, ++ = moderate, + = mild and - = nil

Table 6. Mite count using Skin Scraping on various days in fluralaner treated dogs

Drug	Mite count			
	0th day	28th day	56th day	84th day
Fluralaner	11.37±1.27[b] (9-15)	0.375±0.17[a] (0-1)	0.00±0.00[a] (0-0)	0.00±0.00[a] (0-0)
Efficacy (%)		96.70%	100%	100%

The values in a row with different superscripts a and b vary significantly (P< 0.01)

83.6% on the 21th post-treatment day. Fluralaner @25mg/kg b.wt orally effective for three months, could provide a very convenient and effective treatment option for dogs suffering from mite infestations (Petersen *et al*., 2020). Fluralaner is easily absorbed after a single oral dose and has a long elimination half-life, long mean residence time, relatively high apparent volume of distribution, and low clearance. These pharmacokinetic properties help to explain fluralaner's prolonged activity against fleas and ticks in dogs after a single oral dose. Fluralaner differs from the other three isoxazolines with a prolonged duration up to 12 weeks, which is three times longer than afoxolaner, sarolaner, and lotilaner (FDA 2014). The evaluation of clinical signs of sarcoptic mange-affected dogs pre- and post-fluralaner treatment is given in Table 5.

The mite count of fluralaner treated dog was significantly lower on the 28th day when compared to the 0th day with a drug efficacy 96.70 per cent and a non-significant reduction of mite count was observed on 56th and 84th day in comparison to 28th day with a drug efficacy of 100 per cent. Taenzler *et al*. (2017) found that administering fluralaner orally or topically to naturally infested dogs resulted in a 100% reduction in *Sarcoptes scabiei var. canis* mites and improved clinical signs over a 4-week period. Whereas according to Romero *et al*. (2016) fluralaner was effective in eliminating scabies mites within 14 days and significantly resolving clinical signs associated with sarcoptic mange within 21 days after a single dose. Chiummo *et al*. (2020) documented it an effective and safe treatment of *S. scabiei* var. *canis* infestations that lasted for 84 days (12 weeks) after treatment. The mite count using skin scraping on various days in fluralaner-treated dogs is presented in Table 6

In conclusion, a single treatment of fluralaner chewable tablet proved to be a safe and effective treatment of *Sarcoptes. scabiei* var. *canis* infestations that lasted for 84 days after treatment.

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Therapeutic effects of chewable fluralaner against *Sarcoptes scabiei* infestations in dogs

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Abstract

Scabies, also known as sarcoptic mange, is a potentially contagious ectoparasitic disease that poses a threat to both human and animal health. The majority of conventional medications that are suggested for the treatment of sarcoptic mange require repeated administration over a period of several weeks and are regarded as harmful to both pets and pet owners. A recently developed long-acting oral ectoparasiticide of the isoxazoline class is called fluralaner. Therefore, the current study's objective was to assess the therapeutic effects of a single dose of chewable fluralaner against canine sarcoptic mange. Nine dogs were enrolled who tested positive for *Sarcoptes scabiei* mite infestation and had a pinnal-pedal scratch reflex. The diseased dogs were given a single oral dose of fluralaner chewable at a rate of 25-56 mg/kg body weight. Six more healthy dogs were enrolled as healthy controls. Blood samples were collected from each diseased dog prior to the start of therapy and on day 21 post-therapy, and were used to analyse the haemato-biochemical changes. The haemato-biochemical panels of dogs with sarcoptic mange differed significantly from those of healthy dogs. None of the treated dogs had any *S. scabiei* mites on day 21 post-therapy. On day 21 post-therapy, it was found that the sarcoptes-induced skin lesions (SSLS) in dogs treated with fluralaner had improved by a total of 83.6 percent. Except for alanine aminotransferase (ALT), all altered haemato-biochemical panels of ill dogs returned to normalcy on day 21 following therapy. Fluralaner was found to be effective and safe enough to treat canine sarcoptic mange with a single oral dose. To corroborate the results of this study, additional research involving a large number of dogs with sarcoptic mange is required.

Key words: Acaricide, Dogs, Fluralaner, Sarcoptic mange, Sarcoptes scabiei, Scabies

Sarcoptic mange (Scabies), an intensely pruritic non-seasonal transmissible dermatosis caused by *Sarcoptes scabiei* mite infestation, is a new or re-emerging infectious ectoparasitic disease of humans and animals (Guaguere and Beugnet, 2008; Singh *et al.,* 2011; Miller *et al.,* 2013; Arlian and Morgan, 2017). Sarcoptes scabiei is thought to infect over 300 million people worldwide (Orrico and Krause-Parello, 2010), and the World Health Organization (WHO) has classified scabies as a human neglected tropical disease (El-Moamly, 2021). It is characterised by intense pruritus associated with a vesiculo-papular eruption, deposition of pinpoint yellowish crusts, alopecia, and extensive excoriations and poses a global threat to human and animal health (Pin et al. 2006, Arlian and Morgan, 2017). Clinical manifestations of alopecia, excoriations, and crust deposition primarily affect the periocular skin, ear pinna margins, and pressure points (elbows/hocks) of the body, which eventually spread to other body areas (Scott *et al.,* 1995; Griffin *et al.,* 1993; Curtis *et al.,* 2004). The pinnae and elbow margins are the most commonly affected and are prime locations for obtaining diagnostic scrapings (Walton and Currie, 2007). Direct contact with infected animals or environmental fomites, where the mite can survive for several days, are the two modes of transmission (Fang *et al.,* 2015). The immune status, nutritional status, and oxidative status of the host are all considered to be major risk factors for the development of sarcoptic mange in animals (Dimri *et al.,* 2008; Singh *et al.,* 2014).

A complete clinical and parasitological cure for mite-induced dermatitis takes a very long time. As a result, conventional miticides must be administered on a regular basis. Repeated administrations of conventional medicines predispose parasites to develop resistance to allopathic drugs and are also hazardous to the animals being treated. Traditional sarcoptic mange treatments include the organophosphates imidacloprid and moxidectin

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(Krieger *et al.,* 2005), lime sulphur, milbemycin oxime, and amitraz (Carlotti and Bensignor, 1997; Curtis, 2004). Several avermectins are recommended for the treatment of sarcoptic mange in dogs, with repeated administration (Shanks *et al.,* 2000; Scott *et al.,* 2001; Pin *et al.,* 2006). Therefore, an oral treatment option that is proven, convenient, and safe would be a significant benefit in the care of sarcoptic mange patients. Because of the widespread prevalence of sarcoptic mange in dogs, the growing concern about drug resistance, and the negative side effects of most conventional drugs, researchers are looking for an effective long-acting anti-mite medicine. Isoxazolines are a potent new class of effective ecto-parasiticides that can be used to improve basic ecto-parasite control while also lowering the risk of pathogen transmission. Afoxolaner, sarolaner, lotilaner, and fluralaner are the approved isoxazolines for veterinary use. Isoxazolines are a relatively new class of antiparasitic agents that inhibit gamma-aminobutyric acid (GABA) chloride channels (GABACls) and L-glutamate chloride channels (GluCl) with significant sensitivity for insect neurons, resulting in paralysis and death of ectoparasites (Taenzler *et al.,* 2017).

Fluralaner (Bravecto), the most recent addition to the isoxazoline class of oral ectoparasiticides, is an extremely effective insecticide and acaricide (Petersen *et al.,* 2020). Because of their high efficacy, rapid resolution, and low side effects, isoxazolines may eventually replace traditional treatments for demodectic and sarcoptic mange. Fluralaner, at a dose rate of 25-56 mg/kg body weight orally once, could provide a very convenient and effective treatment option for pet animals suffering from mite infestations (Taenzler *et al.,* 2016; Petersen *et al.,* 2020; Singh *et al.,* 2022). Fluralaner has a 12-week period of persistent efficacy against tick and flea infestations in dogs (Romero *et al.,* 2016). It has a long elimination half-life, a long mean residence time, a relatively high apparent volume of distribution, and low clearance after a single-dose oral administration. These pharmacokinetic properties help to explain fluralaner's prolonged activity against fleas and ticks on dogs after a single oral dose. Fluralaner differs from the other three isoxazolines in that it works three times as long as afoxolaner, sarolaner, and lotilaner after a single administration. To support its clinical recommendation, fluralaner's therapeutic effectiveness against canine sarcoptic mange must be examined. Therefore, the current study's objective was to assess the therapeutic effects of a single dose of chewable fluralaner against canine sarcoptic mange.

Materials and Methods

Study plan

The dogs enrolled in the study were client-owned and were presented with skin disorders at the Teaching Veterinary Clinical Complex (TVCC, Kothari Hospital) of the University for clinical and dermatological examination. Diagnosis of canine sarcoptic mange was made by testing positive *S. scabiei* mite in the skin scrapings samples of diseased dogs. A total nine dogs presented with the skin issued were tested positive for *S. scabiei* mite infestation and revealed pinnal-pedal scratch reflex were enrolled in the present study. The diseased dogs were treated with single oral administration of fluralaner chewable at a dose rate of 25-56 mg/kg body weight. Another six healthy dogs were also enrolled as healthy controls. The treated dogs were re-examined on day 21 post-therapy for the clinical and parasitological cure. On each examination, the severity of sarcoptes induced skin lesions (SSLS) were recorded at day 0 (pre-therapy) and day 21 (post-therapy) (Krieger et al. 2005). Approximately, 5 mL blood samples were aseptically collected from each of the enrolled dogs and used for the haemato-biochemical analysis.

Haemato-biochemical examination

Haematology of enrolled dogs was carried out by using a fully automated haematology analyzer. Total erythrocyte counts (TEC), total leukocyte counts (TLC), differential leukocyte counts (DLC), haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured. Blood biochemistry of diseased and healthy dogs was performed from obtained serum samples with help of an automated biochemistry analyzer using serum biochemistry analysis specific kits. For the assessment of the health profile of each dog, serum glucose, total cholesterol, triglyceride, total protein, albumin, creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were estimated. Globulin level was estimated by deducting the albumin content from total proteins.

Statistical analysis

Statistical analysis was conducted to determine the difference between the groups. The comparison

between the groups was analyzed by the unpaired student's *t*-test. The statistical difference between the pre- and post-therapy data was analyzed by paired student's *t*-test using GraphPad Prism 8 software (San Diego, CA, USA). P values less than 0.05 were considered significant.

Results and Discussion

A total of 9 dogs identified to have clinical *Sarcoptes* mite infestation were included in the present study. Out of 9 dogs, there were 3 female and 6 male dogs. Of these, four were of German Shepherds, four Mongrels (mixed breeds) and one was Rottweiler. The age of the dogs ranged between 1 to 8 years. Out of 9 participated dogs, only 6 dogs could complete the study period, while 3 dogs were dropped from the study due to non-compliance of the pet owner during the COVID-19 pandemic. The skin lesions were mainly distributed on the ear flaps including the ear margins, hock regions, hind legs, thoracic areas, face and the ventral side of abdominal areas (Fig. 1). All dogs revealed intense pruritus and self trauma due to scratching. On day 0, all disease dogs revealed strongly positive pinnal-pedal scratch reflex on clinical examination.

The evaluation of parasitological cure was based on detection of the mite by microscopic examination of skin scraping sample of chewable fluralaner treated diseased dogs at day 21 post-therapy. On day 21 post-therapy none of the treated dog (N=6) was found to have presence of *S. scabiei* mite and/or its developing stages on microscopic examination. Complete parasitological cure was observed in all dogs on day 21 of chewable fluralaner administration. The clinical cure was assessed on the basis of percent improvement in SSLS on day 21 post-therapy. On day 0, dogs with sarcoptic manage revealed the SSLS 9.50 ± 1.2 score. A significant (P=0.002) reduction in the SSLS was revealed by these dogs of day 21 post-therapy, and it was recorded as 1.67 ± 0.4. A total of 83.6 % improvement in the SSLS of fluralaner treated dogs was found on day 21 post-therapy. Marked improvement in the clinical manifestations was observed in all treated dogs on day 21 post-therapy (Fig. 2). Further, the treated dogs were followed-up till the three months post-therapy and complete clinical recovery was observed in all followed-up dogs within three months (Fig. 3). On day 21 post-therapy mildly positive pinnal-pedal scratch reflexes was revealed by the all treated dogs.

Marked alterations in the haemogram panels were observed in dogs with sarcoptic mange as compared to healthy dogs (Table 1). Dogs with sarcoptic mange had a significantly lower (P=0.043) hemoglobin level as compared to healthy dogs. Moreover, a non-significant reduction in TEC (P=0.074) and HCT (P=0.061) was revealed by diseased dogs. The significantly elevated total leukocytes counts (P=0.002) and absolute granulocyte counts (P=0.001) were revealed by dogs with sarcoptic mange as compared to healthy ones (Table 1). The dogs with sarcoptic mange revealed significantly elevated total protein (P=0.016) and globulin (P=0.028) levels as compared to healthy dogs (Table 2). Moreover, significantly elevated AST (P=0.010) and ALT (P=0.038) activities in diseased dogs were estimated. Whereas significantly decreased BUN (P=0.006), and glucose (P=0.004) levels were estimated in diseased dogs (Table

Fig 1: A 18-month-old mogrel dog suffering from sarcoptic mange at day 0 (pre-therapy)

Fig 2: Same dog with marked clinical improvement at day 21 post-therapy of fluralaner administration.

Fig. 3: Same dog with complete clinical recovery at day 90 post-therapy of fluralaner administration.

2). While in other studies, biochemical panels were not significantly altered in diseased dogs when compared with healthy ones (Table 2).

On day 21 post-therapy, a non-significant reduction in TLC and absolute granulocytes count was observed in fluralaner treated dogs as compared to their own day 0 values (Table 3). Moreover, a non-significant elevation in TEC (P=0.179), haemoglobin (P=0.155) and HCT (P=0.099) values was revealed by the treated dogs as compared to their own values on day 0 (Table 3). The hematological panels were found to be ameliorated towards normalcy on day 21 post-therapy in all treated dogs. A non-significant amelioration in the all altered biochemical parameters was observed in dogs treated with fluralaner (Table 4). On day 21 post-therapy, the treated dogs revealed a non-significantly reduction in total protein (P=0.234) and globulin (P=0.462) levels as compared to their own day 0 values. Whereas, a non-significant increase in glucose (P=0.317) and BUN (P=0.527) levels was estimated in these dogs. Moreover, a non-significantly reduction AST (P=0.438)) activities was revealed by the treated dogs on day 21 post-therapy. However, the activities of ALT remained elevated on day 21 post-therapy in fluralaner treated dogs. Except to ALT activities, all studied biochemical panels ameliorated towards normalcy on day 21 post-therapy in fluralaner treated dogs.

Assessment of biochemical markers in serum is crucial for determining the extent of organ and tissue damage (Steinhardt and Thielscher, 2000). In the current study, haematological panels in dogs with sarcoptic mange were found to be significantly altered. Dogs with sarcoptic mange had significantly lower total erythrocyte counts, haematocrit, and haemoglobin values. Low TEC, HCT, and Hb levels in dogs with sarcoptic mange may be caused by hyporexia, restlessness, blood loss from scratching, and/or oxidative damage to the erythrocytes, which lead to apoptosis and membrane injury. The pathogenesis of clinical Sarcoptes mite infestation in dogs has been shown to involve a significant alteration in the oxidant/antioxidant balance (Singh *et al.,* 2011)**.** Decreased levels of haemoglobin may be due to the chronicity and generalized nature of the disease (Ferreira *et al.,* 1987). It might also be due to the significant low erythrocyte count and erythrocyte fragility and low production of erythropoietin due to toxaemia caused by mites. In agreement with our findings, Reddy *et al.* (2014) demonstrated a markedly lower level of mean Hb of

Table 1: Comparison of haematological alterations of dogs with sarcoptic mange and healthy controls.

Parameters	Healthy dogs (N= 6)	Dogs with sarcoptic mange (N=9)	P value
TEC ($\times 10^6/\mu L$)	6.71 ± 0.27	5.75 ± 0.35	0.074
Hb (g/dL)	14.85 ± 0.69	11.97 ± 0.93*	0.043
HCT (%)	45.35 ± 1.62	37.54 ± 2.88	0.061
MCV (fL)	67.75 ± 1.65	64.7 ± 1.61	0.226
MCH (pg)	22.05 ± 0.76	20.64 ± 0.59	0.166
MCHC (g/dL)	32.73 ± 0.57	32 .56 ± 0.68	0.865
Platelets ($\times 10^3/\mu L$)	274 ± 25	435 ± 61	0.063
TLC ($\times 10^3/\mu L$)	8.5 ± 1.12	19.47 ± 2.1*	0.002
Lymphocyte ($\times 10^3/\mu L$)	2.15± 0.54	2.48 ± 0.39	0.615
Monocyte ($\times 10^3/\mu L$)	0.25± 0.03	0.41 ± 0.09	0.197
Granulocyte ($\times 10^3/\mu L$)	6.13 ± 0.61	16.32 ± 2.05*	0.001
Eosinophils ($\times 10^3/\mu L$)	0.07 ± 0.05	0.28 ± 0.09	0.101
Lymphocytes (%)	22.38 ± 4.2	13.67± 2.35	0.075
Monocytes (%)	2.53 ± 0.31	2.01 ± 0.44	0.402
Granulocytes (%)	74.15 ± 4.67	83.06 ± 2.46	0.088
Eosinophils (%)	0.93 ± 0.37	1.24 ± 0.38	0.590

Data presented are Mean ± S.E.M.
*Differed significantly ($P \leq 0.05$), when compared with healthy animals.

dogs with scabies. Similarly, altered hemato-biochemical changes were demonstrated in buffaloes with sarcoptic mange by Dimri *et al.* (2007). Hematological parameters viz. hemoglobin (Hb%) and total erythrocytes count (TEC) of dogs with sarcoptic mange were reported to be significantly decreased (Behera *et al.,* 2011; Singh *et al.,* 2011). A similar finding having low hemoglobin animals with sarcoptic mange has also been demonstrated by other scientific reports (Cimen, 2008; Saleh *et al.,* 2011; Beigh *et al.,* 2013). Sarcoptic mange affected dogs had significantly higher total leukocytes and granulocytes counts than control. *S. scabiei* infestation is often accompanied by hypersensitivity reactions (Noviana *et al.,* 2004). The cytokines secreted from the T lymphocytes play a key role in the immune response of the dogs against parasitic skin infestations (Arlian *et al.,* 1996; Singh and Dimri, 2014).Our finding of elevated total leukocytes and absolute granulocytes counts of dogs with sarcoptic mange might be attributed to the inflammatory immune response of the host. The findings of the present study are in agreement with the earlier scientific reports (Arlian *et al.,* 1995; Behera *et al.,* 2011). Leucocytosis along with granulocytosis of dogs with sarcoptic mange is parallel with the findings of Reddy *et al.* (2014), Narang *et al.* (2015), Beigh *et al.* (2016) and Nwufoh *et al.* (2019).

In the present study, dogs with sarcoptic mange revealed significantly elevated total protein and globulin levels while albumin level was within the reference range of healthy controls. Significantly elevated levels of total proteins and globulins in diseased dogs might be attributed to the consistent immune response of the host against the parasite's antigenic flux. The mite had a long co-evolution with its mammalian hosts and is capable of modulating many aspects of the innate and adaptive protective responses of their host enabling them to survive and thrive (Singh *et al.,* 2014).Due to the chronic pattern of the disease, sustained mite's antigens are presented to the host immune system, which can trigger the continuous production of immunoglobulins and hence conferring hyperproteinemia and hyperglobulinemia in diseased dogs. The observations of hyperproteinemia and hyperglobulinemia in dogs with sarcoptic mange are in agreement with the earlier scientific report demonstrating an elevated level of total proteins (De and Dey, 2010). However, in contrast to our findings, hypoproteinaemia was reported in dogs with sarcoptic mange (Reddy *et al.,* 2014). They reported lowered mean total serum protein and albumin levels, but, elevated serum globulin levels were recorded by them in dogs with scabies (Reddy *et al.,* 2014). Moreover, decreased total protein and albumin in canine and ovine scabies was demonstrated (Chandy *et al.,* 2000; Singh *et al.,* 2011). In these studies, the lowered albumin level would have resulted in decreased total protein (Behera *et al.,* 2011). The clinical presentation, the chronicity of disease condition, areas of the skin lesions and the presence of concurrent skin diseases might be attributed to the differences observed in our study and other reported studies.

Table 2: Comparison of serum biochemical panels of dogs with sarcoptic mange and healthy controls.

Parameters	Healthy dogs (N=6)	Dogs with Sarcoptic mange (N=9)	P value
Triglyceride (mg/dL)	48.32 ± 6.21	52. 40 ± 9.65	0.758
Cholesterol (mg/dL)	227 ± 16	234 ± 15	0.788
Total protein (g/dL)	7.35 ± 0.31	10.62 ± 0.93*	0.016
Albumin (g/dL)	3.15 ± 0.22	3.08 ± 0.23	0.893
Globulin (g /dL)	4.23 ± 0.28	7.53 ± 1.06*	0.028
A /G ratio	0.75 ± 0.08	0.50 ± 0.10	0.098
Aspartate Aminotransferase AST (U/L)	17.96 ± 1.92	29.2 ± 2.76*	0.010
Alanine Aminotransferase ALT (U/L)	33.69 ± 4.39	44.41 ± 2.45*	0.038
Alkaline phosphatase (ALP) (U/L)	70.91 ± 11.14	120.59 ± 27.90	0.187
BUN (mg/dL)	15.69 ± 1.26	11.79 ± 0.50*	0.006
Creatinine (mg/dL)	1.33 ± 0.066	1.82 ± 0.205	0.083
Glucose (mg/dL)	99.92 ± 6.09	69.44 ± 5.86*	0.004

Data presented are Mean ± S.E.M.
*Differed significantly ($P \leq 0.05$), when compared with healthy dogs.

Table 3: Comparison of haematological changes of fluralaner treated dogs with sarcoptic mange at pre- and post-therapy.

Parameters	Pre-therapy (Day 0) (N=6)	Post-therapy (Day 21) (N=6)	P value
TEC ($\times 10^6/\mu L$)	5.98 ± 0.36	6.54 ± 0.40	0.179
Hb (g/dL)	12.5 ± 0.96	14.58 ± 1.15	0.155
HCT (%)	39.08 ± 2.94	46.15 ± 2.88	0.099
MCV (fL)	65.06 ± 1.72	66.4 ± 1.28	0.478
MCH (pg)	20.81 ± 0.72	21.18 ± 0.61	0.649
MCHC (g/dL)	31.98 ± 0.35	31.93 ± 0.54	0.937
Platelets ($\times 10^3/\mu L$)	372.6 ± 74.3	421 ± 73.9	0.607
TLC ($\times 10^3/\mu L$)	18.70 ± 2.30	16.9 ± 2.30	0.658
Lymphocyte ($\times 10^3/\mu L$)	2.73 ± 0.51	2.76 ± 0.45	0.937
Monocyte ($\times 10^3/\mu L$)	0.36 7± 0.143	0.51± 0.11	0.335
Granulocyte ($\times 10^3/\mu L$)	15.26 ± 1.88	13.4 ± 2.39	0.646
Eosinophils ($\times 10^3/\mu L$)	0.28 ± 0.13	0.32 ± 0.09	0.849
Lymphocytes (%)	15.11 ± 2.76	17.96 ± 3.29	0.522
Monocytes (%)	1.93 ± 0.52	3.13 ± 0.62	0.037
Granulocytes (%)	81.50 ± 2.67	77.5 ± 3.60	0.405
Eosinophils (%)	1.45 ± 0.55	1.38 ± 0.51	0.934

Data presented are Mean ± S.E.M.

The significant increase in ALT and non-significant increase in AKLP activities observed in dogs with sarcoptic mange might be attributed to stress induces hepatic impairment in diseased dogs. The role of significant alteration in oxidant/antioxidant balance in the pathogenesis of clinical *Sarcoptes* mite infestation of dogs has been demonstrated (Singh *et al.,* 2011). The hypoglycemia observed in dogs with sarcoptic mange might be due to increased energy demand of the skin during inflammatory reactions, pruritus-induced hyporexia, and inflammation-induced hepatic impairments (Singh *et al.,* 2014). A lower level of BUN might be attributed to hyporexia-induced malnourishment and/or inflammation-induced hepatic impairment. The findings of our study are in agreement with previous scientific reports demonstrating elevated liver-specific enzymes activities in dogs with sarcoptic mange (Singh *et al.,* 2011; Behera *et al.,* 2011).

On day 21 post-therapy, a total of 83.6% clinical recovery (reduction in SSLS) was observed in dogs treated with oral fluralaner. However, mild pruritus and penal-pedal reflex were recorded in all treated dogs at day 21 post-therapy, but the intensity was markedly lowered than day 0 (pre-therapy). The treated dogs were followed up to 3 moths-post of therapy; a complete clinical recovery and no pruritus and penal pedal reflex were observed. Therefore, a single oral dose fluralaner equivalent to 25 to 56 mg/kg was found to be effective for the treatment of sarcoptic mange in dogs. Fluralaner could provide a very convenient and effective treatment option for dogs suffering from mite infestations (Petersen *et al.,* 2020). Fluralaner provides an extended period of persistent efficacy against ticks and fleas for dogs (Romero *et al.,* 2016). In the present study, we demonstrated that fluralaner is effective for the complete treatment of sarcoptic mange in dogs. In agreement with our findings, Romero *et al.* (2016) reported that fluralaner was effective in eliminating scabies mites within 14 days and significantly resolved the clinical signs associated with sarcoptic mange within 21 days after a single dose. The rapid mite killing potential of fluralaner might be associated with a significant reduction in the clinical signs of scabies in treated dogs. Taenzler *et al*. (2016) reported that the fluralaner administered either orally or topically to naturally infested dogs resulted in a 100% reduction in *S. scabiei var. canis* mites and improves clinical signs over a 4-week observation period. Moreover, the similar therapeutic efficacy of a single treatment of client-owned, sarcoptic mange-affected

Table 4. Comparison of serum biochemical panels of fluralaner treated dogs with sarcoptic mange at pre- and post-therapy.

Parameters	Pre-therapy (Day 0) (N=6)	Post-therapy (Day 21) (N=6)	P value
Triglyceride (mg/dL)	47.19 ± 11.74	54.93 ± 11.88	0.451
Cholesterol (mg/dL)	222 ± 20	245 ± 22	0.479
Total protein (g/dL)	10.77 ± 1.33	8.95 ± 0.82	0.234
Albumin (g/dL)	3.13 ± 0.35	2.61 ± 0.28	0.125
Globulin (g/dL)	7.64 ± 1.52	6.33 ± 0.77	0.366
A/G ratio	0.54 ± 0.15	0.44 ± 0.06	0.418
Aspartate Aminotransferase (AST) (U/L)	31.42 ± 3.64	27.14 ± 4.58	0.438
Alanine Aminotransferase (ALT) (U/L)	42.69 ± 3.42	48.58 ± 5.22	0.415
Alkaline Phosphatase (ALKP) (U/L)	115 ± 33	112 ± 14	0.943
BUN (mg/dL)	12.39 ± 0.62	13.22 ± 0.81	0.527
Creatinine (mg/dL)	2.01 ± 0.27	1.65 ± 0.15	0.334
Glucose (mg/dL)	75.11 ± 7.44	84.36±6.79	0.317

Data presented are Mean ± S.E.M.

dogs with either fluralaner chewable tablets or fluralaner spot-on formulation was demonstrated by Chiummo *et al*. *(*2020). Recently, a study demonstrated that a single oral administration of fluralaner was effective in complete clinical and parasitological cures of 12 pet rabbits with sarcoptic mange within 21 days of therapy (d'Ovidio and Santoro, 2021). They reported that all treated rabbits were negative for the presence of mites on day 14 post-therapy (d'Ovidio and Santoro, 2021). In the present study, no major adverse effects for instance ataxia, seizures, and shaking of the body were observed in any of the fluralaner-treated dogs during the study period followed up to day 21. Moreover, except for ALT activities, all studied haemato-biochemical panels shifted toward normalcy in all treated dogs. Therefore, the finding of the present study suggests that fluralaner can be used safely in dogs for therapeutic management of sarcoptic mange. Similar findings were reported for the safe use of isoxazolines in cats (Cavalleri *et al*. 2018a; Cavalleri *et al*. 2018b; Kuntz and Kammanadiminti, 2018) and MDR1-/-collies dogs (Walther *et al.,* 2014). However, Palmieri *et al.* (2020) reported the occurrence of neurological adverse effects on dogs using isoxazolines including 13.7 % seizures, 15.9% ataxia, and 16.7% shaking as overall rates for afoxolaner, fluralaner, and sarolaner, respectively. In our study, only one dog vomited once after an hour of administration of chewable fluralaner during treatment. In agreement with our findings of adverse effects, vomiting was reported as the most common adverse event and no treatment-related serious adverse events were observed in fluralaner-treated dogs by Meadows *et al.* (2014). Further, studies in large no of dogs are needed to validate the major adverse effects of fluralaner chewable in dogs.

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Conflicts of interest/Competing interests:

The authors declare there is no conflict of interest.

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Ultrasonographic examination of rumen in healthy non-descript goats

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Abstract

The present study was conducted to establish ultrasonographic features of rumen in 20 numbers of healthy non-descript goats by using two-dimensional B-mode ultrasound scanner (SonorayDS 50 plus VET) with curvilinear probe. Ultrasonographic examination was performed on both sides of goats in a standing position with a frequency of 2.5-4.5 MHz. The rumen was examined from 8th to 12th intercostal spaces (ICSs) and flank on the left and from 12th ICS, flank on the right. The dorsal and ventral sacs of the rumen are differentiated based on longitudinal groove. The gas layer enveloped by an echogenic rumen wall and reverberation artifacts. The fiber mat appears as an echogenic mass with gas inclusions and ventral fluid layer was hypoechoic. The rumen wall thickness of gas layer, fiber mat and fluid layer were respectively 2.18 ± 0.44mm, 2.69 ± 0.39 mm and 2.34 ± 0.46mm.

Keywords: Ultrasonography, Rumen, Goat, Echogenic line, Hypoechoic and Flank.

Ultrasonography is most commonly used imaging technique in veterinary practices and forms an integral part of clinical diagnosis for many disorders. Ultrasonography is a non-invasive, essentially nontoxic, free from radiation hazards and provides quick and dynamic visualization. Ultrasonography has become a commonly employed diagnostic tool in large animal practice, owing to the increased availability of durable, portable machines and the increased producer demand for early pregnancy detection in many livestock species (Scott and Sargison, 2010). Ultrasonography provides images in real time. Ultrasound displays the findings on the radiographs, as well as the soft tissues textures and dynamics of some organs, e.g. motility of the bowel (Siems, 2005). Ultrasonography has been used to evaluate the rumen in cattle (Braun, 2009). Ultrasonography is relatively inexpensive compared to other modes of investigation, viz. CT or MRI. Ultrasonography findings are not necessarily specific for histopathology diagnosis and however, the ability to distinguish solid masses allows the sonographer to focus differential diagnosis and to formulate management plans (Walter, 2003). Ultrasonography uses high frequency sound viz. ultrasound waves to produce images of internal organs and other tissues. A sound wave travels in a pulse and when it is reflected back it becomes an echo. It is the pulse-echo principles, which is used for ultrasound imaging. Ultrasound can be used to evaluate most soft tissue, including muscles, tendons and ligaments, the heart and abdominal organs. Ultrasound cannot be used to scan gas filled or bony tissues. Sonographic imaging is also limited in regard to the depth of tissues that can be examined. Ultrasonography is an ideal tool for evaluation of the contour and motility of reticulum and adjacent organs i.e. spleen, diaphragm, abomasum, liver etc.

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Materials and Methods

Animal

Twenty numbers of non-descript goats of both sex with age group of 1.5 to 7 years (mean ± sd=3.10 ± 1.69 years) and body weight between 20-30 kg (mean ± sd =24.74 ± 3.46 kg) were selected for present study.

Animal examination

Clinical examinations viz. rectal temperature, heart rate, respiratory rates and rumen motility were performed for all healthy goats under the study. Blood samples were collected for haematology and biochemical examination. Urine samples were examined for colour, transparency, specific gravity and for other characteristics using a test strip. Rumen fluid samples were examined for colour, odour, consistency, sedimentation activity test, pH, iodophilic activity test, microscopic examination of protozoa, grams stain and methylene blue reduction time.

Ultrasonography of the rumen

A two-dimensional B-mode ultrasound scanner (Sonoray DS 50 plus VET) with a 2.5-4.5 MHz curvilinear

probe was used. Ultrasonography examination was performed in standing position without sedation and to prevent artifacts, ultrasonography gel was used.

Statistical Analysis

Data obtained from electronic calipers during ultrasonography were used for descriptive statistical analysis for calculating thickness of ruminal wall viz. Gas layer, Fiber mat layer, and Fluid layer.

Results and Discussion

All goats were found apparently healthy after through clinical examination, haematology and biochemical examination, urinalysis, rumen fluid examination and faecal sample examination. The ruminal wall appears as a thick echoic line. The contents of the rumen are layered as dorsal gas cap, fiber mat layer (middle layer) and fluid layer (ventral layer).Changes in the layering of the ruminal contents was seen during contractions. The gas layer was found became smaller and the fiber mat layer was observed larger. Ultrasonography of the rumen viewed from dorsal left flank the rumen wall appears as an echogenic and reverberation artifact, which reflects the gas layer (Figure I). The fiber mat layer (Fig. II) which was viewed from the mid-region of the left flank was echogenic and the bordering rumen wall is heterogeneous. The fluid layer (Fig. III) was viewed from the ventral region of the left flank was appeared as a hypoechogenic and surrounded by a homogeneous and smooth rumen wall. The dorsal and ventral sacs of rumen are differentiated based on the longitudinal groove, which forms an echoic notch (Fig. IV). In Ultrasonography of 12th intercostal space on the left side dorsolateral region, the spleen and rumen were observed (Fig. V) and on the right side caudal flank region, rumen and small intestinal loop adjacent to the rumen was seen (Fig. VI). The wall thickness of rumen in gas layer, fiber mat layer and fluid layer is presented in Table I.

B-mode ultrasonography of rumen was performed in twenty numbers of healthy non- descript goats by using 2.5 – 4.5 MHz curvilinear probe. Ultrasonography was performed in non-sedated goats in a standing position, ultrasonography in non- sedated goat, cattle and buffalo calves in standing position was also reported earlier by Braun *et al.* (2011), Braun (2003) and Rathore (2007). Rumen wall in all the twenty healthy goats appeared as thick echogenic structure and the contents of the rumen were not visualized because of gas filled composition in the rumen. Similar kind of findings were reported by Kandeel *et al*., 2009; Abu- Seida, 2002 and Braun, 2003. Ruminal contractions could not be directly observed, changes in the layering of the ruminal contents was seen during contractions, finding correlates with the finding reported by Braun *et al.* (2011). Rumen viewed from dorsal left flank rumen wall appears echogenic and reverberation artifact, finding support the findings reported by Tschuor and Clauss (2008), Braun *et al.* (2011). In fiber mat layer bordering rumen wall observed heterogeneous in nature, similar findings were also reported in goats by Tschuor and Clauss (2008), Braun *et al.* (2011). The fluid layer viewed from the ventral region of the left flank appeared as hypoechogenic, the finding was concurred with finding of Braun *et al.* (2011). The dorsal and ventral sacs of the rumen are differentiated based on the longitudinal groove, which support the finding reported by Braun *et al*. (2011).

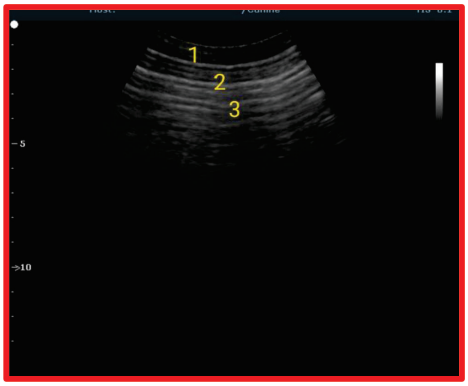

Fig. I: Ultrasonography of the rumen gas layer viewed from the dorsal left flank of a goat using a 2.5-4.5 MHz curvilinear probe. 1. Abdominal wall 2. Rumen wall 3.Reverberation artifacts.

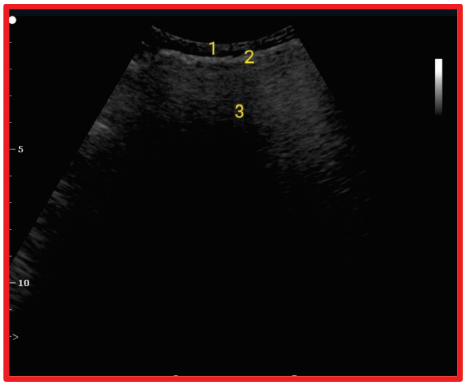

Figure II: Ultrasonography of the fiber mat of the rumen viewed from the mid-region of the left flank using a 2.5-4.5 MHz curvilinear probe. 1. Abdominal wall 2. Rumen wall 3.Fibre mat of the ruminal contents.

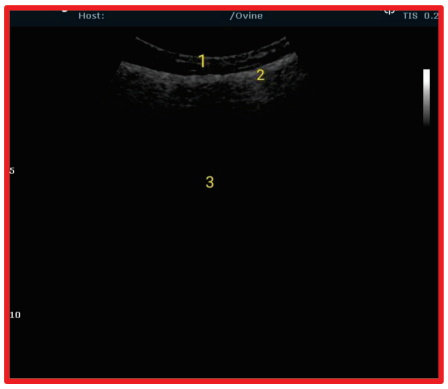

Figure III: Ultrasonography of the fluid layer of the rumen viewed from the ventral region of the left flank using a 2.5-4.5 MHz curvilinear probe. 1. Abdominal wall 2.Rumen wall 3. Fluid layer of the ruminal contents.

Figure IV: Ultrasonography of the rumen of goat showing longitudinal groove view from flank using 2.5-4.5 MHz curviliniear probe. 1. Abdominal wall 2.Dorsal sac of the rumen 3.Ventral sac of the rumen 4.Longitudinal groove.

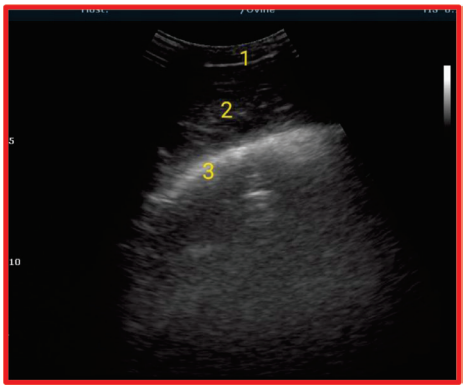

Figure V: Ultrasonography examination of rumen and spleen viewed from 12th intercostal space on left side using a 2.4-4.5 MHz curvilinear probe. 1. Abdominal wall 2. Spleen 3. Rumen wall.

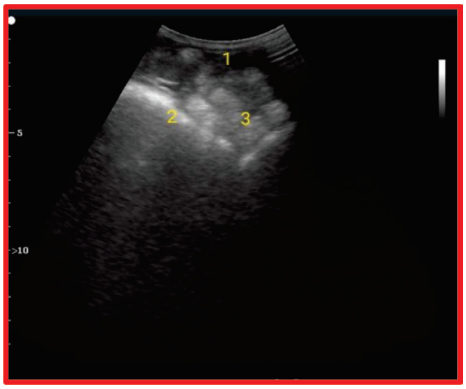

Figure VI: Ultrasonography examination of rumen on right side flank using a 2.4-4.5 MHz curvilinear probe. 1. Abdominal wall 2.Rumen wall 3.Small intestine.

Table I. Rumen wall thickness and echogenicity.

Sl. No.	Layer of the ruminal content	Thickness of the rumen wall (mm) (mean ±sd)	Rumen wall echogenicity
1	Gas layer	2.18 ± 0.44	Echogenic and reverberation artifact.
2	Fiber mat layer	2.69 ± 0.39	Echogenic and bordering rumen wall was heterogeneous.
3	Fluid layer	2.34 ± 0.46	Smooth and homogeneous rumen wall and ruminal contents was hypoechogenic.

Ultrasonography on the left side dorsolateral region of 12th intercostal space the spleen and rumen was seen; similar finding earlier reported by Braun *et al*. (2011) and on the right side caudal flank region, rumen and small intestinal loop adjacent to the rumen was observed, similar observation were also reported by Braun *et al*. (2011).

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Management of Haemorrhagic Cystitis in dogs

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Abstract

Present study was carried out in the Department of Veterinary Medicine, Dr. G C Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India. Detailed clinical evaluation of 6 dogs were carried out based on history and presenting clinical signs. The most common clinical sign noted were haematuria, dribbling of urine, pollakiuria and dysuria. Haematological examination, biochemical analysis, urine examination (urine dipstick, cytology and microbiological), and ultrasonographic studies were performed for the confirmatory diagnosis. Specific therapeutic of choice was advised based on cultural sensitivity test. *E. Coli and Staphylococcus spp.* were the bacteria isolated in the present study. Meropenem, Marbofloxacin and Nitrofurantoin were the most sensitive antibiotic found in the cultural sensitivity test. Complete amelioration of clinical signs was found in 4 dogs, 14 days post treatment whereas in 1 dog, treatment was extended till 21 days for complete recovery.

Keywords: Haemorrhagic Cystitis, Haematuria, *E.Coli*, Meropenem

Haemorrhagic cystitis is described as a diffuse inflammatory disease condition of urinary bladder caused by infectious and non-infectious agents resulting in bleeding from the bladder mucosa (Manikandan *et al.,* 2010). The disease is characterised by the presence of blood in urine (haematuria), dysuria and pollakiuria. The glycosaminoglycan layer that covers the bladder transitional epithelium, normal urethral microflora, frequent voiding of urine, antimicrobial properties of urine, an appropriate host immune response, and a functional urethral sphincter are all factors that restrict bacterial colonization of the bladder (Greene, 2006).

Bacterial pathogens are the common infectious agents, which mostly responds to treatment. Bacterial urinary tract infections are one of the most prevalent infectious disorders in dogs, affecting 14% of them throughout their lives (Brovida, 2003). Prominent bacterial pathogens causing haemorrhagic cystitis are *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis* and *Klebsiella species* (Krane and Levine, 1992). The fungal microbes associated with haemorrhagic cystitis are *Cryptococcus neoformans*, *Candida albicans*, *Aspergillus fumigates* and *Torulopsis glabrata*. Viral agents such as the *Polyma virus*, *Adenovirus* and *Herpes virus* have been reported in haemorrhagic cystitis in pediatric and immunocompromised patients [Storver *et al*. 2004; Hatland and Waschcka 2004]. Echinococcus granulosus infections can also lead to calcified cysts formation that may penetrate the bladder wall resulting in Haematuria and Cystitis (Manikandan *et al.*, 2010).

Chronic and/or recurrent cystitis may also occur due to chemotherapy or exposure to radiation in pelvic malignancies. Haemorrhagic cystitis has been reported in dogs when treated with cyclophosphamide which is used to treat lymphosarcoma, mastocytoma, transmissible venereal tumour, bladder carcinoma, multiple myeloma and auto immune diseases (Marin *et al.,* 1996).

Materials and Methods

Present study was carried on 6 dogs presented to the Department of Veterinary Medicine, Dr. G C Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India. Preliminary screening was done based on History, Clinical manifestations and Urinalysis. Haematological, Biochemical Examination, Ultrasonographic studies, Antimicrobial sensitivity testing and Identification of bacteria in urine were carried out. Treatment was advised based on Antibiotic sensitivity testing.

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Table 1. Clinical parameters Haemorrhagic Cystitis in dogs

S. No.	Parameter	Healthy animals (n = 15) (Mean ± S.E.)	Haemorrhagic Cystitis (n = 6) (Mean ± S.E.)
1.	Rectal Temperature (°F)	101.42 ± 0.21	102.75 ± 0.31**
2.	Respiration rate (per min.)	31.73 ± 0.86	34.05 ± 1.22
3	Heart rate (per min.)	102.53 ± 3.13	105.55 ± 4.53

Results and Discussion

The clinical parameters in dogs suffering from Haemorrhagic Cystitis are depicted in the Table 1. The mean values of Rectal Temperature, Respiration Rate and Heart Rate were 102.75 ± 0.31 ºF, 36.05 ± 1.22 per minute and 105.55 ± 4.53 per minute respectively. The mean of Rectal Temperature was significantly increased when compared with the healthy animals.

Haematuria (gross or microscopic) 83.3% (5/6), Pollakiuria 66.66% (4/6), Dribbling of urine 50% (3/6) and Dysuria 50% (3/6) were the most common clinical signs associated with haemorrhagic cystitis in the present study. Other signs such as oliguria, reduced water intake, anorexia, fever and dehydration were also noticed (Plate 1 and 2).

The haematological parameters of affected dogs are enlisted in Table 2. The mean values of Total Leucocytic Count (TLC), Total Erythrocytic Count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV) and Platelets (PLT) were 26.23 ± 2.56 x 10^9/L, 6.45 ± 0.36 x 10^{12}/L, 12.98 ± 0.51 g/dL, 44.32 ± 0.58 % and 200.8 ± 17.38 x 10^9/L. Sarma and Kalita (2019) and Roopali *et al*. (2018) also reported the increase in TLC in dogs affected with Cystitis. Leucocytosis occurs due to variable extent of stress induced by bacterial organisms in the urinary tract as well as sign of manifestation of body defense mechanisms against bacterial infection.

The plasma Haematobiochemical parameters namely Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total Protein (TP), Blood Urea Nitrogen (BUN) and Creatinine (Cr) were 46.12 ± 7.8 U/L, 71.64 ± 12.27 U/L, 101.18 ± 31.3 U/L, 0.26 ± 0.1 mg%, 6.19 ± 0.47 g%, 30.1 ± 4.27 mg% and 1.1 ± 0.17 mg% respectively. The mean values of AST, ALP and BUN were significantly increased when compared with healthy animals. These finding were in accordance with Punia *et al*. (2018). Increase in ALP values indicated inflammation and muscle tissue damage.

Ultrasonography

Ultrasonographically all the 6 dogs were diagnosed with inflamation and urinary bladder wall

Tabel 2. Haematobiochemical parameters in Haemorrhagic Cystitis in dogs

Parameter	Healthy animals (n=15) (Mean ± S.E.)	Haemorrhagic Cystitis (n=6) (Mean ± S.E.)
Total Leucocytic Count (x 10^9/L)	11.92 ± 0.32	26.23 ± 2.56**
Total Erythrocytic Count (x 10^{12}/L)	6.67 ± 0.29	6.45 ± 0.36
Haemoglobin (g/dL)	13.96 ± 0.40	12.98 ± 0.51
Packed Cell Volume (%)	41.22 ± 1.01	44.32 ± 0.58
Platelets (x 10^9/L)	312.4 ± 27.49	200.8 ± 17.38
Alanine aminotransferase (U/L)	30.77 ± 1.58	46.12 ± 7.8
Aspartate aminotransferase (U/L)	38.59 ± 2.19	71.64 ± 12.27*
Alkaline Phosphatase (U/L)	72.21 ± 8.63	101.18 ± 31.3**
Total Bilirubin (mg%)	0.32 ± 0.02	0.26 ± 0.1
Total Protein (g%)	6.46 ± 0.24	6.19 ± 0.47
Blood Urea Nitrogen (mg%)	18.45 ± 1.44	30.1 ± 4.27**
Creatinine (mg%)	0.89 ± 0.06	1.1 ± 0.17

**Significant at 1% level of significance, *Significant at 5% level of significance

thickness, where mean wall thickness was 5.82 mm (4.6 mm - 7.1mm) (Plate 3 and Plate 4). Irregular bladder was evident in 2 dogs where urinary bladder was collapsed. These findings were in accordance with Fromsa and Saini (2019).

Urine Examination

Dipstick examination

Upon Urine examination blood in urine (50 to 250 RBC/µL) was evident, along with Proteinuria (100 to 1000 mg/dL), Glucosuria (Negative to 500 mg/dL) and Leucocytes (Negative to 50 WBC/µL). The mean values of Urine pH and Specific gravity 6 ± 0.27 and 1.033 ± 0.002 (Table 3). The mean value of urine specific gravity was significantly increased when compared with healthy animals. Increased urine specific gravity may be due to over saturation of urine, proteinuria, reduced water intake and infection of the bladder.

Urine examination showed the presence of RBC's, WBC's, struvite, and bilirubin crystals, bacterial rods and cocci, in the urine sediment in the present study (Plate 5 and Plate 6). These findings were in accordance with Martinez et al. (2003). Presence of blood/RBCs in urine either occult or clinical haematuria in the sediment of the centrifuged urine sample is indicative of hemorrhage. Increase in number of leucocytes in urine were indicative of inflammation of urinary tract.

Cultural sensitivity test revealed that urine samples were sensitive to Meropenem (6/6), Marbofloxacin (5/6), Nitrofurantoin (4/6), Enrofloxacin (4/6), Amoxyclav (3/6), Ciprofloxacin (3/6) and Doxycycline (3/6) (Plate 7). These findings were contrary to Ukwueze (2013) who reported Gentamicin as the most sensitive antibiotic on Antibiogram. Bacterial pathogens isolated in haemorrhagic cystitis dogs were *E. coli (n = 5)* and *Staphylococcus spp., (n = 1).*

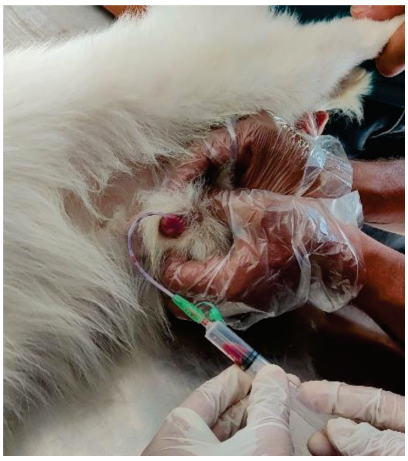

Plate 1: Blood in urine during Catherization

Plate 2: Blood in urine

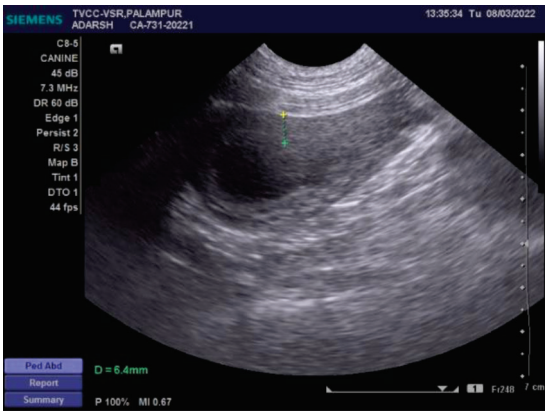

Plate 3: Collapsed, thickened urinary bladder with debris/ sludge

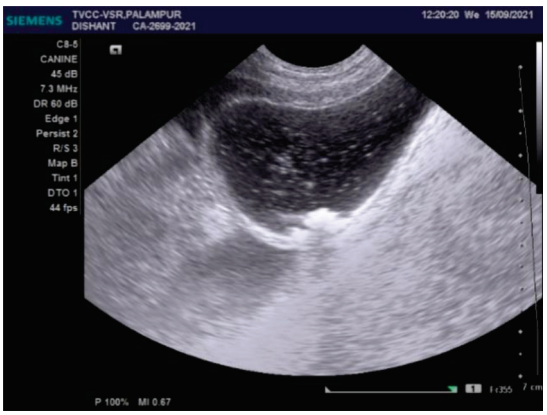

Plate 4: Thickened Urinary bladder filled with echogenic debris

Table 3. Urine Dipstick Analysis in Haemorrhagic cystitis in dogs

Parameter	Healthy animals (n=15) (Mean ± S.E.)	Haemorrhagic Cystitis (n=6) (Mean ± S.E.)
Blood	Negative	50 to 250 RBC/µL
Bilirubin	Negative	Negative
Urobilinogen	Normal	Normal
Ketones	Negative	Negative
Protein	Negative	100 to 1000 mg/dL
Nitrite	Negative	Negative
Glucose	Negative	Negative to 500 mg/dL
pH	5.83 ± 0.16	6 ± 0.27
Specific Gravity	1.021 ± 0.001	1.033 ± 0.002**
Leucocytes	Negative	Negative to 50 WBC/µL
Clarity	Clear	Clear

***Significant at 1% level of significance*

Therapeutics

The mean values of rectal temperature, respiration rate and heart rate were 102.75 ± 0.31 ºF, 36.05 ± 1.22 per minute and 105.55 ± 4.53 per minute respectively on Day 0, whereas post treatment values were 101.22 ± 0.41 ºF, 36.05 ± 1.22 beats/minute and 105.55 ± 4.53 beats/minute (Table 4). The mean post treatment value of rectal temperature was significantly decreased on day 15, when compared with day 0 and healthy animals. These finding were similar to the findings recorded by Weese *et al*. (2011).

The Pre and Post treatment values of haemato-biochemical parameters in Haemorrhagic Cystitis are listed in the Table 5. The mean post treatment values of TLC, TEC, Hb, PCV and PLT were 15.58 ± 2.96 x 10^9/L, 6.63 ± 0.45 x 10^{12}/L, 14.78 ± 0.87 g/dL, 38.92 ± 2.8 % and 220.4 ± 28.67 x 10^9/L. The mean post treatment value of TLC was significantly decreased when compared with the day 0 and healthy animals. The mean post treatment values all the above parameters varied non-significantly when compared with healthy animals. The post treatment biochemical parameters of Alanine aminotransferase, Aspartate aminotransferase, Alkaline Phosphatase, Total Bilirubin, Total Protein, Blood Urea Nitrogen and Creatinine were 49.82 ± 4.15 U/L, 40.12 ± 9.12 U/L, 194.44 ± 15.87 U/L, 0.34 ± 0.14 mg%, 6.21 ± 0.43 g%, 27.22 ± 6.87 mg% and 0.79 ± 0.18 mg%. The post treatment values of all the above parameters varied non-significantly when compared with day 0 and healthy animals.

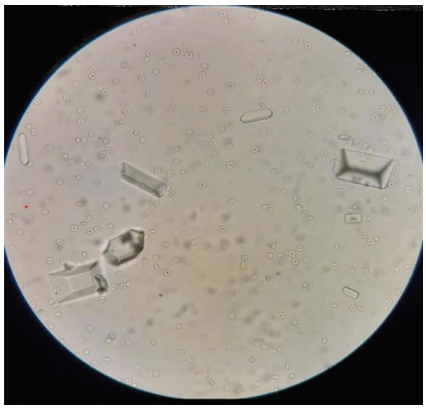

Plate 5: RBCs and Triple phosphate crystals in unstained slide, 40x

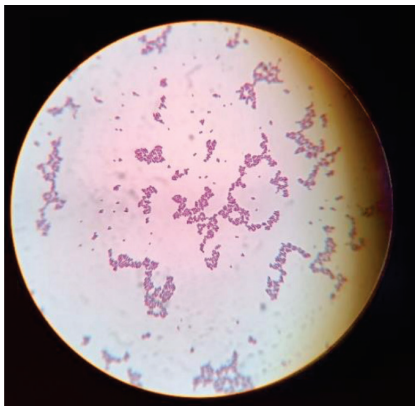

Plate 6: Grams Staining showing Gram positive cocci, 100x

Plate 7: Different zones of inhibition according to the sensitivity of antibiotic discs

Table 4. Pre and Post treatment values of Clinical Parameters in Haemorrhagic Cystitis in dogs

S.No.	Parameter	Healthy animals (n = 15) (Mean ± S.E.)	Haemorrhagic Cystitis (n = 6)	
			Day 0 (Mean ± S.E.)	Day 15 (Mean ± S.E.)
1.	Rectal Temperature (°F)	101.42 ± 0.21[a]	102.75 ± 0.31[b]	101.22 ± 0.41[a]
2.	Respiration rate (per min.)	31.73 ± 0.86[a]	34.05 ± 1.22[a]	32.05 ± 1.45[a]
3	Heart rate (per min.)	102.53 ± 3.13[a]	105.55 ± 4.53[a]	100.28 ± 3.21[a]

Values with same superscripts in a row differ non-significantly in each stage

Table 5. Pre and Post treatment values of Haemato-Biochemical parameters with Haemorrhagic Cystitis in dogs

Parameter	Healthy animals (n=15) (Mean ± S.E.)	Haemorrhagic Cystitis (n=6)	
		Day 0 (Mean ± S.E.)	Day 15 (Mean ± S.E.)
Total Leucocytic Count (x 10^9/L)	11.92 ± 0.32[a]	26.23 ± 2.56[b]	15.58 ± 2.96[a]
Total Erythrocytic Count (x 10^{12}/L)	6.67 ± 0.29[a]	6.45 ± 0.36[a]	6.63 ± 0.45[a]
Haemoglobin (g/dL)	13.96 ± 0.40[a]	12.98 ± 0.51[a]	14.78 ± 0.87[a]
Packed Cell Volume (%)	41.22 ± 1.01[a]	44.32 ± 0.58[b]	38.92 ± 1.8[a]
Platelets (x 10^9/L)	312.4 ± 27.49[a]	200.8 ± 17.38[b]	220.4 ± 28.67[ab]
Alanine aminotransferase (U/L)	30.77 ± 1.58[a]	46.12 ± 7.8[a]	49.82 ± 4.15[a]
Aspartate aminotransferase (U/L)	38.59 ± 2.19[a]	47.64 ± 12.27[a]	40.12 ± 9.12[a]
Alkaline Phosphatase (U/L)	72.21 ± 8.63[a]	101.18 ± 31.3[a]	94.44 ± 15.87[a]
Total Bilirubin (mg%)	0.32 ± 0.02[a]	0.26 ± 0.1[a]	0.34 ± 0.14[a]
Total Protein (g%)	6.46 ± 0.24[a]	6.19 ± 0.47[a]	6.21 ± 0.43[a]
Blood Urea Nitrogen (mg%)	18.45 ± 1.44[a]	30.1 ± 4.27[a]	27.22 ± 6.87[a]
Creatinine (mg%)	0.89 ± 0.06[a]	1.1 ± 0.17[a]	0.79 ± 0.18[a]

Values with same superscripts in a row differ non-significantly in each stage

Table 6. Pre and Post treatment Urine Dipstick Analysis in Haemorrhagic cystitis

Parameter	Healthy animals (n=15) (Mean ± S.E.)	Haemorrhagic Cystitis (n=6)	
		Day 0 (Mean ± S.E.)	Day 15 (Mean ± S.E.)
Blood	Negative	50 to 250 RBC/µL	Nil to 50 RBC/µL
Bilirubin	Negative	Negative	Negative
Urobilinogen	Normal	Normal	Normal
Ketones	Negative	Negative	Negative
Protein	Negative	100 to 1000 mg/dL	Nil to 100 mg/dL
Nitrite	Negative	Negative	Negative
Glucose	Negative	Negative to 500 mg/dL	Negative
pH	5.83 ± 0.16[a]	6.0 ± 0.27[a]	6.2 ± 0.25[a]
Specific Gravity	1.021 ± 0.001[a]	1.033 ± 0.002[b]	1.025 ± 0.002[a]
Leucocytes	Negative	Negative to 50 WBC/µL	Negative
Clarity	Clear	Clear	Clear

Values with same superscripts in a row differ non-significantly in each stage

The mean Pre and Post treatment values of Urine Dipstick Analysis in Haemorrhagic cystitis are listed in the Table 6. Post treatment Urine dipstick examination revealed reduced Blood in urine and decreased proteinuria. The mean post treatment values of pH was 6.2 ± 0.25 and specific gravity was 1.025 ± 0.002. The mean post treatment values of urine Specific gravity was significantly decreased when compared with day 0, but varied non-significantly when compared with healthy animals. The above findings were in accordance with Martinez *et al*. (2003) and Merkel *et al*. (2017).

Out of six dogs suffering from haemorrhagic cystitis, based on cultural sensitivity test suitable antibiotic was advised. Four dogs were advised with Tablet Marbofloxacin @ 2.5 mg/kg b.wt and two dogs were advised with Tablet Nitrofurantoin @ 5 mg/Kg b.wt. Fifteen days post treatment five dogs recovered successfully whereas in one dog partial amelioration of clinical signs was noted, hence antibiotic was extended upto twenty-one days. After twenty-one days complete recovery was observed. However, the use of Gentamicin @ 5mg/kg b.wt was proved to be effective in treating Haemorrhagic cystitis, based on culture sensitivity testing Ukwueze, 2013.

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Short Communications

Field trial assessment of feeding amino-acids, enzymes and probiotics (Netzyme-strong) on the performance of broilers

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Performance of broilers is very much dependent upon nutrition in which amino acids, probiotics and enzymes play a major role. The purpose of adding enzymes into poultry feeds is to increase the efficiency of production of broiler's meat and inclusion of enzyme preparation resulted in positive effect in FCR, which was noted by dietary addition of enzymes in the growth phase by enhancing digestibility (Saleh *et al*., 2018). Enzyme supplementation in poultry feeds enhances bio-nutrient availability and utilization along with reducing ill-effects of toxins and anti-nutritional factors on poultry feed production (Jadhav and Siddiqui, 2007). The inclusion of amino acids, probiotics, prebiotics, enzymes, liver extracts, vitamins, minerals, protein constituents, herbal products, yeasts in broiler feeds nullify stress, increase resistance and performance of poultry birds (Jadhav, 2022). Probiotic feeding helps in advancement of resistance to infection by developing microflora in intestinal tract (Bansal *et al*., 2011).

One thousand day old broiler chicks were randomly divided into two groups of 500 each and reared for 41 days under the standard and identical managemental conditions for the trial. The control group was fed the plain commercial broiler feed whereas the treatment group was supplemented with a product containing amino acids, enzymes and probiotics (Netzyme-strong @ dose rate of 0.5 grams per kg of feed) for the entire trial period. During the trial, group-wise average weekly body weight, weekly feed consumption, weekly FCR of broilers and assessment of disease incidence was observed and recorded. The gross analysis of these parameters was done with normal mathematical average calculations group-wise and compared. The observations recorded are presented in Table 1 to 4.

From the tabulated results and analysis, it is assessed that the supplementation of Netzyme-strong numerically improved the body weight gain of broilers to the tune of 110 grams in treatment group as compared to the control. Both the groups were fed *ad-libitum* and equal quantity of the feed which is tabulated in Table no. 2 for feed consumption. The results agreed with the findings of Haben *et al*. (2021) who reported that the chickens supplemented with probiotics showed the higher body weights than control. Further, the FCR revealed numerical superiority by 0.10 indicating the saving of 100 grams of feed per kg body weight in the product supplied group. Results of feed intake in the starter phase were in the line with Abdel-Raheem and Abd-Allah (2011) who observed that the feed intake was improved by supplementation of probiotics and prebiotics. The final results in those trials revealed that the body weight, body weight gain, relative growth rate percent and FCR were significantly superior in treatment groups.

The economic evaluation (Table 4) showed the additional benefits of the supplementation of Netzyme-strong brought in enhanced net worth per kg of live weight gain to the tune of Rs. 9.60. Those results tallied with the research observations of Kamel *et. al*. (2016) who noted the important role of feed additives like probiotics, prebiotics and feed enzymes in elevating productive and economic efficiency of broiler chicks. Similarly, Koushal *et al.* (2019) also observed superior performance and economics in broiler chickens through incorporation of dietary enzymes and probiotics which corroborates with present outcome in broiler supplementation of amino acids, probiotics and enzymes (Netzyme-strong) in feed.

Table 1: Weekly Weight of broilers supplemented with Netzyme-strong

S. No.	Age (in days)	Weekly Weight (in kg)	
		Treatment	Control
1.	7	0.187	0.185
2.	14	0.380	0.375
3.	21	0.770	0.765
4.	28	1.33	1.32
5.	35	1.66	1.65
6.	41	1.96	1.85

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Table 2. Feed Consumption in broilers supplemented with Netzyme-strong

S. No.	Age (in days)	Weekly Feed Consumption (in kg)		Cumulative Feed Consumption (in kg)	
		Treatment	Control	Treatment	Control
1.	7	40.7	40.8	40.7	40.8
2.	14	125.2	125.3	165.9	166.1
3.	21	251.2	251.8	417.1	417.9
4.	28	361.7	361.8	778.8	779.7
5.	35	496.2	496.3	1275	1276
6.	41	382.3	382.7	1657.3	1658.7

Table 3. FCR in broilers supplemented with Netzyme-strong

S. No.	Age (in weeks)	Weekly FCR		Cumulative FCR	
		Treatment	Control	Treatment	Control
1.	1st	0.43	0.44	0.43	0.44
2.	2nd	1.29	1.31	0.87	0.88
3.	3rd	1.28	1.29	1.08	1.09
4.	4th	1.29	1.30	1.17	1.18
5.	5th	3.0	3.0	1.53	1.55
6.	6th	2.54	3.8	1.69	1.79

Table 4. Economics of supplementation of Netzyme-strong per bird

S.No.	Particulars	Savings/Expenses
1.	Additional weight gain per bird	110 grams
2.	Additional income from benefit of weight gain @ Rs. 86/kg	**(+)** Rs. 9.46
3.	Saving of feed (100g/kg live weight) with improved FCR	196 grams
4.	Saving of cost of feed per bird @ Rs. 50 per kg of feed	**(+)** Rs. 9.8
5.	Feeding cost of the product (1.65 g of product @ Rs. 200 per kg)	**(-)** Rs. 0.35
6.	Net benefit per kg of live weight gain with all standard and stable costs	Rs. 9.6

There was no difference in the mortality pattern of both the groups. The overall enhancement in performance of broilers can be attributed to better availability of nutrients, their improved assimilation and absorption with better conversion of feed into weight gain. Further, beneficial microbes in probiotics might have resulted into decreased gastric emptying time leading to higher feed intake and utilization, suppression of adverse effects of pathogens in gut, balancing microbe load in it leading to efficient processing of feed stepping-up weight gain (Rahman *et.al*., 2020).

Conclusion

The overall assessment of results of trial at our farm indicated promising beneficial effects on performance of broilers in respect of live body weight gain, FCR and economics of broiler rearing. However, the scientific field trials are proposed for confirmation of the accuracy of improvement in performance and economics at different locations by other institutes for better conclusion.

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Therapeutic management of canine oral papilloma (COP) using *Thuja occidentalis*

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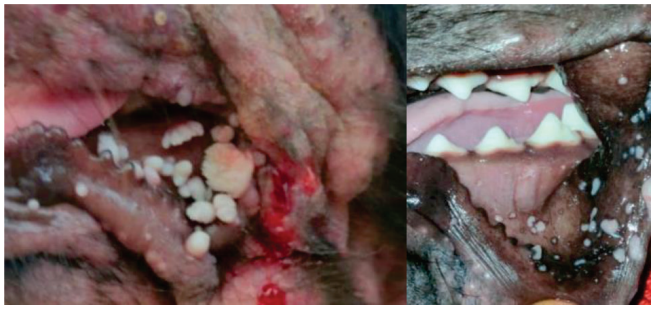
Canine oral papillomatosis (COP) in dogs is a frequently reported neoplastic, self-limiting disease caused by canine oral papilloma virus (Raj *et al*., 2020). The lesions vary in appearance from large, gray, pedunculated masses to small, white, smooth nodules. It causes physical difficulty in food intake, anorexia, drooling, halitosis, bleeding and purulent discharge from papilloma due to secondary bacterial infection. The Papilloma virus commonly affects the older dogs, puppies and immunocompromised dogs (Sundberg *et al*., 1994). The medicine for treatment of papilloma in veterinary practice is very limited and few medicines available for treatment have unpredictable affects. *Thuja occidentalis* has shown promising results against oral papilloma as per available literature, therefore, it was selected for the treatment of oral papilloma in dogs.

A total of 6 naturally infected dogs with history of multiple pedunculated or cauliflower like growth in oral cavity, salivation, bleeding and foul smell from mouth were presented in the Teaching Veterinary Clinical Complex. All the dogs were between age group of 6 months to 4 years and pedunculated or cauliflower like growth were present for more than 30 days in all cases. The body weight of dogs varied from 12 kg to 40 kg. The papillomatous growth were present in the buccal cavity to commissure of gum and around the face of dogs. Their size varied from small pedunculated mass to cauliflower like growth.



(a) (b)

Fig. 1 (a) Cauliflower like growth in COP (b) Recovered dog

The number of papilloma growth in these dogs varied from 2 to uncountable. The growth was interfering in the food and water intake by dogs. The biopsy sample was collected for histopathological examination using standard procedure.

Histopathological examination of biopsy materials revealed the presence of multiple papillary projections with fibrovascular core which is composed of hyperplastic squamous epithelium on HE staining indicative of papilloma (Fig 2).

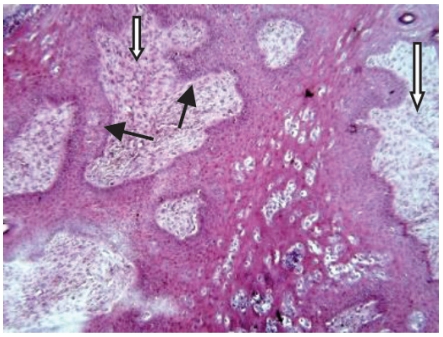


Fig. 2: Note the papillary hyperplasia of stratum corneum (black arrow) with abundant fibrovascular core (white arrow). HE x 50

The dogs were treated with *Thuja occidentalis* (M/s SBL Pvt. Ltd.) @ 30 c, 8 pellets twice daily alone irrespective of age and body weight for 15 days (Saibaba *et al*., 2016). The all-treated dogs responded well to the treatment and all of them recovered between 10-15 days of institution of treatment. None of the treated dog showed any adverse effects during the course and after the treatment.

No recurrence of papilloma was observed during the follow-up period of 12 months in all treated dogs. The *Thuja* (*T. occidentalis*) was selected for present study as it is immunomodulatory with antiviral properties which cause B and T lymphocyte proliferation and differentiation into CD4+ cells and induces production of interleukin (IL)-2, IL-1, IL-6, tumor necrosis factor-α and interferon-γ production *in vitro* and *in vivo* (Nasser *et al*., 2005). Agnihotri *et al*. (2015) reported that treating

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canine oral papilloma with *Thuja* alone for 2 months was not effective contrary to our study. Raj *et al.* (2020) reported combination of homeopathic drugs (Sulfur 30+*Thuja* 30+Graphites 30+Psorinum 30) was very effective in the treatment of COP and observed complete regression of papilloma lesions between 7 and 15 days after initiation of treatment. It is observed that Thuja alone is also have potential to completely regress the oral papilloma in dogs. In conclusion, *Thuja occidentalis* has potential to regress the canine oral papillomatosis without any side effects and is useful as alternative drug to surgical intervention and toxic drugs like vincristine for the treatment of oral papillomatosis.

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Symmetric Dimethyl Arginine: A Prognostic Marker in Early Diagnosis of Canine Chronic Kidney Disease

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Kidney diseases are most common clinical problems, occurring in dogs (Katoch *et al*., 2018) and considered as the third leading cause of death. Indiscriminate use of certain antimicrobial, non-steroidal, anti-inflammatory or analgesics and anti-neoplastic drugs have been reported to cause renal damage in dogs (Legatti *et al.*, 2018). The emergence of several infectious (*Ehrlichia canis, Babesia gibsoni, Leptospira*,etc.) and metabolic diseases (diabetes) have further aggravated the incidence rate of renal disorders among dogs in India. Early diagnosis of kidney diseases is essential to stabilise renal function and prevent the rapid progression of the disease. In general, the markers of renal disease recognized from haematological and serum biochemical evaluations, urinalysis, or imaging or pathology studies (Polzin, 2011) have low sensitivity and specificity in the early stages which limit their use in screening of renal diseases. Biomarkers that are capable of early detection, risk stratification and prognostication such as SDMA would represent a tremendous focus (Hall *et al*., 2016). Symmetric dimethyl arginine (SDMA) is primarily removed from the body by the kidneys, and unlike creatinine it is not influenced by muscle mass, age and breed (Yerramilli *et al.,* 2016). The key to successful management of patients with renal disease lies in the early detection of varied etiology and initiate aggressive therapy. Timely intervention and systematic approach will slow down the progress of disease process in kidneys. Therefore, the present study was conducted for early detection of chronic kidney disease in dogs with SDMA biomarker and to see its association with other serum biochemical parameters.

In the present study, a total of 9,347 dogs of different breeds, age groups of either sex were screened for kidney diseases. A total of 103 dogs were selected based on history, clinical signs and diagnosis of CKD confirmation by estimation of SDMA. The study also included apparently healthy dogs (n=10) as control. Apart from imaging studies, quantitative estimation of canine symmetric dimethyl arginine (SDMA) was done using sandwich ELISA method as described by manufacturer using the kits supplied by M/s. Bioassay Technology Laboratory, Shanghai and expressed in µg/dL. The other serum biochemical parameters estimated using assay kits and standard procedures were BUN, creatinine, total protein, albumin, globulin, ALT and ALP. The dogs with CKD were categorized into 4 stages based on SDMA, viz., stage I, II, III and IV according to IRIS guidelines (modified 2019). The data was subjected to statistical analysis by using SPSS (SPSS 20.0, Chicago, IL, USA). Tukey's multiple comparison post hoc test was also used to find the differences between groups. All the data was presented as Mean±SE and $P < 0.05$ was considered significant (Snedecor and Cochran, 1994).

In the present study, the means ± SEs of SDMA in dogs with stage I, II, III and IV CKD were 16.59±0.53 µg/dL, 25.07±1.30 µg/dL, 46.89±1.09 µg/dL and 87.51±3.11 µg/dL, respectively and these were compared with other biochemical parameters in healthy dogs and dogs with CKD (Table 1).

Majority of the dogs (44.67 %) in the current study were found to be in stage IV and their values range from 54-146 mg/dL with Mean± SE as 87.51±3.11mg/dL. These findings were in agreement with those of Sharma *et al*. (2015), who graded affected dogs into different stages and recorded that majority of the dogs were in stage III and IV. It is evident from this study that the mean serum creatinine level in stage I of CKD dogs was 0.84 ± 0.07 mg/dl, in stage II 1.83±0.09, in stage III 2.85±0.15 and in stage IV 8.93 ± 0.51mg/dL. Though serum SDMA was above 14 µg/ dL, serum creatinine was below 1.4 mg/dL in 13 dogs of CKD stage I and 3 dogs of stage II (Table 1), which was in agreement with Yerramilli *et al.* (2016) who reported creatinine as insensitive for the detection of early renal disorders and also opined that SDMA was more sensitive biomarker for early detection of renal dysfunction even in non-azotemic dogs that are not influenced by muscle mass, age and breed (Hall *et al.*, 2016). SDMA, a new biomarker for the early detection of

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Table 1. Serum biochemical parameter in healthy and dogs with different stages of CKD (Mean± SE)

Sr. No	Parameter	Healthy (n=10)	CKD-I (n=17)	CKD-II (n=15)	CKD-III (n=25)	CKD-IV (n=46)
1.	SDMA (µg/dL)	9.12± 0.89^{a}	16.59±0.53^{b}	25.07±1.30^{c}	46.89±1.09^{d}	87.51±3.11^{e}
2.	Creatinine (mg/dL)	0.69±0.10^{a}	0.84±0.07^{a}	1.83±0.09^{b}	2.85±0.15^{c}	8.93±0.51^{e}
2.	Blood Urea Nitrogen (mg/dL)	16.36±1.07^{a}	14.9±0.39^{a}	37.76±3.75^{ab}	55.87±6.01^{b}	127.25±16.4^{d}
3.	Total Protein(g/dL)	7.83±0.66^{d}	6.49±0.29^{d}	6.93±0.33^{cd}	5.60±0.28^{a}	5.54±0.23^{a}
4.	Albumin (g/dL)	3.25±0.07^{c}	2.86±0.04^{b}	2.85±0.30^{b}	2.54±0.24^{a}	3.11±0.12^{c}
5.	Globulin(g/dl)	3.71±0.68^{d}	3.73±0.31^{d}	4.08±0.28^{d}	3.06±0.19^{b}	2.43±0.21^{a}
6.	A/G ratio	0.87±0.23^{a}	1.32±0.07^{a}	0.79±0.13^{a}	0.97±0.12^{a}	1.27±0.87^{b}
7.	Alanine amino-transferase (U/L)	17.81±3.92^{a}	19.24±0.28^{a}	22.63±3.69^{a}	30.18±3.35^{b}	27.98±1.49^{b}
8.	Alkaline phosphatise (U/L)	48.37±5.77^{a}	51.88±0.77^{a}	61.28±8.38^{ab}	87.22±7.91^{b}	145.52±20.3^{c}

Means bearing different superscripts within a row differed significantly (P<0.05)

kidney dysfunction, is an endogenous methylated form of the arginine that is released into circulation during normal protein catabolism. Dahlem *et al.* (2017) stated that SDMA is excreted by the kidneys into urine in an unchanged form and hence, this metabolite accumulates in the course of renal dysfunction, which increases earlier than serum creatinine in animals with progressing kidney dysfunction. Pelander *et al.* (2015) also stated that SDMA estimation in addition to creatinine might improve the diagnostic value and avoid false positives as with estimation of serum creatinine alone which was in agreement with present study.

Significant elevation of BUN and creatinine values in dogs with CKD were recorded in this study similar to earlier observations (Sonu *et al*., 2019). Elevated levels of creatinine and blood urea nitrogen could be due to diminished renal excretion and enhanced tubular absorption of urea which would be elevated only when there was loss of up to 75 % of functional renal mass, whereas SDMA would be elevated even with loss of 10 % functional nephrons (Dahlem *et al.*, 2017).

Significantly lower (P<0.05) mean values of serum total protein (g/dL) were recorded in dogs with chronic kidney disease stage III (5.60±0.28) and stage IV (5.54±0.23) as compared to Stage II, I and healthy control group. Similar was the trend for globulin and inverse trend for A/G ratio in dogs with CKD (Table 1). These findings were in accordance with the reports of Kandula and Karlapudi (2015), who opined that hypoproteinemia might be due to gastrointestinal bleeding and proteinuria.

Significant elevation of alanine amino transferase and alkaline phosphatase in CKD stage III and IV was recorded in the present study. Elevated alkaline phosphatase in CKD might be due to secondary renal hyperparathyroidism and was associated with increased mortality (Beddhu *et al.*, 2009).

Although novel markers like SDMA provide exciting clues into the pathophysiology of diseases and enable us to improve diagnostic capabilities, the high cost involved is still prohibitive for widespread clinical application.

Conclusions

Our study demonstrated that SDMA is a sensitive biomarker in early detection of kidney diseases, which further helps in slowing down the progression of disease.

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Clinical Articles

Therapeutic Management of Canine Babesiosis along with Identification of Vector: Case Study

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Abstract

Present study was conducted in two Labrador male dogs and one non-descript bitch with history of off feed, fever, vomiting and diarrhea, dullness, ocular discharge, bleeding from nostrils in bitch and presence of ticks on body surface. Based on history and clinical findings, disease was diagnosed tentatively as babesiosis which was further confirmed by blood smears examination. Blood smears examination showed presence of small dot shape *B. gibsoni* protozoal parasite in large number of RBCs. Babesiosis affected dogs were treated with imidocarb dipropionate, ivermectin and oxytetracycline along with other supportive therapy. Treated dogs showed marked improvement after 2 days of treatment and complete recovery was on day 4th of treatment in non-descript indigenous dog and on day 6th of treatment in Labrador dogs. For identification of vector, ticks were collected in screw cap bottles contain 80 parts ethanol, 05 parts glycerin and 15 ml distilled water and identified on the basis of the morphological characters of the gross specimens and permanent slides of the specimens by mounting process. The isolated ticks were identified as *Rhipicephalus, Hyalloma* and *Haemaphysalis* spp.

Key Words: Canine babesiosis, *B. gibsoni,* Imidocarb, Ticks

Babesiosis is caused by intraerythrocytic protozoal parasite of genus *Bebesia* which are a diverse group of tickborne, obligate, intra-erythrocytic Apicomplexan parasites (Susan and Michael, 2016). Although the major economic impact of babesiosis is on the cattle industry, infections in other domestic animals, including horse, sheep, goat, pigs and dogs assume varying degrees of importance throughout the world. In dogs, babesiosis is caused by *B. canis, B. vogeli, B. rossi and B. gibsoni*. Disease is usually transmitted by tick vectors including *Rhipicephalus (Boophilus), Hyalloma* and *Haemaphysalis* spp. of ticks. In ticks both transovarial or trans-stadial transmission of babesia spp. has been suggested. A carrier state of babesiosis in recovered dogs has also been suggested. Besides it, this disease can also transmitted by parenteral injection of infected blood (Chakrabarti, 2011). Intrauterine infection has also been reported but is rare.

Infection of a vertebrate host is initiated by inoculation of sporozoite stage parasites into the blood stream during the taking of a blood meal by infected ticks (Radostitis *et al*., 2007). After inoculation most babesial sporozoites directly invade circulating erythrocytes without a tissue stage of development. Clinically, canine babesiosis may be manifested in two different forms; the uncomplicated and the complicated forms (Welzl *et al*., 2001; Lobetti, 2005). Uncomplicated babesiosis refers to the clinical manifestation which is mainly attributable to haemolytic anaemia (Jacobson and Clark, 1994) while in the complicated form, the observable clinical signs are those which cannot be directly linked to haemolysis but appear to result from the host's inflammatory response (Jacobson and Clark, 1994). Multiple organ dysfunctions in babesiosis are believed to be the end result of tissue inflammation that is initiated by several factors which include hypotension, septic shock and infectious organisms (Lobetti, 2005).

Case History and Observations

Two Labrador male dogs and one non-descript bitch which were brought in Veterinary Clinical Complex of Apollo College of Veterinary Medicine, Jaipur, Rajasthan with history of off feed, fever, vomiting and diarrhea, dullness, ocular discharge, bleeding from nostrils in bitch and presence of ticks on body surface. The clinical findings observed in these dogs were anorexia, intermittent fever, vomiting, diarrhea, tachycardia (average 113/min), dyspnea and occasional coughing, muscular weakness, emaciation (Fig. 1), epistaxis in bitch, keratitis, paler or whitish conjunctiva (Fig. 2) and presence of ticks

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on body surface (Fig 3). Based on history and clinical findings disease was diagnosed tentatively as babesiosis. Confirmatory diagnosis was based upon blood smears examination.

Blood samples from affected dogs were collected from cephalic vein in EDTA containing and without anticoagulant vacutainers and were immediately transported to laboratory in ice box. The EDTA containing blood samples were used for estimation of haematological parameters on the same day and without anticoagulant blood samples were used for separation of serum by standard processes for biochemical analysis. Blood smears were prepared and stained with Giemsa's stain for confirmation of babesiosis by microscopic examination. Blood smears were examined under on 100x under oil immersion.

For identification of vector, axillary, inguinal, neck and brisket region of dogs were examined carefully for presence of ticks and were randomly collected in screw cap bottles contain 80 parts ethanol, 05 parts glycerin and 15 ml distilled water. Precaution was adopted during collection of ticks since their mouth parts were firmly embedded in the skin. For this purpose, the capitulum of ticks was gripped firmly and lightly with the help of forceps and then turn the tick over on to its back and taken out sharply, perpendicularly away from the body. Isolated ticks were identified on the basis of the morphological characters of the gross specimens and permanent slides of the specimens by mounting process as per the keys described by Walker *et al.* (2003).

For mounting, the ticks were kept in 10 per cent KOH and subsequently heated intermittently to boil for two minutes for liquefying the internal tissues of ticks. For engorged female ticks, the posterior margin of the

Fig 1: Emaciated condition of bitch

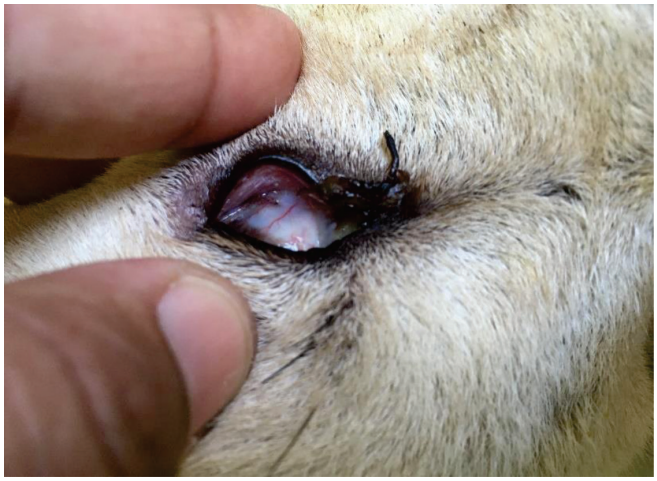

Fig. 2: Whitish conjunctiva of bitch

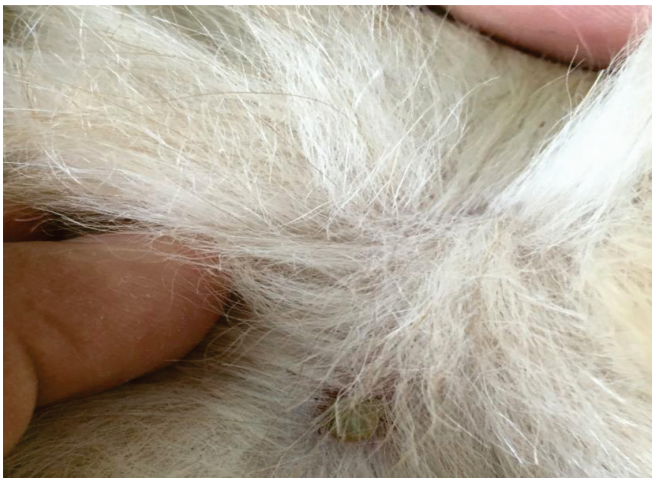

Fig. 3: Presence of ticks on body surface of dog

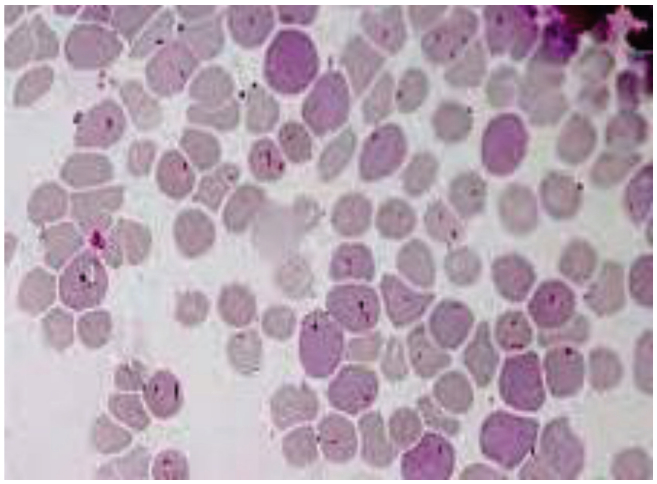

Fig. 4: *B. gibsoni* protozoal parasite in large number of RBC

Table 1: Average haemato-biochemical parameters in babesiosis affected dogs

Parameters	Results	Normal Values
Hb	7.7 gm/dl	12-18
PCV	25.4 %	37-55
TEC	3.4 x 10^6/µl	5.5-8.5
TLC	24.6 x 10^3/µl	5-17
Neutrophils	76 %	60-70
Eosinophils	11 %	2-10
Platelets	1.69 x 10^5/µl	2-9
Albumin	1.60 gm/dl	2.6-4.0
ALT	144.90 U/L	8-57
AST	62 U/L	9-49
Creatinine	1.92 mg/dl	0.5-1.6
BUN	29 mg/dl	8.8-25.9

body was punctured at 3-4 places to ensure effective penetration of KOH. Subsequently, the specimens were removed from KOH and internal liquefied tissues were removed from the body by pressing dorsal surface slowly with the help of a pin head. Dehydration of specimens was done by keeping for at least 10 minutes twice in each 30 per cent, 50 per cent, 70 per cent, 90 per cent and absolute alcohol and then were cleared in cedar wood oil at least for 24 hours and placed in xylene for 1 minute before mounting. The ticks were mounted in Canada balsam mountain on a glass slide.

Treatment and Discussion

These dogs were treated with inj. ringer lactate @ 20 ml/kg b.wt., i.v., inj. normal saline @ 20ml/kg b.wt., i.v., inj. Imidocarb dipropionate @ 6.6 mg/kg b.wt., s/c., inj. oxytetracycline @ 10 mg/kg b.wt., i.v. slow, inj. vetalgin @ 1ml/kg b.wt., i.m., s.o.s., inj. ondansetron @ 0.2 mg/kg b.wt., i.v. x 3 days, inj.ivermectin @ 0.2 mg/kg b.wt., s/c once, inj. ferritas @ 1ml/kg b.wt., i.m. 3 times on alternate day, inj. Tribivet (vit. B-complex) @ 1ml/20 kg. b.wt., i.v. x 3 days.

Haemato-biochemical examination revealed decreased haemoglobin, packed cell volume, total erythrocytes count, platelets and albumin whereas total leucocytes count, neutrophils count, eosinophils count, serum ALT, AST, creatinine and BUN were found increased than the normal reference values (Table 1).

Decreased level in Hb and TEC are in agreement with the reports of Selvaraj *et al.* (2010); Wadhwa *et al*. (2011); Andoni *et al*. (2013) and Nalubamba *et al.* (2015). The destruction of circulating RBC by auto antibodies is directed against infected and non-infected red cell membranes resulting into intravascular and extravascular haemolysis (Day, 1999; Pederson, 1999). Anaemia may be due to the direct damage of RBC caused by parasites as documented by Taboada and Lobetti (2006) or due to macrophages causing erythrophagocytosis and the damage due to the formation of anti-erythrocytic membrane antibodies induced by secondary immune system (Salem and Farag, 2014). Leucocytosis and neutrophilia in canine babesiosis may be due to the marked systemic inflammatory response. The mechanisms of the thrombocytopenia are not yet fully understood in babesiosis; multiple mechanisms, including platelet sequestration in the spleen, immune-mediated platelet destruction and development of disseminated intravascular coagulation are possible. In spite of thrombocytopenia no single dog in this study showed any haemorrhages on the surface of the body. Increased serum activity of Alanine transaminase (ALT), Aspartate transaminase (AST), creatinine and blood urea nitrogen (BUN) and decreased albumin are resulted from hepatopathy and renal failure. Centrilobular hepatitis with hypoxic liver damage could be the possible mechanism that resulted in significant changes in hepatic enzymes and decreased albumin levels (Taboada and Lobetti, 2006). Renal impairment noticed due to damage of renal cells caused by inflammatory mediators or possibly due to the development of refractory hypotension resulting in reduced renal tissue perfusion and glomerular filtration rate (Zygner and Wedrychowicz, 2009).

Confirmatory diagnosis of babesiosis was based upon blood smears examination which showed presence of small dot shape *B. gibsoni* protozoal parasite in large number of RBCs (Fig. 4). Treatment was started after confirmatory diagnosis of babesiosis and all three dogs showed marked improvement after 2 days of treatment. Complete recovery was on day 4th of treatment in non-descript indigenous dog and on day 6th of treatment in Labrador dogs.

Isolated ticks from these dogs were identified as *Rhipicephalus (Boophilus)* spp.*, Hyalloma* spp., and *Haemaphysalis* spp. on the basis of the key described by Walker *et al.* (2003). The *Rhipicephalus (Boophilus)* spp. species are inornate. Anal groove is absent in female and faint in male. Eyes are present but festoons are absent. The *Hyalloma* spp. species are inornate, rarely ornate. Eyes are present and round. Spiracles are comma shaped in males and triangular in female. The *Haemaphysalis* spp. species are small ticks with short mouthparts. The basis capitulum is rectangular and the base of the second palpal segment is expanded, projecting laterally beyond the basis capitulum. Eyes are absent.

Conclusion

Blood smear examination is simple, rapid and conventional method for confirmatory diagnosis of babesiosis in animals. Haemato-biochemical alteration are much important tools for indication of prognosis in babesiosis affected animals and correlation with blood smears examination would be important for early diagnosis of disease. Imidocarb dipropionate is effective for treatment of canine babesiosis. In early diagnosis and treatment prognosis is good but severely affected or untreated animals may die. *Rhipicephalus (Boophilus)* spp.*, Hyalloma* spp., and *Haemaphysalis* spp. were identified as principal vector in canine babesiosis.

Conflict of Interest - None

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Sproadic incidence of *B. melitensis* associated abortion storm in a sheep flock

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Abstract

Small ruminants are considered as moving banks of poor farmers and landless labourers in India. Diseases affecting small ruminants are the major factor limiting the productivity and profits of small ruminant farming system in India. Incidence of reproductive diseases associated with *Brucella melitensis* is rarely reported in the country. The present report is on the incidence of abortion storm in a sheep flock in Namakkal region of Tamil Nadu. Liver and spleen from one aborted foetus and blood samples (31 samples) were collected from sheep in an unorganised sheep farm with the history of abortion in ten ewes within a week time. Seropositivity of 77.42 % to brucellosis was detected by RBPT (Rose bengal plate test) and *B. melitensis* infection was confirmed by AMOS – PCR in the aborted foetal and liver samples. High seropositivity and occurrence of abortion storm in the rural sheep farm in India indicated the underlying unnotified circulation of *B. melitensis* infection in sheep. Periodical serosurveillance, test and slaughter policy, regular immunization of small ruminants with *B. melitensis rev* 1 vaccine are the possible primary and secondary preventive strategies to limit the occurrence and spread of brucellosis in small ruminants in India.

Key words: Brucellosis, Sheep, AMOS-PCR, RBPT

Small ruminants are considered as moving banks and socio economically important livestock species in the country like India which holds 10 per cent of small ruminant population of the world with 135.17 million goat and 65.07 million sheep and leading producer of goat milk and second in chevon and goat skin production in the world (Dixit *et al*., 2017). Small ruminants are the main source of income to the farmers holding less than two hectares and land less labourers who are rearing more than 70 per cent of sheep and 76 per cent of goats in the country (Shome *et al*., 2015).

Almost 99 per cent of small ruminants in the country are depending on grazing lands as their sole source of nutrition with poor management system including lack of health coverage like vaccination and deworming. Lack of health coverage is acting as predisposing factor for the incidence of infectious and contagious diseases affecting sheep and goats.

Brucellosis is a chronic bacterial disease of ruminants caused by a small aerobic, non - motile, gram - negative coccobacilli of the genus Brucella (Rajendran, 2021). Infertility and abortion are the characteristic clinical features of brucellosis in animals and is reported to be a serious zoonotic disease in the world (Memish and Balkhy, 2004). It is endemic in Latin America, Asia, some Mediterranean regions and Africa including India (OIE, 2022).

Brucella species predominantly affects small ruminants is *Brucella melitensis,* which causes characteristic clinical entity like abortion, stillbirths, birth of weak offspring, infertility etc., and also a leading cause of zoonotic brucellosis. *Brucella ovis* infection causes epididymitis and orchitis in rams (Rossetti *et al*., 2022). Economic losses due to *B. melitensis* infection in sheep and goats in India is estimated as INR. 2122 and 1818 respectively (Sulima and Venkataraman, 2010). Even though economic losses due to brucellosis is high, it is a neglected disease in India and paucity of information on the occurrence of brucellosis particularly in small ruminants in the country (Shome *et al.,* 2021).

Sporadic occurrences of brucellosis often unnoticed both by the farmers and veterinarians due to lack of awareness, inaccessibility of field based confirmatory diagnostic tests and high economic costs associated with absence of effective treatment/control techniques. Although *B. abortus* infect cattle and *B.melitensis* infect sheep and goats but this can occur vice versa also. Therefore, brucella species infecting animals has to be identified in order to advocate sound control measures in the maintenance host. Blood and tissues serve as a good source of nucleic acid for the diagnosis of Brucella infection. Serum based detection

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and AMOS PCR based detection are considered as important diagnosis of brucella species in ruminants hence this study was designed.

Clinical History and Observations

Aborted foetus (one) and clotted blood samples of sheep (31 samples) were collected from an unorganised sheep farm, Namakkal, Tamil Nadu, India with the history of abortion of ten pregnant sheep (3-4 months of gestation) within one week time, absence of health cover (deworming, vaccination etc.), poor nutritional management (Grazing on barren lands and absence of concentrate feeding) with a flock size of forty eight animals.

Serum samples were subjected to RBPT using *B. abortus* coloured antigen procured from IVRI, Izat Nagar as per the standard procedure (OIE, 2022). Liver and spleen samples were collected from aborted foetuses and subjected to DNA extraction by using DNeasy blood and tissue kit (Qiagen, India). Bru - AMOS PCR for *B.abortus, B.melitensis*, *B.ovis* and *B.suis* species identification was carried out as per Bricker and Halling (1994). The PCR reaction (25 µl) mixture consists of 12.5 µl of PCR master mix (Qiagen India), cocktail of the five primer sets including *B. aboruts, B. melitensis, B. ovis*, *B. suis and IS 711* (20 pmol/ 1µl each) (Table -1.) and 2.5 µl of template DNA. Amplified product of AMOS-PCR reaction was electrophoresed in 1.5 % agarose gel and documented.

Discussion

Species of the bacterial genus Brucella, principally targeting the reproductive system of domestic animals causes abortion, infertility, retained foetal membranes and is a serious zoonotic disease reported worldwide (Natesan *et al*., 2021). It is an economically important disease of small ruminants and the disease in goats is usually caused by *B. melitensis* and less frequently by *B. abortus* and in sheep, rough strain of Brucella, *B. ovis* is less frequently involved in addition to *B. melitensis* and *B. abortus* (Corbel *et al*.,2004).

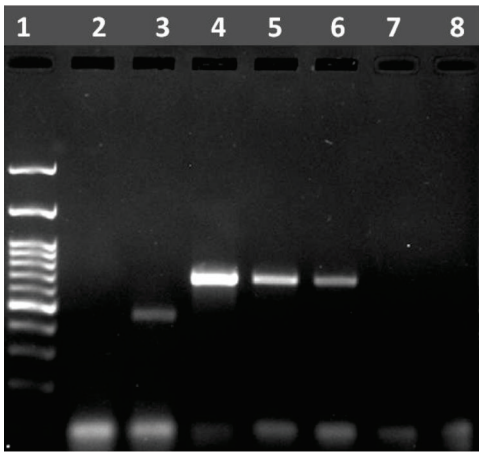
B. melitensis infection in sheep and goats has been neglected for long time, as the small ruminant production was considered as generally low-income activity practiced by landless farmers and marginalized communities in the developing countries (Kirshnakumar *et al*., 2018). Reasons for neglection also include absence of typical pathognomonic clinical signs, lack of field based confirmatory tests for diagnosis, inadequate laboratory facilities for handling and confirmation of potential public health significance of *B. melitensis* infection.

In the present study, abortion in ten pregnant ewes (3-4 months of gestation) was reported within one to two weeks time in the sheep flock consisting of 48 animals including 31 ewes and lambs under 5-6 months in Namakkal region of Tamil Nadu, India. Abortion in ewes is the most commonly reported clinical sign of *B. melitensis* infection which was also reported by various authors including Shome *et al*. (2015).

As per OIE (2022) buffered *Brucella* antigen tests are suitable for screening of herds/flocks and individual small ruminants and could be used as a screening test in unvaccinated population for detection of prevalent status. This study reported 77.42 %, 24 out of thirty one ewes, were seropositive for brucellosis by rose Bengal plate agglutination test (RBPT). High seropositivity of 19.25 % in unvaccinated sheep flock was also reported by Sundar *et al*. (2020) in Tirunelveli region of Tamil Nadu and it indicates silent unnotified infection of ewes with brucella species. The circulation of Brucella species infection in small ruminants of the country also proved by detection of 11.55 % seropositivity in sheep in twenty seven states of India during the year 2017-18 by Shome *et al.* (2021). Significant presence of Brucella antibodies in the unimmunized sheep population of the country in the majority states of India by Shome *et al.* (2021) proves the presence of silent/underreported Brucella infection.

Table.1. Primer sequence for AMOS-PCR

***Brucella* species**	Primer Sequence	Target	Size (bp)
B. abortus	GAC GAA CGG AAT TTT TCC AAT CCC	*IS 711*	498
B. melitensis	AAA TCG CGT CCT TGC TGG TCT GA		731
B. ovis	CGG GTT CTG GCA CCA TCG TCG GG		976
B. suis	GCG CGG TTT TCT GAA GGT GGT TCA		285
IS 711	TGC CGA TCA CTT AAG GGC CTT CAT		



Lane-1 - 100 bp DNA ladder
Lane-2 - Negative control
Lane-3 - B. abortus positive control
Lane 4 - B. melitensis positive control
Lane 5 - Foetal liver
Lane 6 - Foetal spleen

Plate.1. *Brucella melitensis* detection by AMOS-PCR

The AMOS PCR for detection of *B. abortus, B. melitensis, B. ovis and B. suis* was first published in 1994 (Bricker and Halling (1994). This method could detect biovar 1, 2 and 4 of *B. abortus*, biovars 1,2 and 3 of *B. melitensis* and biovar 1 of *B. suis* and *B. ovis* and could not differentiate individual biovars within a species. In this study foetal liver and spleen were used for the extraction of DNA to detect brucellosis based on AMOS PCR. Several studies have also documented the presence of brucella pathogen in blood (Saravanan *et al*.,2021) and foetal abomasal contents (Parthiban *et al*., 2021). All the samples produced specific amplicon of 731 bp by AMOS PCR indicating the involvement of *B. melitensis* infection only. The AMOS PCR was utilised as a single step identification technique by various authors for the past two decades including Ntivuguruzwa *et al*. (2022).

Sporadic occurrence of ovine brucellosis associated abortion storm in a sheep flock of rural Tamil Nadu warrants a routing screening and surveillance of this bacterial infection which is a limiting factor for successful small ruminant production system.

The present report of abortion storm due to *B. melitensis* shows the tip of an iceberg of the underlying prevalence of small ruminant brucellosis. Presence of highly zoonotic *B. melitensis* infection in sheep is of high public risk for sheep farmers, animal handlers, slaughter house workers and people who consume unpasteurized milk and milk products of affected animals.

Hence, periodical screening of small ruminants for brucellosis using OIE recommended screening tests like RBPT/BPAT, test and slaughter policy, improving the flock immunity by vaccination with *B. melitensis* Rev-1 vaccine, strengthening of disease reporting and development of on-site diagnostic systems are the possible primary prevention and secondary prevention strategies for ovine and caprine brucellosis in the country.

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An outbreak investigation of Chlamydial abortion and infectious keratoconjunctivitis in goats of Punjab

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Abstract

Chlamydiosis is a highly contagious abortive disease of sheep and goat caused by *Chlamydia abortus,* and characterized by necrotic placentitis, metritis, retention of placenta and abortion. Chlamydia spp. is also a major cause of keratoconjunctivitis in small ruminants. This study investigated an outbreak of abortion and keratoconjunctivitis associated with chlamydial infection in a goat flock in Punjab. Nine goats (7 does and 2 kids) were clinically affected among which 5 does had aborted the fetus in their last month of gestation. All the affected goats had corneal opacity and bilateral keratoconjunctivitis. Evaluation of haematological parameters revealed reduced haemoglobin (7.44±0.13 g/dL) and increased TLC (16518±1050 x10^3/µl) and relative neutrophil count (55.33±3.59 %), suggestive of bacterial inflammation. Cytological examination of conjunctival smear revealed intracytoplasmic basophilic elementary bodies indicating chlamydial infection. Furthermore, numerous degenerated neutrophils along with few lymphocytes indicated ongoing inflammation of conjunctiva. The affected does and pregnant does in the farm were administered long acting oxytetracycline @ 20 mg/kg, intramuscularly, for two times at fifteen days interval. The kids were treated with 150 mg of oral chlortetracycline powder. Remission of clinical signs were observed within 15 days of treatment, without any subsequent abortion in pregnant does. In conclusion, cytological examination of conjunctival smear confirms the diagnosis of chlamydial infection, and oxytetracycline stood very effective against chlamydiosis in goats.

Keywords: Chlamydiosis, Keratoconjunctivitis, Abortion, Elementary body, Oxytetracycline

Chlamydia is an obligatory intracellular gram-negative bacterium that causes several disease conditions in large and small ruminants (Tejedor-Junco *et al.,* 2019). Diseases caused by this agent include abortion, pneumonia, gastroenteritis, encephalomyelitis, keratoconjunctivitis, and polyarthritis etc. (Giannitti *et al.,* 2016). Among various chlamydial species, *C. abortus* and *C. pecorum* are two important species affecting small ruminants. Chlamydial abortion caused by *C. abortus* is one of the most important abortive diseases in small ruminants causing severe economic losses. In an affected flock, nearly one third of pregnant ewes and more than 60% of pregnant does may abort in the late gestation or give birth to dead or weak newborns (Rodolakis *et al.,* 2015). Genital secretions prior to abortion or during parturition and the products of abortion including infected placentae, fetuses, and the coats of neonates are the main sources of infection (Longbottom and Coulter, 2003). It is one of the zoonotic chlamydial species causing abortion due to its ability to colonize the human placenta in pregnant women, who acquire the pathogen from exposure to infected tissues or fluid from affected animals during pregnancy (Cheong *et al.,* 2019). Infectious keratoconjunctivitis is a multifactorial, contagious disease in sheep and goat caused by *Mycoplasma conjunctivae., Chlamydia abortus* and *Chlamydia pecorum.* Although *C. abortus* has higher affinity for placental tissue, it also affects the ocular tissues leading to development of conjunctivitis in which epiphora, conjunctival hyperemia, corneal opacity etc. are the predominant clinical signs observed. This disease has been reported to cause significant economic losses to livestock farmers due to treatment costs, decrease in production and death of animals. Transmission of infection occurs by direct contact and also mechanically by flying insects (Gelormini *et al.,* 2017).

When an outbreak of chlamydiosis occurs in a flock, it is essential to achieve a proper diagnosis, in order to adopt the suitable control measures for preventing spread of infection. A presumptive diagnosis of chlamydial infection can be made on the basis of history, clinical signs, gross pathological abnormalities and examination of smear from conjunctiva in keratoconjunctivitis cases and from infected placental tissue. This study describes an outbreak of chlamydial abortion and keratoconjunctivitis in a goat flock, and its successful management by antimicrobial therapy.

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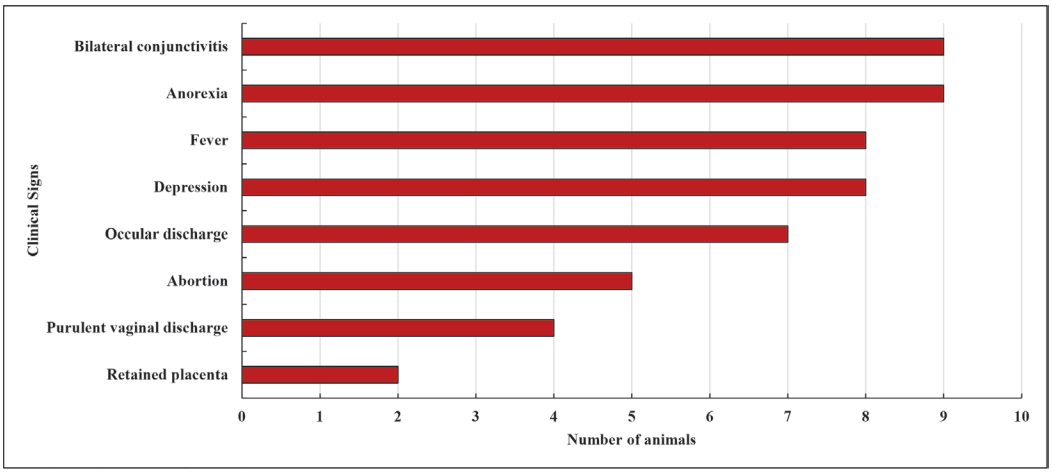

Fig. 1. Clinical signs exhibited by goats affected with chlamydiosis

Clinical History and Observations

Animal selection and clinical observation

The study was carried out in a goat farm in Ludhiana district of Punjab and the laboratory work was done in Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. This study included total of thirteen goats (9 clinically affected and 4 pregnant does without any clinical signs) in the flock. The affected animals (n=9, 7 does and 2 kids) were suspected for chlamydiosis and screened based on the history, clinical signs and cytological examination of conjunctival smear.

Sample collection and processing

Two milliliters of whole blood were collected in a vacutainer containing ethylene diamine tetra acetate (EDTA) from the affected animals (n=9). After properly mixing, the blood sample was sent to Teaching Veterinary Clinical Complex, GADVASU for hematological examination.

The conjunctival swab was taken from all the affected animals using a sterile cotton swab and smear was prepared by rolling the swab on a clean glass slide. The slide was air dried and stained with Giemsa stain and seen under high power microscope.

Treatment and Follow-Up

The affected does (n=7) and unaffected pregnant does (n=4) were treated with long acting oxytetracycline @ 20 mg/kg, IM for two doses at 2 weeks interval. The kids were given 150 mg of oral chlortetracycline powder for fifteen days. The animals were examined for remission of clinical signs on 4th and 15th day post-treatment.

Treatment Outcome and Discussion

Various clinical signs exhibited by the affected animals were mentioned in Figure 1. Among the seven affected does, 5 had aborted the fetus in their last month of gestation, 4 were having purulent vaginal discharge indicating metritis, and two does had the history of retained placenta. *Chlamydia abortus* most commonly infect the trophoblast layer of placentomes from which the bacteria enter the inter-cotyledonary junction causing

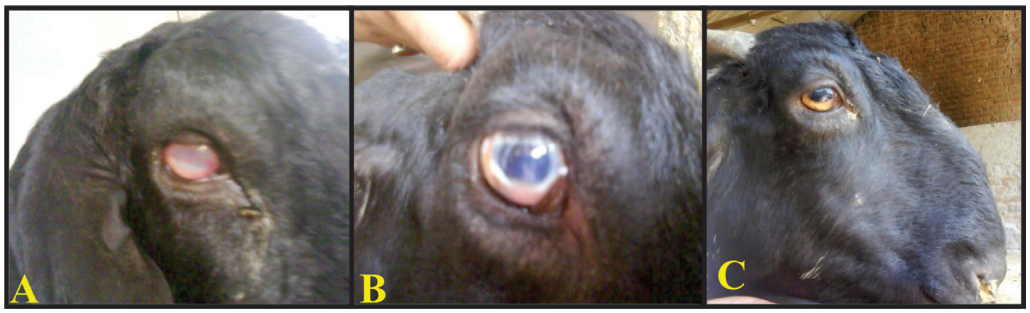

Fig. 2. (A) Corneal opacity and keratoconjunctivitis in a goat. (B) Reduction in extent of corneal opacity after 4th day of treatment. (C) Complete remission of eye lesions after 15th day of treatment.

Table 1. Haematological parameters of affected goats before and after treatment.

Parameters	Before treatment (n=9)	15th day post-treatment (n=9)	Reference range
Hemoglobin (g/dL)	7.44±0.13	9.78±0.40	8-12
Total leukocyte count (TLC) (x10^3/µl)	16518±1050	8904±340	4000-13000
Neutrophil (%)	55.33±3.59	38.67±1.73	30-48
Lymphocyte (%)	43.56±3.28	60.67±1.70	50-70
Eosinophil (%)	0.88±0.48	0.67±0.33	1-8

necrotic placentitis, leading to inhibition of nutrition and oxygen exchange between fetal and maternal blood, resulting in fetal death (Buxton *et al.,* 2002; Longbottom *et al.,* 2013). This might be the reason for abortion in affected does in this study. Similar pathogenesis is also involved with *Chlamydia pecorum infection,* which causes severe necrosuppurative chorionitis with vasculitis, and fetal pyelonephritis causing abortion in small ruminats (Westermann *et al.,* 2021). *Chlamydia* spp., especially *C. pecorum and C. abortus* (Gupta *et al.,* 2015), were also involved in the development of keratoconjunctivitis in goats (Nietfeld, 2001). Initially, there are hyperemia of the conjunctiva and sclera followed by serous lacrimation, and blepharospasm (Radostits *et al.,* 2007). All the affected does and kids in this study were having corneal opacity and kerato-conjunctivitis (Figure 2). Anorexia, fever, and depression were other common clinical signs observed in affected does and kids.

The hemoglobin concentration of the affected animals (7.44±0.13 gm/dl) were slightly lower than the normal range indicating mild anemia. Total leucocyte count (TLC) (16518±1050 x10^3/µl) and relative neutrophil count (55.33±3.59 %) of the affected animals were higher than the normal range (table 1). Thus, hematologic parameters interpreted neutrophilic leukocytosis along with mild anemia.

Exfoliative cytology is an important diagnostic tool in infective and degenerative ocular diseases especially keratoconjunctivitis (Cakir *et al.,* 2014). The microscopic examination of the conjunctival cytosmear in our study revealed numerous degenerated neutrophils and lymphocytes, along with few hyperplastic cells (Figure 3) characteristics of ongoing inflammatory process. Predominance of degenerative neutrophils in a conjunctival cytosmear indicates bacteria as the most common underlying causes of ocular disease (Atkins, 2002; Gilger, 2006). Thus, conjunctival smear in this study indicated a bacterial inflammatory process. Binucleated and multinucleated epithelial cells were also reported

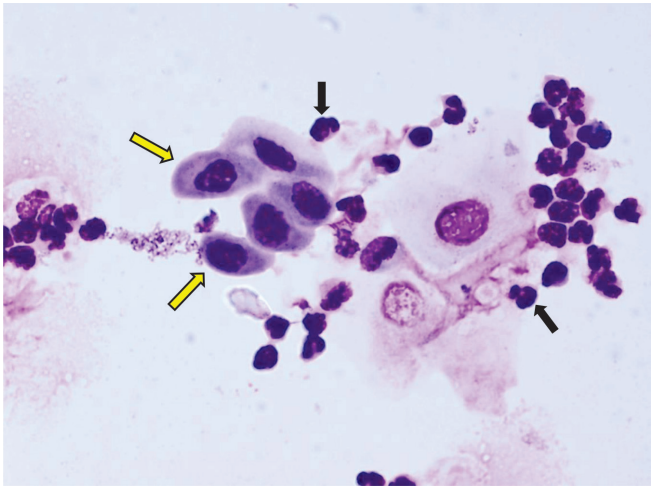

Fig. 3. Microscopic picture (100X) of conjunctival smear showing degenerated neutrophils (black arrow), hyperplastic epithelial cells (yellow arrow) and few lymphocytes.

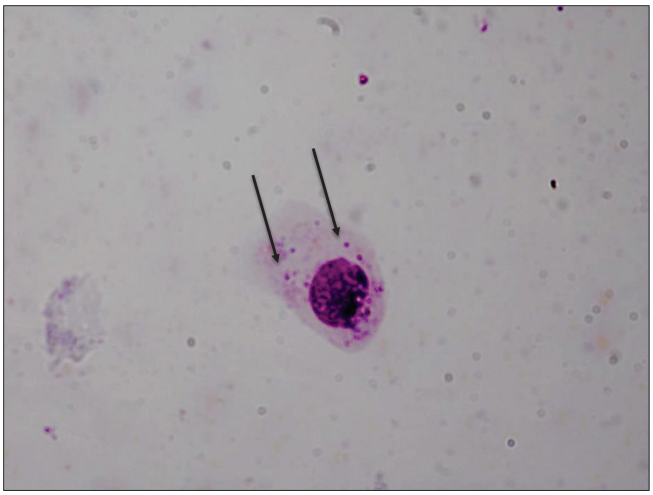

Fig. 4. Microscopic picture (100X) of conjunctival smear showing purple-colored basophilic chlamydial elementary bodies.

to be commonly found in conjunctival smear of human (Sharma, 2012; Yağmur *et al.,* 1997) and dogs (Çakır *et al.,* 2011) affected with keratoconjunctivitis. However, no such findings were evidenced in this study.

Interestingly, basophilic chlamydial elementary bodies characterized by purple colored coccoid structures were found inside the conjunctival epithelial cells (Figure 4). Other reports also found these characteristic intra-cytoplasmic inclusion bodies inside the trophoblast cells of fetal placenta and liver of aborted fetus died of chlamydial infection (Osman *et al.,* 2010).

Affected and pregnant does were treated with parenteral oxytetracycline and kids with oral chlortetracycline. All the affected goats were responded well to the treatment and remarkable elimination of clinical signs was observed. Corneal opacity and keratoconjunctivitis disappeared gradually after 15 days of treatment (Figure 2). Animals were started taking their feed after 4-5 days of treatment. The mean value of hemoglobin (9.78±0.40 g/dL) and TLC (8904±340 $x10^3/\mu l$) were within the normal range on 15th day post-treatment (table 1). Topical, systemic, and subconjunctival administration of antibiotics and anti-inflammatory drugs especially corticosteroids are the commonly adopted treatment protocol used since a long time. Antibiotics reduce the bacterial load, whereas, corticosteroids reduce the inflammation in the eye. Oxytetracycline is a broad-spectrum bacteriostatic antibiotic that is effective against *Mycoplasma*, *Chlamydia*, and *Rickettsia* (Borghi and Palma, 2014). Long acting oxytetracycline reduce the severity of chlamydial infection, both in form of ocular lesions and losses resulting from abortion (Longbottom and Coulter, 2003). To prevent further abortion in an outbreak, the treatment should be given soon after 95th to 100th day of gestation as this is the time when pathologic changes start to occur (Stuen and Longbottom, 2011). Oxytetracycline given in this study proved to be effective against chlamydial keratoconjunctivitis as well as prevent further abortion in pregnant does as no subsequent abortion was found in unaffected pregnant does. Although such therapy inhibits the shedding of infectious organisms, it does not eliminate the infection nor reverses any pathologic abnormalities already occurred in the placenta, thus delivery of stillborn or weak lambs can still occur (Stuen and Longbottom, 2011). However, no such incidents were evidenced in this study. Although corticosteroid act as a potent anti-inflammatory agent, no such treatment was given in this study as the steroids can impair formation of the vasculature, which would otherwise help in the healing process. In another study, topical chloramphenicol and sub-conjunctival administration of oxytetracycline and dexamethasone was found very effective against chlamydial keratocunjunctivits (Dibyendu and Saifuddin, 2017).

Conclusion

Chlamydia abortus infection is a highly contagious abortive disease of sheep and goats. Chlamydiosis should be suspected when abortion occurs in multiple pregnant goats in a short span of time in a flock. Cytological examination of conjunctival smear should be done in keratoconjunctivitis cases to examine intracellular elementary bodies suggestive of chlamydial infection. Long acting oxytetracycline stood effective against chlamydial infection in goats. When an abortion storm occurs in a newly affected farm, all the pregnant animals should be treated with antibiotics in order to prevent the losses due to abortion.

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Conflict of interest

Authors declare there is no conflict of interest.

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Clinico-therapeutic and diagnostic aspect of *Dipyllidium caninum* infection in a dog

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Abstract

A 2.5 month old Bhotia pup was presented to Department of Veterinary Clinical Complex, College of Veterinary and Animal Sciences, with the history of dullness, anorexia, loose faeces and melena since last 4 days. White cucumber seed shaped segments in faeces and scooting behaviour was also noticed. Faecal sample with proglottids were collected in a sterile vial and was sent to Department of Veterinary Parasitology for coproscopic examination. The proglottids were processed by standard protocol for the identification of the endoparasite. Proglottids were noticed in chain and single. The direct faecal sample examination revealed large number of typical egg capsules which were round in shape containing large number of eggs, characteristic of *Diphyllidium caninum*. Pup was treated with praziquental @ 5 mg/kg body weight orally and animal recovered completely.

Key Words: Dipylidium caninum, Dog, Therapy, Diagnosis

Dipylidium caninum infection is an ubiquitous infection among the dogs and cats. It is reported mainly from dogs and cats infected with fleas or louse (Wani *et al*., 2015), but cases in humans are also reported (Taylor *et al*., 2007). The intermediate host is the larval stage of *Ctenocephalides* species of dog or cat flea. Dogs and cats accidently swallow the infected fleas and acquires infection. It is one of the commonest cestode infection in pets.Canines which are infected will shed the segments of *D. caninum* in faeces and contaminate the surrounding with this proglottids which acts as a source of infection (Yasuda *et al*., 1971). It is a disease of public health importance as children usually are in close contact with pets and they can easily contract infection. Proper diagnosis, flea control and deworming is very essential to prevent the infection in humans and animals. Children will catch infection through accidental consumption of dog fleas and in most of the cases there won't be any apparent clinical manifestations. Clinical signs in affected dogs are reduced growth rate, pot-belly, diarrhoea and anal scooting due to pruritis (Taylor *et al*., 2007). Proglottid shedding can be noticed usually from 2 weeks after infection in dogs and cats. Diagnosis of infection can be done by direct examination of proglottid or direct faecal sample examination. The therapeutic management of disease is always successful in dogs and humans which can be done with any anticestode drugs. In this paper we are discussing a case of *Diphyllidium* infection in dog, its clinical manifestations, diagnosis and treatment.

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Case History and Clinical Observations

A 2.5 month old Bhotia pup was presented to Department of Veterinary Clinical Complex, College of Veterinary and Animal Sciences, SVPUAT, Meerut (U.P) with the history of dullness, anorexia, loose faeces and melena since last 4 days. White cucumber seed shaped segments in faeces and scooting behaviour was also noticed. Anamnesis revealed that the pup was due for deworming. Thorough physical examination revealed presence of fleas on skin coat (Fig. 4) On clinical examination, animal was unthrifty with poor hair coat infested with fleas. Mucus membranes were pale and temperature was 101.5°F. Heart rate and pulse was in normal range. No abdominal pain was elicited during palpation. Faecal sample with proglottids were collected in a sterile vial and was sent to Department of Veterinary Parasitology for coproscopic examination and identification of endoparasite. The proglottids were processed by standard protocol for the identification of the endoparasite (Soulsby, 1982).

Proglottids were noticed in chain and single (Fig. 2). It was white and cucumber seed shaped which was characteristic of *D. caninum.* Microscopic confirmation of the endoparasite was done in Department of Veterinary Parasitology, COVAS, SVPUAT, Meerut. The direct faecal sample examination on microscopy revealed large number of typical egg capsules of *D. caninum*. These egg

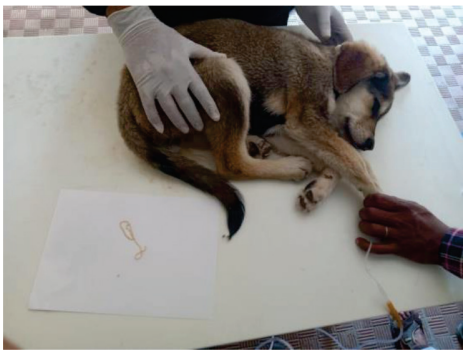

Fig.1- Affected Dog

Fig.2- Adult Worm from faecal sample

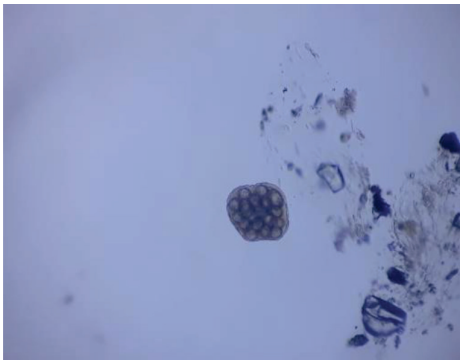

Fig.3- Egg Capsule (x40)

Fig.4- Dog Flea (*Ctenocephalides canis*)

capsules were round in shape and contain large number of eggs in it (Fig. 3). Dog was treated with Tab. Praziquental @ 5 mg/kg body weight orally for 3 days with supportive fluid therapy.

Discussion

D. caninum is very common cestode infection of dogs contracted by ingestion of fleas containing the infective cysticercoid stage (Gopinath *et al*., 2018). It is also known as flea tapeworm, cucumber tapeworm and double-pored tapeworm. Apart from the signs of worm infestation, animal frequently shows signs of flea infestation such as skin pruritis, dermatitis and anaemia (Yaphe *et al.*, 1993). In the present study, fleas were observed on the skin of dog and dog shows typical clinical manifestation of *D. caninum* infection such as anal scooting which is due to the irritation of movement of the proglottids in the anal region. The eggs were round in shapes and were inside egg capsules which is very typical of *D. caninum*. Clinical signs was successfully managed by anticestode therapy and animal recovered completely after five days of treatment. Proper and timely diagnosis of the case helps in successful management of the case. Diagnosis can be easily done at every level due to its peculiarity of eggs and proglottids. Praziquantel and epsiprantel are considered as the drug of choice for therapy against *D.caninum*.

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Unilateral renal subcapsular abscess associated with *Escherichia coli* infection in a Dog

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Abstract

Occurrence of renal abscess is uncommon in canine practice. A sixteen-month-old female Saint Bernard dog was presented to the medicine unit of University Veterinary Hospital, Kokkalai, Kerala with a complaint of anorexia, fever, reduced urination and black coloured watery faeces for the past 3 days. On clinical examination, dog was found dull, dehydrated, emaciated and evinced pain on palpation of sub-lumbar region. Blood picture showed leucocytosis, anaemia and azotemia. Ultrasonogram revealed a round hypoechoic structure within the capsule of right kidney suggestive of abscess. On ultrasound-guided percutaneous aspiration a blood-tinged purulent material was obtained which on culture produced *Escherichia. coli* organisms. The case was diagnosed as unilateral renal subcapsular abscess due to *Escherichia. coli* infection. Antibiotic treatment and supportive therapy were unsuccessful in saving the patient. This report describes a rare case of renal failure and unilateral renal subcapsular abscess associated with *Escherichia. coli* infection in a dog.

Key words: Dog, Renal subcapsular abscess, *E. coli,* Ultrasonography

In dogs, occurrence of renal and perirenal abscess are uncommon. Renal subcapsular abscess could develop from hematogenous systemic infection, or from ascending infection of the urinary tract (Hutchison and Kaysen, 1988). Clinical signs were usually non-specific, such as abdominal pain, depression, fever, and loss of appetite (Faucher *et al.,* 2017).

Renal subcapsular abscess in dogs had been reported by Cola *et al,* (2020) in Italy and by Hwang *et al,* (2020) in Korea. But, on a through literature review and to the best of author's knowledge, this is the first report in dogs in India.

Case History and Clinical Observations

A 16 months old female Saint Bernard weighing 30 kg was presented to the University Veterinary Hospital, Kokkalai with a complaint of anorexia, fever, reduced urine output and black coloured watery faeces for past 3 days. Clinical examination revealed dehydration, dullness, pale mucous membranes, tachypnoea (56 breaths/min), tachycardia (136 beats/min), pyrexia (103.2°F) and pain on palpation of the sub-lumbar region. Complete blood count, serum biochemistry, blood gas analysis (Table 1-2) revealed leucocytosis with lymphocytosis and monocytosis, anaemia, thrombocytopenia, an elevation in serum creatinine, BUN and phosphorus and a reduction in blood pH and $HCO3^-$ level. Urine dipstick test revealed the presence of proteinuria and high level of RBCs and WBCs (Table 3). The urine protein-creatinine ration was 0.63.

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Table 1. Complete blood count

Parameters	Measured Value	Reference Range
WBC ($x10^3/\mu L$)	20.4	6-17
Lymphocyte ($x10^3/\mu L$)	9.2	0.7-5.1
Monocyte ($x10^3/\mu L$)	3.0	0.2-1.7
Granulocyte ($x10^3/\mu L$)	8.3	4.4-12.6
RBC ($x10^6/\mu L$)	2.30	5.5-8.5
Hct (%)	18.6	37-55
Hb (g/ dL)	6.1	11-19
MCV (pg)	69.4	62-72
MCHC (g/ dL)	30.6	30-38
Platelets ($x10^3/\mu L$)	169	160-525

Abdominal ultrasonography identified rounded hypoechoic subcapsular lesions in the right kidney (Fig. 1) though poor corticomedullary differentiation and loss of architecture were noticed in both the kidneys. Renal resistive index (RRI) of left and right kidney detected by doppler ultrasonography was 0.78 and 0.76, respectively and is shown in Fig. 2 (Reference interval: 0.56-0.67).

Percutaneous ultrasound-guided aspiration of

Table 2. Serum biochemical results

Parameters	Measured Value	Reference Range
Urea(mg/dl)	59.53	9.2-29.2
Creatinine (mg/dL)	10.37	0.5-1.7
Phosphorus (mg/ dL)	16.09	2.5-6
Blood pH	6.58	7.36-7.44
$HCO3^-$ (mmol/L)	5.3	24.0-26.0
Potassium (mmol/L)	2.1	3.9-4.9
ALT (U/L)	38.44	10-109
ALP (U/L)	46	5-55

Table 3. Urinary dipstick and physical examination findings

Parameters	Measured value
Colour	Yellow
Consistency	Turbid
Protein (g/ dL)	1.0
Specific gravity	1.015
pH	Acidic
RBC(/µl)	200
WBC (/µl)	125
Urine spot protein (mg/ dL)	49.5
Urine spot creatinine (mg/ dL)	78.9
UPC	0.63

renal subcapsular lesion on the right kidney (Fig. 3) revealed a thick, blood-tinged purulent fluid (Fig. 4). Cytological examination of aspirated sample revealed bacterial cocci, numerous pus cells and few RBCs (0-2/ HPF). Bacterial culture of both aspirated sample and urine produced heavy growth of *E. coli*. Azotemia was considered renal due to a lack of response to diuresis by fluid therapy and frusemide administration. Based on these findings, the case was diagnosed as renal failure and unilateral renal subcapsular abscess due to *E. coli* infection. Prognosis of the case was grave.

Treatment and Discussion

Treatment was initiated with intravenous injection of amoxycillin-sulbactum @ 12.5 mg/kg BW twice a day, pantoprazole @ 1 mg/kg BW, once a day and ondansetron @ 0.5 mg/kg BW, once a day and intramuscular injection of 2 ml polybion (B-complex). Though treatment was continued the next day, the dog

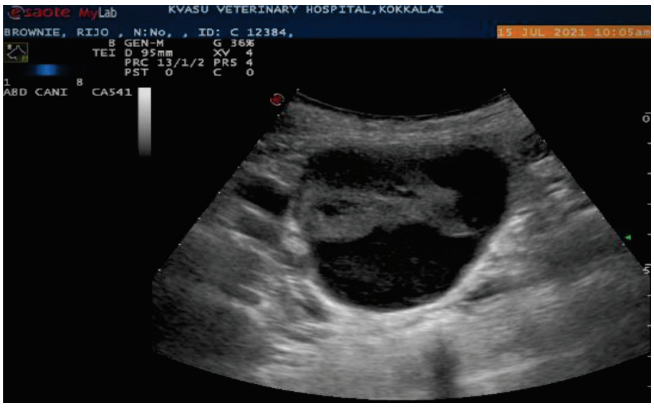

Fig. 1. Ultrasonogram of right kidney with a hypoechoic subcapsular lesion suggestive of abscess

passed away even before the bacterial culture results arrived. The owner was unwilling for a post mortem examination.

Renal subcapsular abscess is defined as a suppurative process between the renal parenchyma and the renal capsule (Lee *et al.,* 2010). A renal subcapsular abscess differs from a renal abscess in which the latter is located in the cortical or cortico-medullary parenchyma (Dembry and Adriole, 1997). Intrarenal cortical abscesses develop secondary to hematogenous bacterial infection, whereas corticomedullary abscesses are considered to result from an ascending infection of the urinary tract (Lee *et al.,* 2008). The pathogenesis of the unilateral renal subcapsular abscess in this case remains less clear. The concomitant positive culture for *E. coli* of the urine and subcapsular pus possibly suggests an ascending UTI. Anderson and McAninch (1980) reported a high correlation between organism grown in urine culture and the abscess and repeated urine cultures were positive for the causative organism in almost all cases. In this case both renal subcapsular abscess and urine yielded heavy growth of *E. coli* organisms which was similar to the findings of Hess and Ilan (2003). *Staphylococcus* spp was isolated by Cola *et al.* (2020) in dog with renal subcapsular abscess.

Clinical signs were non-specific. Fever, lethargy, anorexia and diarrhoea were similar to the findings of Lewis *et al.* (1998) and Hess and Ilan (2003).

Sampling of renal subcapsular fluid using ultrasonic guidance was important to differentiate abscess, haemorrhage, urine leakage, and neoplasia. Percutaneous abscess drainage under ultrasound guidance or surgical treatment had shown better results (Hwang *et al*., 2020).

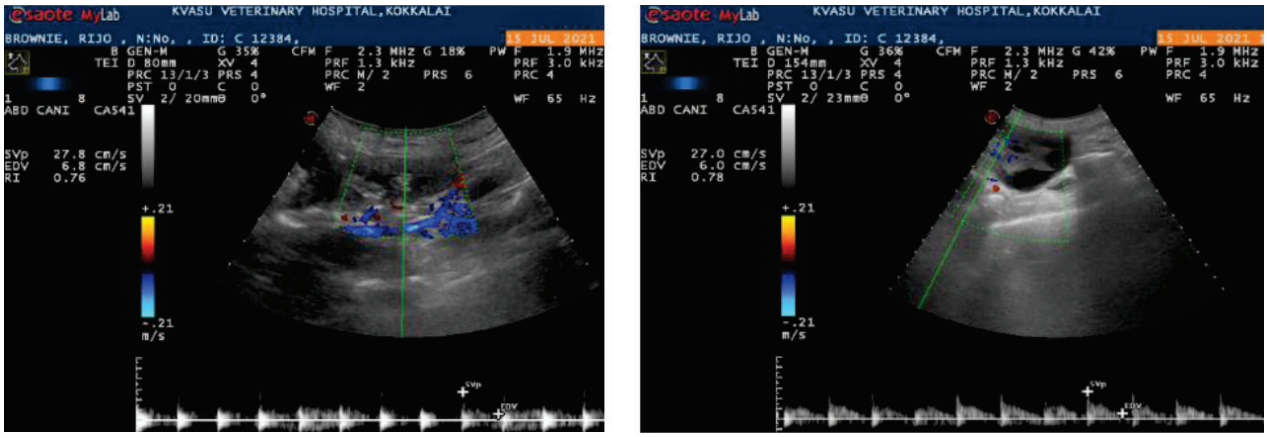

Fig.2. Doppler ultrasonogram showing high RRI in both kidneys.

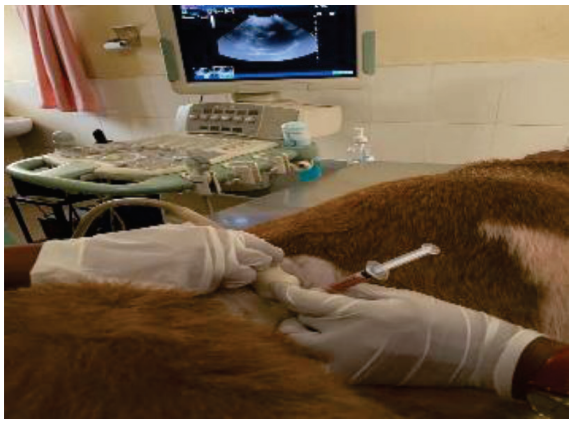

Fig. 4. Blood-tinged purulent material (left) and yellowish turbid urine (right)

Fig. 3. Percutaneous ultrasound-guided aspiration of the renal subcapsular abscess.

Treatment of renal abscess in humans required at least 4 weeks of antibiotics depending on the etiological agent or an empirical therapy for gram negative bacteria when the causative agent is not isolated. A sub-optimal response to antibiotics required surgical intervention. In dogs, nephrectomy was indicated in renal or perirenal abscess owing to severe damage to the kidney parenchyma or the deep location of the lesion (Lewis *et al.,* 1988; Agut *et al.,* 2004).

Conclusion

Renal abscess is a rare renal pathology in dogs. Abdominal ultrasound and ultrasound guided aspiration of the abscess are helpful in establishing an early diagnosis. This article reports a case of renal failure and unilateral renal subcapsular abscess due to E.Coli infection in a Saint Bernard.

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Clinical investigation and therapeutic management of hypoglycaemic seizures in a pug dog

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Abstract

A male pug of age 3 years (body weight 12 kg) was presented to the Veterinary Clinical Complex, Nagpur with a history of anorexia, muscle twitching, blindness, and lateral recumbency for 2 days with a recurrent rate of seizures within a period of 10 to 15 min. The dog has mild to no response to pain stimulation (mild to no withdrawal reflex), pupils were dilated, and on complete blood count, leucocytosis was observed and random blood glucose revealed a blood glucose level of 28mg/dl(60-111mg/dl), calcium was within the normal range(60-110mg/dl) and with SpO_2 87%. The case was treated with dextrose 10% IV, injection ringers lactate IV, dexamethasone IM, injection vitamin B complex IM bid for 3 days and oxygen supplementation were given.

Key words: Hypoglycemia, Seizures, Muscle twitching, Dextrose 10%, Oxygen

Hypoglycaemic seizure is not a common disease in dogs. In this condition, there is a severe fall in blood glucose levels. In clinically normal dogs, glucose is maintained within a narrow range of 60 mg/dl to 111 mg/dl (Smith, 2004), and clinical signs of hypoglycemia do not usually develop until glucose concentrations are <50 mg/dl (Koenig, 2009) and hypoglycemia are defined as glucose less than 50 mg/dl. (Koenig, 2009; Nelson, 2014). There are 4 mechanisms due to which hypoglycemia may arise: i) Glucose and other substrates used in hepatic gluconeogenesis deficit diet; ii) Sometimes normal or neoplastic cells may cause an increase in glucose uptake and utilization due to an increase in demand or secondary to hyperinsulinism; iii) Disturbance in hepatic glycogenolytic or gluconeogenic pathways; and iv) A deficiency of counter-regulatory hormones such as cortisol due to endocrine abnormalities (Koenig, 2009; Nelson, 2014). Clinical signs may include altered mentation behaviour changes and other signs such as seizures, muscle twitching, lethargy, collapse, incoordination, weakness, and impaired vision. These clinical signs are mainly due to neuroglycopenia or cerebral hypoglycemia (Nelson, 2014).

Case History and Clinical Observations

A male pug of age three years was presented to the Veterinary Clinical Complex, Nagpur, with a history of anorexia, muscle twitching, blindness, and lateral recumbency for two days with a recurrent rate of seizures within a period of 10 to 15 minutes. On clinical examination, the dog was observed to have mild to no response to pain stimulation (no to slight withdrawal reflex). Pupils were dilated, and on complete blood count, leucocytosis was observed. Random blood glucose revealed a blood glucose level of 28mg/dl, with Spo2 of 87%.(normal->95%). The case was relatively confirmed for hypoglycaemic seizures based on history, clinical signs, and blood biochemistry.The case was treated with injection dextrose 10%,injection ringers lactate 100ml (7ml/kg/hr), glucocorticoid dexamethasone 1ml (0.5-1mg/kg) bid for 3days and oxygen supplementation

Discussion

Smith (2004) stated that in dogs, the average glucose level is 60mg/dl to 111 mg/dl, and Koenig(2009) said that clinical signs appear when glucose goes below 50mg/dl. The dog has 28mg/dl of blood glucose in this case. That's why clinical signs were manifested in this case. Koenig (2009) and Nelson (2014) mentioned There are 4 Main reasons which may include poor dietary intake of glucose and other substance used in hepatic gluconeogenesis, increased glucose uptake and utilization by neoplastic cells, dysfunctional hepatic glycogenolytic or gluconeogenesis pathway or endocrine abnormalities resulting in a deficiency of counter-regulation hormones such as cortisol and Nelson (2004) stated that signs will be observed in the hypoglycemic dog which has blood glucose less than 50mg/dl hence in the current case the dog has clinical manifestations like seizures, muscle twitching, lateral recumbency, loss of vision, and

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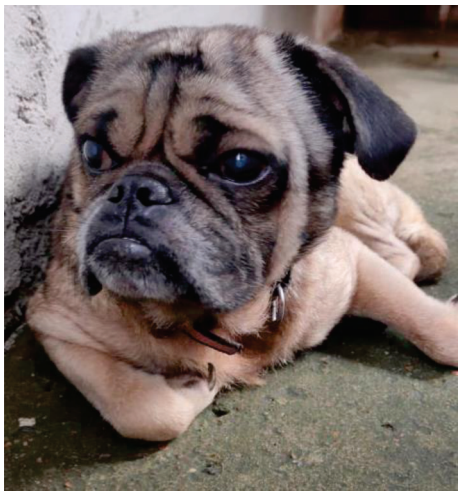
During Treatment



After Treatment

Table 1. Pre and post-therapy Hematological analytes and biochemical parameters

Parameters	Day 1	Day 2	Day 3	Day15	Normal range
		Erythrogram analytes			
TEC (× 10 6 /µl)	7.60	7.50	7.90	8.1	5.5-8.5
Hb (gm/dl)	14.2	14.1	15.0	14.9	12-18
PCV (%)	45.8	50	51.5	52	37-55
MCV um^3	60.0	61	60	63	62-77
MCH (Pcg)	18.7	18.9	19	23	21-26.2
MCHC (gm/dL)	31.1	34.0	34.5	34.7	32-36
		Leucogram analytes			
WBC (/µL)	52200	36000	23000	15000	6000-17000
Neutrophils (%)	62.5	63	66	70	62-80
Lymphocytes (%)	24.5	22.6	18.2	15.2	10-28
Monocytes (%)	13.0	10	7	5	3-9
Eosinophils (%)	4.6	5.0	5.3	5.8	2-12
		Random blood glucose			
glucose(g/dl)	28.0	104	134.2	105	70-120
Spo2 (%)	87-92	95-96	96	96-98	98-100

anorexia, hemo-biochemical parameters before initiation of treatment revealing leukocytosis and low random glucose value. During therapy as well as the recovery period, all the parameters were restored within the normal range (Table 1)

The case was successfully treated with an injection of dextrose 10% and after 1st day of administration, and there was a significant increase in glucose level and improvement in feed intake. Koenig (2009)& Harmon(2016) stated that Care must be taken and that dextrose must be administered slowly. As it was said by Kuo *et al.* (2015), Glucocorticoids promote gluconeogenesis in the liver. In contrast, skeletal muscle and white adipose tissue decrease glucose uptake and utilization by antagonizing insulin response. Hence, in this case, dexamethasone is used. Datte and Guillaumin (2016), in their study of 9 dogs, successfully used glucagon CRI to raise blood glucose concentrations in all dogs

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Effective use of polyvalent antivenom in snake bite dogs – A review of three cases

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Abstract

Three dogs with snake bite presented to the critical care unit, Madras Veterinary college were taken up for this study. Affected dogs were treated with antivenom along with supportive care consisting of intravenous fluid therapy and pain management. In the present study, additional antivenin was administered in one dog with persistent clinical signs. The dose varies from 10 to 50 ml administered intravenously. It also depends on the severity of symptoms, lapse of time after the bite, size of snake and size of patient. Additional doses should be given every 2 hours as required, if symptoms such as swelling and pain persist or recur. Non-venomous snakebites are generally treated with wound cleaning, antibiotics, anti-histamines and anti-inflammatory medications.

Key words: Dog, Snake bite, Antivenom, Treatment

Snake bite in animals generally occurs during grazing or hunting or while playing in the garden. Most of the cases of snake bite have been reported in dogs and horses (Garg, 2000). Among the domestic animals dogs are most frequently attacked and killed by the snakes (Osweiler, 1996). Poisoning from snake venom in animals is an emergency which requires immediate attention or otherwise delayed and inadequate treatment may lead to untoward consequences. The present paper describes snake bite in canines and its therapeutic approach.

Case History and Treatment

Case 1

Six years old non-descript female dog was presented with the history of snake bite and excessive salivation. Fangs marks were noticed in the frontal region and swelling in the face, representing multiple bites. Clinical examination revealed temperature 102.9ºF, respiratory rate 40bpm, heart rate 50bpm, congested mucus membrane and SPO_2-92%. Haematology was normal except leukocytosis. Serum biochemical values were within the normal limits. To assess the type of envenomation about 2ml of whole blood collected for checking 20 minute whole blood clotting time. The blood was clotted within 6 mins which indicates the bite was negative for hemotoxic envenomation. Based on clinical findings and history of the owner it was diagnosed as case of snakebite and therapeutic measures were undertaken immediately. The fang mark area of the skin was thoroughly cleaned with povidone iodine. Then 10 ml of polyvalent anti-snake venom was administered along with NSS IV over a period of one hour. In addition, Cefotaxime @ 30mg/kg body weight IV and tetanus toxoid were also given. The dog was kept under observation and did not show any clinical improvement. After four hours again the animal was treated with 10 ml of polyvalent anti-snake venom along with normal saline intravenously over a period of one hour. After second dose the clinical signs reduced and the next day, animal recovered uneventfully. Further the antibiotic therapy was continued for 5 days along with supplements.

Case 2

Two year old Greatdane male dog was presented with the history of snake bite and excessive salivation. Fangs marks were noticed in the left upper lip and swelling also noticed. Clinical examination revealed temperature 101.7 ºF, respiratory rate 38bpm, heart rate 72bpm, congested mucus membrane and SPO_2-96% with normal haematology. Serum biochemical values were within the normal limits. Twenty minute whole blood

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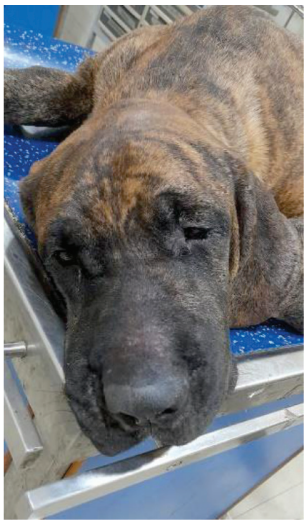
clotting time was done and the blood clotted within 5 minutes. It indicates the bite was negative for hemotoxic envenomation. Based on these findings and history, the case was diagnosed as neurotoxic snakebite and treated with 10 ml of polyvalent anti-snake venom with normal saline intravenously over a period of one hour. In addition, Cefotaxime @ 30mg/kg body weight intravenously and tetanus toxoid were also given subcutaneously. The animal recovered on the next day. Further antibiotic therapy was continued for next 5 days along with supplements.



Case 3

Three year old Labrador male dog was presented with the history of snake bite and swelling in the mouth region. Fangs marks were noticed inside the upper lip and swelling around the face. Clinical examination revealed temperature 101.7 ºF, respiratory rate 32bpm and heart rate 82bpm. Haemato-biochemical parameters were within the normal limits. The owner brought the snake photo which was identified as Indian rat snake and it is a common non-venomous snake species. The dog was treated with Inj. Dexamethasone alone for swelling without administration of any polyvalent anti-snake venom but kept under observation for another one day. The dog recovered and became apparently normal.

Discussion

Snake envenomation causes a combination of local and systemic clinical signs with considerable variations, depending on the snake species (Bolon *et al*., 2019). Local manifestations such as edema at the bite site, infection, and tissue necrosis are the most common. The systemic manifestations include vomiting, nausea, dizziness, rhabdomyolysis due to muscle necrosis, renal dysfunction, and various other clinical conditions that could lead to death (Debono *et al*., 2019).

The hematologic finding of leukocytosis suggest an inflammatory reaction caused by the snake bites. These results are similar to those of a previous study that suggested stress as a contributing factor (Lobetti and Joubert, 2004). The use of tetanus toxoid provides protection against the tetanus spore that might have entered animal body from contaminated snake mouth (Shukla, 2009). Blaylock (2001) opined that broad spectrum antibiotics were administered to the dogs, as snake fangs are contaminated with different types of bacteria which are mainly gram negative enterobacteriaceae. Further he suggested that the increased leucocytes count is attributed to systemic infection as snake fangs and oral cavity has bacterial contaminants.

Polyvalent snake anti-venom was preferred in the present case as it provides protection against the venom of big four (common cobra, common krait, saw scaled viper and russell's viper) species of the snakes. A standard treatment protocol for snake envenomation is lacking for dogs; however, the only accepted treatment is the administration of antivenom along with supportive care consisting of intravenous fluid therapy and pain management (Armentano and Schaer, 2011). Antivenom limits clinical signs and reverses coagulopathy (Riffer *et al,* 1987).

In the present study, additional antivenin was administered in one dog with persistent clinical signs. The dose varies from 10 to 50 ml (1 to 5 vials) administered intravenously. It also depends on the severity

Table 1: Hematobiochemical values of snake envenomated dogs

	Case 1	Case 2	Case 3
Hemoglobin (g/dl)	10.5	11.2	10.3
PCV %	38	42	40
RBC (millions/cmm)	6.0	5.5	5.8
WBC (numbers/cmm)	27100	12000	10500
BUN (mg/dl)	38	32	328
Creatinine (mg/dl)	1.2	1.0	0.89
ALT (U/L)	77	86	95

of symptoms, lapse of time after the bite, size of snake and size of patient. Additional doses should be given every 2 hours as required, if symptoms such as swelling and pain persist or recur (Peterson, 2009). When no envenomation occurs, or if the bite is inflicted by a non-venomous snake, the bite should be treated as a puncture wound. Non-venomous snakebites are generally treated with wound cleaning, antibiotics, anti-histamines and anti-inflammatory medications.

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Shell rot infection in a red eared turtle

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Abstract

A Six month old red-eared turtle weighing 400gm was brought to the Department of Veterinary Medicine, College of Veterinary and Animal Sciences, MAFSU, Parbhani with a history of gradually increasing discoloration of shell since a week. After proper restrain detailed clinical and physical examination was carried out that revealed irregular white spots spread across the dorsal aspect of the carapace (shell) although the shell was free from cracks and wound. The turtle had a normal appetite and was fed commercial turtle feed. Swabs were taken from the carapace and water of the terrarium for culture that revealed mixed infection of *Escherichia coli* and *Staphylococcus* spp. As per the antibiotic sensitivity testing and recommendations of the other researchers therapeutic management was carried out with Oxytetracycline @ 25 mg/kg BW IM for 3 days, Metronidazole oral suspension 50mg/kg BW PO for 10 days and povidone-iodine applied over the shell for 10 minutes daily followed by topical application of silver sulfadiazine ointment on white spots for 15 days. The treatment showed marked clinical improvement with respect to improvement in shell texture and appearance.

Keywords: Shell Rot, Turtle, Mixed infection, Therapeutic management

Red-eared turtle also known as red-eared slider or red-eared terrains is one of the most popular pet turtle in recent years that belong to *Emydidae* family of *Trachemys scripta elegans* species. These freshwater turtles are native of southeastern United States (Teillac-Deschamps *et al.,* 2008). Shell of the turtle being the primary defense needs necessary care and management and dermatological problems have been reported in captive turtle due to improper management and lack of knowledge related to their housing pattern. Environmental factors (high humidity, low temperatures), poor nutrition, improper husbandry practices, dirty environment in the form of dirty water or moldy bedding and injured skin are potential factors that compromise the health of shell in turtles (Das *et al.,* 2018).

Shell rot or Septicemic Cutaneous Ulcerative Disease (SCUD) is a common problem in aquatic turtles and some reptiles caused by a bacterial or fungal infection that can come from dirty environment in the form of dirty water or moldy bedding. Red-eared turtles remain around water all the time, and any previous wound on the shell predisposes them to shell infections mostly by gram-negative bacteria that are normally present in the environment (Kasim *et al.,* 2017).

Clinical History and Observations

A Six month old red-eared turtle weighing 400gm was brought to the Department of Veterinary Medicine, College of Veterinary and Animal Sciences, MAFSU, Parbhani with a history of gradually increasing discoloration of shell since a week. After proper restrain detailed clinical and physical examination was carried out that revealed irregular white spots (Fig. 1) spread across the dorsal aspect of the carapace (shell) although the shell was free from cracks and wounds. The turtle had a normal appetite and was fed commercial turtle feed.

Swabs from the carapace and the water sample from terrarium were collected for the identification of infectious agent. Culture and antibiotic sensitivity test were performed to formulate treatment protocol. The result of culture showed mixed infection of *E.coli* and *Staphylococcus* spp.

As per the antibiotic sensitivity testing and findings of the other researchers, therapeutic management was carried out with Oxytetracycline @ 25 mg/kg BW IM for 3 days, Metronidazole oral suspension 50mg/kg BW PO for 10 days and povidone-iodine applied over the shell for 10 minutes daily followed by topical application of silver sulfadiazine ointment on white spots for 15 days (Harms *et al.,* 2004; Kasim *et al.,* 2017).

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Fig. 1. (Before treatment) Irregular white spots on the dorsal aspect of the shell

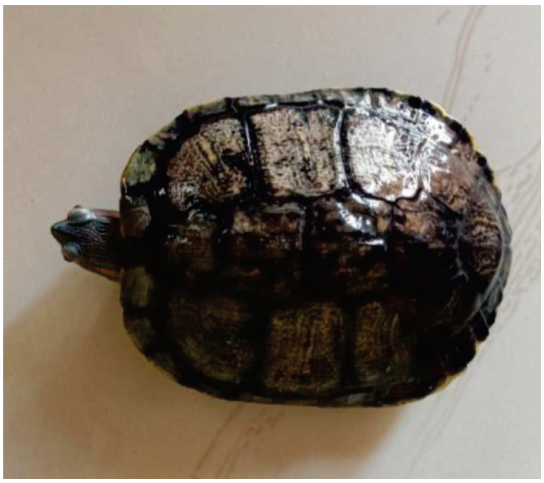

Fig. 2. (After treatment) Disappearance of white spots and healing of the dorsal aspect of the shell

Discussion

The therapeutic management of shell rot carried out for 15 days showed marked clinical effect with respect to improvement in shell texture and appearance (Fig. 2). Shell is the primary defense of turtles against any attack by predators and also gives protection from environmental stressors (Polocavia *et al.,* 2008). Traumatic injuries caused by bites of other turtles or any other predators compromises the outer keratin layer of the shell and burns. Bacterial or fungal infections can also lead to ulcers on the shell (Wyneken 2006). Shell rot or SCUD is a common problem in aquatic turtles frequently reported due to managemental faults or dirty environment in the form of dirty water or moldy bedding leading to the predisposition of the shell to fungal or bacterial infections especially gram-negative bacteria that are normally present in the environment (Kasim *et al.,* 2017). Clinical findings observed such as soft spot of the shell and hyperemia on the turtle carapace have been also reported by Fowler *et al.* (2008) who has mentioned that the initial stages of infection causes soft spots on the shell and later on cutaneous ulceration and fibrin deposit on the shell are observed. Microbial analysis of the swabs taken from the lesions for culture have revealed presence of *E. coli* and *Staphylococcus* spp. and similar findings have been also reported by other researchers such as Harvey-Clark (1995) who reported the gram positive cocci from skin and foot lesions of turtles whereas Kasim *et al.* (2017) reported *E. coli* from the shell of a turtle. As per the antibiotic sensitivity testing and findings of the other researchers, therapeutic management was carried out that resulted in marked clinical improvement with respect to improvement in shell texture and appearance (Fig. 2). Hoppmann (2007) recommended the use of povidone-iodine solution soaked in cotton guaze, twice daily until lesions resolved. Application of povidone-iodine and silver sulfadiazine for treating shell rot with good results fall in agreement with Jadhav *et al.* (2020), Kasim *et al.* (2017) and Kramer (2002). Jadhav *et al.* (2020) successfully treated shell rot in one-year-old female red-eared turtle with gentle scrapping of the shell in areas of the lesions followed by soaking of the shell with povidone-iodine daily for 10 minutes followed by application of silver sulfadiazine ointment topically. Khan *et al* (2019) successfully treated shell rot in a turtle with enrofloxacin bath and topical application of chlorhexidine gel for 7 days. Kasim *et al.* (2017) successfully treated shell rot in a red-eared turtle with a combination of amikacin and metronidazole oral suspension for 10 days along with the topical application of diluted povidone-iodine for 14 days.

Conclusions

The present case report discusses the successful therapeutic management of shell rot infection in a turtle. Clinical findings such as soft spots or hyperemia on the shell and cutaneous ulceration and fibrin deposit on the shell should be checked upon regularly and need to be taken seriously by employing therapeutic management comprising of local application of cotton soaked povidone-iodine, followed by topical use of silver sulfadiazine ointment, parenteral or oral antibiotics, and appropriate managemental practices. If left untreated,

the infection can rot through the bone and into the body cavity and therefore can prove fatal.

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Author Index

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Clinical case reports of interesting and rare nature are published under this heading. The article sent for publication under this head, should contain between 1000-1500 words but not more than three typed pages including references and illustrations and should be marked 'Clinical Article' at the right upper corner of the first page of manuscript. An abstract (not more than 100-150 words), keywords (not more than 3 words). The manuscript should contain history and important clinical observations of the case, tentative diagnosis and its confirmation, line of treatment used and fate of the case. At last, it should have a brief discussion on the line of diagnosis and treatment. All these can be given in separate paragraphs sequentially and sub-heading is not required. The acknowledgement, if necessary, may be given but it should be as short as possible. The reference should be given as per format for the research articles

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I, Swaran Singh, Department of Veterinary Medicine, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated: December, 2022 **Swaran Singh**

To Our Contributors

The Editorial Team is highly thankful to esteemed ISVM members and veterinary fraternity for contributing over-whelmingly for each and every issue of "Indian Journal of Veterinary Medicine". We are very hopeful that scientists and clinicians will contribute their publications with more enthusiasm and also spare their valuable time to be a part of reviewer panel for the journal.

A website of the society (www.isvm.org.in) is functional and includes many features, such as online submission and viewing status of manuscripts, online membership forms etc. Please send your articles using this website.

With your scientific contributions, we could able to bring the Journal NAAS rating to 4.57 and aspire that your consistent support will further help us to improve the journal rating to a suitable level.

Editorial Team

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