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From the Editorial Desk....

Dear All,

Indian journal of veterinary medicine have witnessed its regular publication of 33 years with active contribution of all members of Indian Society for veterinary Medicine, Reviewers, authors and secretarial office .This journal has special significance for meeting the needs for continuing veterinary medicine education, sharing valuable scientific information, clinical experience and expertise. During last year we received good number of articles from abroad that indicates the importance of the journal. However, to maintain continuous improvement and scientific excellence we need quality research papers, clinical case reports and newer diagnostic and therapeutic approaches for successful diagnosis and management of animal diseases. To maintain scientific standard of this journal stereotype articles required to be limited. We are receiving articles in the area of nuclear medicine. In India numbers of tropical diseases are prevalent which are not extensively researched by the developed world; articles of these areas are encouraged. New potential treatment approaches such as regenerative medicine and nanomedicine are critical areas to appraise its efficacy as objectively as possible, recognize side effects. Clinical data are lacking on number of cells should ideally be delivered, species incompatibility, biological and ethical issues. Nano medicine is another such areas where we welcome articles for this journal. We are receiving quite good number of articles from wild life diseases and hope in coming issues we will be able to cover some aspects of animal welfare. Our goal is to cover all aspects of veterinary medicine across the boundaries of discipline which has linked with welfare of animal and farming communities. To overcome the problems in delivery of journals to all members of Indian Society for Veterinary Medicine, we are thinking to deliver soft copy of the journal to all life members by E mail. In this endeavour the editorial office solicits active cooperation of members and requests the members to send their mail ID.

Contents

Research Articles

Short Communication

Contents

Clinical Articles

Indian J. Vet. Med. Vol. 33, No. 2, 2013 pp. 79-86

Research Article

Development of package of practice for management of endemic goitre in goats

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Abstract

To overcome endemic goitre oriented perplexity of farmers of subhimalyan Tarai, a field package of practice has been developed. A controlled therapeutic study of four iodine compounds, namely aqueous iodine, sodium thyroxine, Lugol's iodine and potassium iodide in standard doses in four groups of goitrous goats having six goats, in each were conducted up to 120 days . Aqueous iodine given @ 0.1 mg/kg body weight orally was found most effective on the basis of thyroid hormone profile and restoring of clinical parameters. Based on these studies, 50 goitrous goats were treated with aqueous iodine for 120 days, then they were divided in 5 groups A, B, C, D and E. Goats in groups A, B, C and D were given chemoprophylactically treatment of iodized oil injections @ 1 ml SC at 4 months interval, iodized salt (15 ppm) @ 1.5 % as feed additive, iodized mineral mixture @ 4-10 gm (containing 1.6 - 4 mg iodine and Lugol's iodine solution @ 2 -4 drops /goat/day, respectively for 15 months. Group E served as control. During and post chemoprophylaxis, the recovery rate in production, reproduction, overt clinical signs and thyroid hormone profile was maximum in goats receiving iodized oil injection followed by iodized salt fed goats (B). However, the practical acceptability of iodized salt was more as compared to iodized oil injections. In group E, there was relapse of Goitre in 4 -6 months time. Therefore, use of colloidal Iodine @ of 0.1mg/kg body weight orally for 120 days and then subsequent chemoprophylactic medication of either iodized salt (15ppm) @ 1.5 % as a feed additive medication or alternatively, 4 monthly SC injections of iodized oil injection @ 1.0 ml (375 mg) to each goat uptil reared in endemic areas has been recommended.

Keywords: Endemic, Package of practice, Goiter, Goat.

Iodine deficiency in abiotic environment is the main cause of simple endemic goitre (Southcott, 1945; Brand *et al.*, 1963), though up to 4% of the total incidence of non toxic goitre may be due to other causes such as presence of goitrogens, viz thiourea, thiouracil, isothiocyanates, thiocyanates, nitrites and oxazolidinethiones in feeds. Though goitre as a clinical syndrome in goats was recognized as early as 1935 (Ramakrishna, 1994), but reports are available regarding its occurrence in animals in endemic areas of India (Singh *et al*., 2001, 2003, 2005, 2006, Shiva Kumar *et al*., 2009).

Diagnosis of iodine deficiency disorders is primarily based on the assay of iodine derivative hormones in thyroid and different biological samples along with observation of clinical signs. Treatment of iodine deficiency disorder must be undertaken with care as over dosing of iodine causes toxicity in animals (Radostits *et al.,* 2007). For treatment of iodine deficiency disorders in goats, various iodine compounds like potassium iodide, Lugol's iodine, aqueous iodine, iodized oil injection and synthetic thyroxine have been recommended with variable effect (Singh *et al*., 2001, 2004). However, this information is not sufficed for the management of endemic goitre in goats. Looking in to perplexity of goat keepers associated with high mortalities of neonatal kids/ still births and their lack of interest in goat farming, development of a field package of practice was planned to overcome these disorders.

Materials and Methods

The clinical and physical examinations of various herd/flock of the goats in the area was made. On the basis of palpation of enlarged thyroid glands, the goitrous goats were graded on 0 to 3 stages, as per standard scale of Perez *et al.* (1960). Total 24 goats suffering from stage-3 goitre, were purchased and reared in experimental animal house of the college for one month for acclimatization and dewormed with fenbendazole @ 5 mg/kg body weight orally and reared on pasture grazing basis. The blood and fecal examinations were conducted to rule out the possibility of concurrent infection. For comparative therapeutic evaluation, these goats were divided in 4 equal groups A, B, C and D. Six apparently healthy goats in group E served as healthy control. Goats of group A received aqueous iodine @ 0.1 mg/kg body weight orally, group B sodium thyroxine @ 0.2 mg/day with 10 ml distilled water orally, group C Lugol,s iodine solution @ 4-6 drops per day and group D potassium iodide @ 50 mg /day. The treatment was continued for 120 days. Blood samples were collected from all the goats on day 0, 20, 40, 60, 80, 100 and 120 for thyroid hormone profile. Serum triiodothyronine (T_3) and serum thyroxine (T_4) were estimated by RIA technique using Gamma scintillation counter and specific RIA kit as per method described by Chopra (1972).

The goitre affected goats revealed hard swelling of variable size (walnut to duck egg size) palpable on both side of the neck, still birth/abortion, anoestrous, retention of placenta among females, emaciation, coarseness of hairs and whitish discoloration of mucous membrane. However, distribution of major signs in congenital goitrous kids born out of goitrous doe were enlargement of thyroid gland, letharginess, waddling gait, hydrocephalus, prognathic face, flexion of limb joints, arched back, thyroid thrill and partial alopecia.

Based on findings of therapeutic trial, another therapeutic management protocol was designed for confirmation of therapeutic finding. For it, 50 clinically goitre affected goats were procured and acclimatized as described earlier and divided into 5 groups of 10 each. Each goat in all the groups received aqueous iodine @ 0.1 mg/kg body weight orally daily for 120 days.

A chemoprophylactic treatment protocol of post aqueous iodine treatment was carried out for 15 months using various agents. Goats of group I received iodized oil injection (I-FER-H) @ 1 ml sc repeated at 4 months interval, group II received iodized salt (containing 125 ppm iodine) as 1.5% feed additive, group III iodized mineral mixture (containing 1.6-4 mg iodine)b @ 4-10 gm orally daily and group IV Lugol's iodine lotion @ 2-4 drops per goat daily. Group V served as control. During the trial period, three monthly sampling of blood was made to analyze various parameters to assess the status of recovery. The results were analyzed statistically to record any significant change in different parameters between the groups and within the group using two factors complete randomized design and paired 't' test as described by Snedecor and Cochran (1975).

Results

The values of total T_3 and T_4 before

Table 1: Alterations in Total T_3 and T_4 profile (Mean ± SE) at various days of goitrous goats medications

Parameter	Groups (N=6)	Days of Observation					
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
Total $T_{3(n\ mol/l)}$	A	1.34^{bb} ± 0.062	1.62^{aabb}±0.058	1.93^{aabb}±0.034	2.17^{aabb}±0.056	2.31^{aabb}±0.062	2.42^{aabb}±0.073	2.60^{aabb}±0.043
	B	1.35^{bb}±0.054	1.44^{abb}±0.060	1.62^{aabb}±0.049	1.81^{aabb}±0.041	2.00^{aabb}±0.055	2.19^{aabb}±0.059	2.52^{aabb}±0.061
	C	1.45^{bb}±0.036	1.63^{aabb}±0.053	1.75^{aabb}±0.036	1.86^{aabb}±0.022	1.98^{aabb}±0.019	2.10^{aabb}±0.033	2.26^{aabb}±0.022
	D	1.20^{bb}±0.078	1.33^{aabb}±0.092	1.51^{aabb}±0.095	1.71^{aabb}±0.086	1.94^{aabb}±0.086	2.08^{aabb}±0.078	2.26^{aabb}±0.039
	E	2.51±0.157	2.42±0.193	2.56±0.075	2.65±0.107	2.69±0.069	2.64±0.095	2.72±0.054
Total $T_{4(n\ mol/l)}$	A	40.41^{bb}±3.216	46.54^{aabb}±3.239	50.98^{aabb}±3.412	53.73^{aabb}±3.439	60.57^{aabb}±2.928	72.97^{aabb}±4.370	88.62^{aabb}±3.515
	B	47.67^{bb}±2.249	53.55^{aabb}±1.557	56.68^{aabb}±1.007	60.66^{aabb}±0.817	61.70^{aabb}±0.504	64.37^{aabb}±0.711	92.86^{aa}±5.807
	C	51.41^{bb}±4.623	53.06^{bb}±5.042	56.09^{aabb}±5.200	59.24^{aabb}±4.848	61.83^{aabb}±4.556	67.50^{aabb}±3.858	77.34^{aabb}±2.084
	D	40.06^{bb}±4.602	48.17^{aabb}±6.020	52.01^{aabb}±5.061	55.21^{aabb}±3.682	63.96^{aabb}±4.259	70.58^{aabb}±3.941	81.03^{aabb}±1.872
	E	102.98±5.133	89.55±5.433	89.01±2.668	93.96±3.613	95.56±4.174	97.69±3.675	95.28±3.691

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column

Table 2: Alterations in Free T_4 (Mean ± SE) at various days of goitrous goats medications

Parameter	Groups (N=6)	Days of Observation						
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
Free T_4(p mol/l)	A	4.91^{bb}±0.694	5.43^{abb}±0.583	5.79^{aabb}±0.574	6.42^{aabb}±0.507	7.11^{aabb}±0.363	7.64^{aabb}±0.303	8.07^{aabb}±0.235
	B	4.85^{bb}±0.630	5.52^{abb}±0.609	6.06^{aabb}±0.583	6.62^{aabb}±0.501	7.12^{aabb}±0.386	7.48^{aabb}±0.354	8.22^{aabb}±0.246
	C	5.02^{bb}±0.420	5.36^{bb}±0.419	5.74^{aabb}±0.357	6.10^{aabb}±0.321	6.52^{aabb}±0.220	6.89^{aabb}±0.153	7.25^{aabb}±0.089
	D	5.43^{bb}±0.360	5.69^{bb}±0.333	5.86^{bb}±0.376	6.11^{aabb}±0.344	6.36^{aabb}±0.345	6.79^{aabb}±0.248	7.30^{aabb}±0.125
	E	11.08±1.529	10.14±0.553	10.79±0.765	9.61±0.661	9.88±0.482	9.40±0.609	9.54±0.493

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Table 3: Alterations in Free T_3 (Mean ± SE) at various days of goitrous goats medications

Parameter	Groups (N=6)	Days of Observation						
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
Free T_3(p mol/l)	A	1.59^{bb}±0.282	2.05^{aabb}±0.226	2.53^{aabb}±0.231	3.00^{aabb}±0.222	3.44^{aabb}±0.163	3.74^{aabb}±0.129	4.20^{aabb}±0.119
	B	1.55^{bb}±0.256	1.86^{abb}±0.210	2.39^{aabb}±0.222	2.73^{aabb}±0.205	3.05^{aabb}±0.182	3.35^{aabb}±0.109	3.58^{aabb}±0.101
	C	1.56^{bb}±0.221	1.85^{abb}±0.139	2.25^{aabb}±0.147	2.59^{aabb}±0.142	2.88^{aabb}±0.070	3.05^{aabb}±0.050	3.32^{aabb}±0.035
	D	1.69^{bb}±0.211	1.97^{abb}±0.176	2.25^{aabb}±0.168	2.49^{aabb}±0.147	2.85^{aabb}±0.116	3.12^{aabb}±0.072	3.40^{aabb}±0.046
	E	4.20±0.463	4.51±0.509	4.91±0.410	4.78±0.320	4.63±0.443	4.65±0.444	4.68±0.300

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Table 4: Alterations in TSH profile (Mean ± SE) at various days of goitrous goats medications

Parameter	Groups (N=6)	Days of Observation						
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
TSH(μ mol/L)	A	24.33^{bb}±2.021	21.63^{aabb}±1.797	19.16^{aabb}±1.546	16.83^{aabb}±1.255	14.56^{aabb}±1.045	12.37^{aabb}±0.548	10.57^{aa}±0.373
	B	24.17^{bb}±2.051	21.10^{aabb}±1.648	17.82^{aabb}±1.263	15.21^{aabb}±1.048	13.67^{aabb}±0.946	11.91^{aab}±0.463	10.50^{aa}±0.260
	C	23.22^{bb}±1.495	20.80^{aabb}±1.411	18.46^{aabb}±1.290	16.41^{aabb}±1.063	14.79^{aabb}±0.865	13.04^{aabb}±0.522	11.55^{aab}±0.118
	D	23.06^{bb}±1.532	20.32^{aabb}±1.307	17.62^{aabb}±1.193	15.24^{aabb}±0.961	13.38^{aabb}±0.553	12.20^{aabb}±0.285	11.08^{aa}±0.070
	E	10.87±1.090	10.45±0.298	10.48±0.663	10.57±0.605	10.51±0.576	10.48±0.417	10.21±0.339

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

treatment in goitre affected goats of group A, B, C and D were significantly low as compared to group E (Table 1,2,3). After treatment, these values progressively increased in all the treated groups and reached to almost normal level by day 120. The recovery was comparatively better in goats of group A and B receiving aqueous iodine and sodium thyroxine, respectively (Table 1).The level of free T_3 and T_4 in goitre affected goats of group A, B, C and D were low before start of treatment. However, these values gradually increased following treatment. The increase in free T_3 and T_4 was more appreciable in goats of group A and B which received aqueous iodine and thyroxine sodium, respectively (Table 2). Pre-treatment values of TSH in goitrous goats were significantly high which declined progressively and reached towards normalcy after 120 days of treatment. The decline in TSH was more appreciable in goats treated with aqueous iodine and thyroxine sodium (Table 4).

Pre-treatment value of serum glucose in clinically goitre affected goats was significantly low in comparison to healthy goats. Post medication, value of glucose was significantly low in comparison to healthy goats. However, following treatment, value of glucose was significantly increased in groups A, B and C in comparison to group D (Table 5).

Before treatment values of total cholesterol and total lipid in goitre affected goats were significantly high as compared to healthy control group. After 120 days of medication, these values almost returned to normal. However, changes were more appreciable in goats of group A and B (Table 5). During chemoprophylactic medication trial, there was no significant alteration in values of glucose, total cholesterol and total lipids in groups I, II, III, IV before and 15 months after chemoprophylactic trial (Table 6). However, in group V, glucose level reached and total cholesterol and total lipids concentration increased after 15 months (Table 6).

Comparative therapeutic evaluation revealed that there was variable regression in the size of enlarged thyroid gland in different treatment. However clinical signs like glistening of hairs, alertness in looking, and improvement in health status and regularization in estrous cycle in females were almost similar in different groups of treated goats. Treatment with aqueous iodine showed restoration to normalcy of maximum parameters followed by sodium thyroxine, Lugol's iodine and Potassium iodate treatment.

Table 5: Alterations in biochemical parameters in pre and post treated goitrous goats

S.No.	Parameters	Groups(N=6)	Pretreated goats(0 day)	Post treated goats(120 days)
1.	Serum Glucose(mg/dl)	A	46.67^{bb}± 4.43	89.17^{aabb}± 8.51
		B	39.67^{bb}± 2.03	81.33^{aabb}± 8.41
		C	42.67^{bb}± 2.17	64.67^{aa}± 6.32
		D	38.54^{bb}± 3.12	47.67^{aabb}±7.86
		E	69.50± 3.36	68.33± 3.32
2.	Total Serum Lipid(mg/dl)	A	915.34^{bb}± 50.12	412.84^{aabb}±35.24
		B	912.62^{bb}± 80.02	512.36^{aab}±62.48
		C	921.35^{bb}± 65.72	530.46^{aa}±45.34
		D	918.64^{bb}± 72.26	532.42^{aab}±56.78
		E	508.46± 30.84	522.68± 28.64
3.	Total Serum cholesterol(mg/dl)	A	291.25^{bb}± 16.38	109.82^{aabb}±7.73
		B	293.67^{bb}± 11.93	101.34^{aabb}±6.86
		C	290.54^{bb}± 13.25	132.56^{aabb}±8.98
		D	294.25^{bb}± 10.87	154.64^{aabb}±9.68
		E	116.17± 9.85	120.24± 12.36

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Table 6: Alterations in biochemical parameters in pre and post chemoprophylactic medication in goitrous goats

S.No.	Parameters	Groups (n=10)	Pre-chemophylactic medication (0 day)	Post-chemophylactic Medication (15 mth.)
1.	Serum Glucose(mg/dl)	I	82.17^{b}± 8.51	84.54^{bb}±7.32
		II	81.33^{b}± 8.41	82.34^{bb}±4.56
		III	80.67^{b}± 6.32	80.75^{bb}±3.24
		IV	79.24± 5.54	79.52^{bb}±4.56
		V	75.33± 3.32	47.67±7.86
2.	Total Serum Lipid(mg/dl)	I	518.84±35.24	516.42^{bb}±15.62
		II	520.36±62.48	518.56^{bb}±20.46
		III	525.46±45.34	524.21^{bb}±24.72
		IV	528.42±56.78	526.47^{bb}±21.56
		V	522.68± 28.64	712.46±32.65
3.	Total Serum cholesterol(mg/dl)	I	119.82±7.73	116.64^{bb}±3.43
		II	121.34±6.86	118.34^{bb}±6.86
		III	126.56±8.98	124.56^{bb}±8.98
		IV	124.64±9.68	123.64^{bb}±9.68
		V	116.17± 9.85	208.24± 12.36

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Post therapeutic medication of all the goats of groups A to E with aqueous iodine revealed regression of enlarged thyroid, disappearance of thyroid thrill, still birth/ abortion, improvement in milk production and fertility status of the goats. The look of the skin coat was shining. There was significant improvement in the thyroid hormone profile of all the treated groups of goats in respect to their zero day values (Table 7, 8, 9). Post medication values of Glucose, total cholesterol and total lipids also restored normalcy in comparisons to their pretreatment values.

Chemoprophylactic medication in groups I to IV goats was carried out for 15 months which revealed progressive improvement in the production and reproduction performance in the goats of groups I and II up to 12 months of the chemoprophylaxis and then finding of consistency in these parameters. The findings of thyroid hormones profile in group I and II did not defer significantly after 15 month compared to their respective zero day value. There has been significant lowering of thyroid hormone profile in group III and IV in comparisons to group I and II. Moreover, clinical recovery in respect to vital signs and symptoms was more in group I and II in comparisons to other groups. However significant reduction in profile of TT4 and TT3 and significant increase in TSH value was recorded in control group of goats after six month, in respect to

Table 7: Alterations in Total T_4 profile (Mean ± SE) at various days of goitrous goats with Aq. iodine medication

Parameter	Groups (N=10)	Days of Observation					
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
Total $T_{4(n\ mol/l)}$	A	46.57^{bb}±2.294	54.55^{aabb}±1.575	56.34^{aabb}±1.070	61.66^{aabb}±0.871	66.70^{aabb}±0.540	75.37^{aabb}±0.711	92.95^{aa}±5.870
	B	42.41^{bb}±3.216	48.54^{aabb}±3.293	51.88^{aabb}±3.421	60.71^{aabb}±3.493	65.54^{aabb}±2.982	73.95^{aabb}±4.370	91.92^{aa}±3.515
	C	41.06^{bb}±4.602	49.17^{aabb}±6.020	54.01^{aabb}±5.061	58.21^{aabb}±3.682	68.96^{aabb}±4.259	78.58^{aabb}±3.941	89.73^{aa}±1.872
	D	52.41^{bb}±4. 263	54.06^{bb}±5. 402	57.09^{abb}±5. 020	63.24^{aabb}±4. 488	69.83^{aabb}±4.565	79.50^{aabb}±3.758	93.34^{aa}±2.184
	E	102.98±5.133	89.55±5.433	89.01±2.668	93.96±3.613	95.56±4.174	97.69±3.675	95.28±3.691

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Table 8: Alterations in Total T_3 profile (Mean ± SE) at various days of goitrous goats with Aq. iodine medication

Parameter	Groups (N=10)	Days of Observation						
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
Total $T_{3(n\ mol/l)}$	A	1.38^{bb}±0.045	1.47^{abb}±0.063	1.66^{aabb}±0.094	1.83^{aabb}±0.014	2.10^{aabb}±0.055	2.29^{aabb}±0.095	2.62^{aabb}±0.016
	B	1.35^{bb}±0.062	1.63^{aabb}±0.058	1.92^{aabb}±0.034	2.15^{aabb}±0.056	2.34^{aabb}±0.062	2.52^{aabb}±0.073	2.64^{aabb}±0.043
	C	1.22^{bb}±0.078	1.38^{aabb}±0.092	1.57^{aabb}±0.095	1.78^{aabb}±0.086	2.08^{aabb}±0.086	2.28^{aabb}±0.078	2.60^{aabb}±0.039
	D	1.44^{bb}±0.036	1.61^{aabb}±0.053	1.73^{aabb}±0.036	1.84^{aabb}±0.022	1.98^{aabb}±0.019	2.40^{aabb}±0.033	2.66^{aabb}±0.022
	E	2.51±0.157	2.42±0.193	2.56±0.075	2.65±0.107	2.69±0.069	2.64±0.095	2.72±0.054

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

their zero day values and other groups six month value (Table 10).

Discussion

There was significant decrease in T_3 and T_4 and increase in the TSH levels of pretreated goats affected with goitre compared to normal healthy goats. Similar findings of T_3 and T_4 values were also recorded by Raheja *et al.* (1979), Mano *et al.* (1985), Reddy *et al.* (1996), Toda *et al*. (2001), Singh (2001), Guyot *et al*. (2007) in various species of clinical goitrous animals. Significant decrease in thyroid hormone in goitre affected goats could be due to severe deficiency of iodine. Takahashi *et al.* (2001) found lowered serum T_4 levels in calves with endemic goitre but T_3 levels were similar to those of healthy ones. However Doi *et al*. (1997) recorded low level of serum thyroxine (T_4) and significantly high level of triiodothyronine (T_3), resulting in significantly lower thyroxine/ triiodothyronine ratio in diffuse hypoplastic goitre (DHG). This ratio was significantly higher in colloid goitre (CG). In aqueous iodine treated goitrous goats, there was progressive increase in thyroxine and triiodothyronine value but T_3 increased earlier. However, its reverse was found in thyroxine sodium treated goitrous goats. After 120 days of treatment, almost similar values of triiodothyronine and thyroxine were recorded in both the treated groups.

The reduction in both T_4 and T_3 hormones was due to decreased bioavailability of iodine from different sources of intake. In addition to this even prevalency of various types of goitrogens which act as competitive antagonist of iodine traping by thyroid follicle, might be the contributory factor to reduced synthesis of thyroid hormone.

Synthesis and secretion of T_4 and T_3 are under control of TSH from the anterior lobe of the pituitary gland since TSH stimulates iodide transport from the blood into thyroid cells, oxidation of iodide to iodine, and iodine binding to tyrosine. Synthesis of thyroid hormones is regulated by the levels of circulating free T_4 and T_3 as a negative feedback mechanism. Drug may be stimulating hypothalamo-hypophyseal system to secrete more amount of TSH ultimately stimulating the thyroid gland. It may be true because of regulatory capacity of central nervous system (Singh and Malviya, 1978). TSH causes hyperplasia of the thyroid cells, and goitre, in an attempt to compensate for iodine deficiency by increasing the turnover in the gland (Hetzel, 1991). Panda and Kar (1997 and 1999) reported only serum

Table 9: Alterations in Total T_3 profile (Mean ± SE) at various days of goats with goitre with Aq. iodine medication

Parameter	Groups (N=10)	Days of Observation						
		0th Day	20th Day	40th Day	60th Day	80th Day	100th Day	120th Day
TSH (μ mol/L)	A	22.06^{bb}±1.532	20.32^{aabb}±1.307	16.62^{aabb}±1.193	14.24^{aabb}±0.961	13.18^{aabb}±0.553	12.02^{aabb}±0.285	10.44^{aa}±0.070
	B	24.33^{bb}±2.021	21.63^{aabb}±1.797	19.16^{aabb}±1.546	16.83^{aabb}±1.255	14.56^{aabb}±1.045	12.37^{aabb}±0.548	10.52^{aa}±0.373
	C	22.22^{bb}±1.495	20.80^{aabb}±1.411	18.46^{aabb}±1.290	16.41^{aabb}±1.063	14.29^{aabb}±0.865	12.04^{aabb}±0.522	10.55^{aa}±0.118
	D	24.07^{bb}±2.015	21.20^{aabb}±1.684	16.82^{aabb}±1.236	15.61^{aabb}±1.084	13.27^{aabb}±0.964	11.41^{aab}±0.436	10.61^{aa}±0.206
	E	10.87±1.090	10.45±0.298	10.48±0.663	10.57±0.605	10.51±0.576	10.48±0.417	10.21±0.339

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
Values with superscript 'b' differ significantly (P<0.05) and 'bb' differ significantly (P<0.01) in a column.

Table 10: Alterations in Thyroid hormone profile (Mean ± SE) at various months of Aq. Iodine treated post chemoprophylactic medication with Iodized salt, Iodized oil injection, Iodized mineral mixture& Lugols Iodine.

Parameter	Groups (N=10)	Months of observation					
		0th Month	3rd Month	6th Month	9th Month	12th Month	15th Month
Total $T_{4(n\ mol/l)}$	A	92.95±5.870	93.23±2.543	94.12^{bb}±1.564	95.04^{bb}±1.548	95.16^{bb}±1.68	95.23^{abb}±1.12
	B	91.92±3.515	92.56±3.234	93.34^{bb}±4.213	94.85^{bb}±2.321	94.96^{bb}±1.45	95.01^{abb}±1.42
	C	89.73±1.872	89.67± 1.678	91.86^{bb}±3.234	93.82^{bb}±4.215	93.96^{bb}±2.23	94.02 ^{abb}±2.13
	D	93.34±2.184	93.64±2.547	93.72^{bb}±4.234	94.81^{bb}±3.266	94.94^{bb}±2.46	94.98^{bb}±2.58
	E	95.28±3.691	88.26^{aa}±4.174	81.12^{aa}±3.613	72.01^{aa}±2.668	64.52^{aa}±2.83	62.62^{aa}±2.78
Total $T_{3(n\ mol/l)}$	A	2.62±0.016	2.64^{bb}±0.034	2.68^{bb}±0.025	2.71^{bb}±0.039	2.73^{bb}±0.024	2.74^{abb}±0.021
	B	2.64±0.043	2.65^{bb}±0.021	2.67^{bb}±0.036	2.70^{bb}±0.047	2.72^{bb}±0.032	2.73^{abb}±0.023
	C	2.60^{b}±0.039	2.63^{bb}±0.032	2.65^{bb}±0.078	2.68^{bb}±0.068	2.70^{bb}±0.024	2.71^{bb}±0.012
	D	2.66±0.022	2.68^{bb}±0.042	2.69^{bb}±0.086	2.70^{bb}±0.078	2.71^{bb}±0.031	2.71^{bb}±0.023
	E	2.72±0.054	2.15^{aa}±0.107	1.86^{aa}±0.075	1.58^{aa}±0.193	1.42^{aa}±0.123	1.32^{aa}±0.112
TSH(μ mol/L)	A	10.44±0.070	10.36^{bb}±0.213	10.30^{bb}±0.456	10.22^{bb}±0.634	10.12^{bb}±0.234	10.12^{abb}±0.321
	B	10.52±0.373	10.42^{bb}±0.352	10.36^{bb}±0.364	10.28^{bb}±0.758	10.20^{bb}±0.421	10.16^{abb}±0.231
	C	10.55±0.118	10.47^{bb}±0.452	10.39^{bb}±0.642	10.34^{bb}±0.643	10.30^{bb}±0.241	10.28^{bb}±0.213
	D	10.61±0.206	10.52^{bb}±0.362	10.44^{bb}±0.527	10.38^{bb}±0.537	10.31^{bb}±0.412	10.27^{bb}±0.231
	E	10.21±0.339	13.45^{aa}±0.298	15.48^{aa}±0.663	18.57^{aa}±0.605	18.78^{aa}±0.712	19.21^{aa}±0.361

Values with superscript 'a' differ significantly (P<0.05) and 'aa' differ significantly (P<0.01) in a row.
fer significantly (P<0.01) in a column.

T_4 increase with little or no effect on T_3 concentration, but in the present investigation both serum T_4 and T_3 were increased along with increased free T_4 and free T_3 concentrations following treatment but the increase in T_3 was proportionally less than increase in T_4 concentration. Disorders in the synthesis, storage and secretion of thyroid hormones provide the molecular basis of abnormalities in the thyroid growth or thyroid dishormonogenesis which results in congenital hypothyroidism (Vijender, 2003).

Hypoglycemia in goats with goitre could be due to poor intestinal glucose absorption and hypophagia (McDonald, 1980 and Ettinger, 1995). Kaneko (1997) mentioned that thyroxine improves the intestinal glucose absorption along with glucose turn over and these functions have been adequately reflected in the present study as well, after thyroxine therapy.

Total lipid concentration in goats with goitre was significantly increased as compared to normal healthy goats. Recio-Visedo *et al.* (1986) and Angelov *et al.* (1996) had also recorded similar findings but Tsuneyoshi *et al*. (1995) reported no change in lipid concentration in goitrous calves.

There was significant increase in pre treatment value of cholesterol in goitrous goats as compared to normal healthy goats as observed by Nasseri and Prasad (1987), Ramakrishna *et al.* (1994) and Kececi and Kocabatmaz (1994) also. In spontaneous or induced hypothyroidism of different animals and man high cholesterol has been reported (Lascelles and Setchell, 1959; Sreekumaran and Rajan (1978); Prasad *et al.* (1989); Sasikumar, (1999) and Singh *et al.* (2002b). Cholesterol synthesis is increased in the presence of excess or reduced thyroxine deficiency. These contradictory results have been attributed to decreased biliary excretion of cholesterol in hypothyroid animals causing increased cholesterol, despite reduced synthesis (Dickson, 1993). In addition to that, increased TSH in hypothyroidism could also account for hypercholesteremia (Asboc-Hansen,1958) and

decreased lipoprotein lipolysis, reduced hepatic utilization and augmented hepatic production contribute significantly to increased cholesterol in blood (Weinberg, 1987). Progressive significant reduction in serum cholesterol level was recorded in treated goitrous goats.

The total body fat content of the body is eventually decreased in animals that have a marked thyroid deficiency, and there is little evidence to support the concept of thyroid obesity. In hypothyroid state, triglyceride and cholesterol catabolism is decreased to a lesser extent than the synthesis of cholesterol. These changes result in a relative net increase in serum cholesterol and triglyceride value (Kaneko, 1997).

By the use of iodized oil, absorption into the blood occurs slowly so that most of it remains at the site for weeks or months. Once in the blood stream, the iodine can be taken up by the thyroid, excreted in the urine, and stored in the fatty tissue of the body (Buttfield and Hetzel, 1967; Dunn, 1987). Hetzel (1991), observed that iodized salt is used by all sections of community irrespective of social and economic status while Waghorn *et al*. (1992) found that ruminants, particularly dairy cows, may benefit from iodine supplementation after introduction of iodized salt.

Findings of iodized oil injection were supported by Potter *et al*., (1984), Clark (1998) and Clark *et al.* (1998). Also, Clark (1998) and Clark *et al.* (1998) reported that perinatal mortality of lamb was nullified and there was decrease in thyroid body weight ratio after the use of iodized oil injection as observed in present study as well.

There was least response of mineral mixture compared to others iodine agents. Travnicek *et al*. (2002) also reported that there was no significant impact on the T4 and T3 contents in the blood after use of mineral licks in cattle and sheep.

On the basis of recovery in clinical signs, biochemical and thyroid hormone profile, the aqueous iodine was found as best choice of therapeutic agent for endemic goitre in goats. Iodized oil injection was found best among the chemoprophylactic agent. However, iodized salt was found runner to chemoprophylactic agent with ease of its medication.

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Haemato biochemical alteration in enterotoxigenic *Escherichia coli* affected diarrhoeic calves in an organized dairy farm

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Abstract

The present study was undertaken on a total of 73 clinical cases of diarrhoea in calves of either sex, aged upto 45 days, in an organised dairy herd of IVRI, Izatnagar. A total of 18 diarrhoeic calves diagnosed for entertoxigenic *Escherichia coli* were taken to investigate the clinical and hemato-biochemical profiles. Six clinically normal healthy calves of the same herd were taken as control. The diarrhoeic calves appeared dull, depressed and lethargic with reduced appetite. The faeces were semisolid to watery with offensive odour, greenish to yellow white in colour and sometimes even blood stained. Mild to moderate dehydration was found to be a constant feature in diarrhoeic calves. Rectal temperature, heart and pulse rates were within the normal range. Analysis of blood and serum samples of the diarrhoeic calves revealed significant increases in PCV, TSP, serum A: G ratio, serum urea nitrogen, serum albumin, creatnine and potassium and significant decreases in serum glucose, sodium and chloride. As regards haemoglobin, serum SGPT, SGOT and globulin, no significant alteration were observed between the diarrhoeic calves and healthy control calves.

Keywords: Biochemical, Calf diarrhoea, E.coli, Enterotoxin, ETEC, Hematology.

Diarrhoea in calves is one of the major causes of economic loss to the cattle industry (Radostits *et al.* 2000). It is the leading cause of death in dairy heifer and beef calves aged less than four months with annual mortality rates of 6.6% and 5.6% respectively (USDA, 1996a and 1996b). In India, the economic importance has long been recognized. Losses to the tune of 45 per cent of the total losses have been attributed to this malady (Dhanda and Khera, 1957). It is one of the most common diseases reported in calves up to 3 months old (Svensson *et al*., 2003).

The clinical-biochemical alterations in diarrhoea are complex in nature comprising of serious imbalances of fluid, electrolyte and acid-base status threatening the life of the patient. The effect of diarrhoea on fluid, electrolyte and acid-base equilibrium depends on the type, duration and severity of the diarrhoea as well as the host response. Diarrhoea leads to excess loss of intestinal fluid resulting in severe dehydration (Tennant *et al.* 1972; Demigne *et al.* 1980)., electrolyte imbalances like hyponatraemia, hypochloremia, hyperkalaemia (Demigne *et al.* 1980; Constable *et al.* 1996), and acid-base imbalances like low blood pH, loss of bicarbonate and development of metabolic acidosis (Groutides and Michell, 1990a). Diarrhoeic calves also develop hypoglycaemia (Fettman*et al.* 1986) as the calves become depressed and anorectic, also due to reduced conversion of lactate to glucose in diarrhoeic claves (Tennant *et al.* 1972). Death of calves caused by diarrhoea is attributed to severe dehydration and metabolic acidosis (Groutides and Michell, 1990c).

In order to evolve most effective clinico-therapeutic measures for calf diarrhoea in Indian managemental conditions it is essential to define the influence of specific calf diarrhoea on full range of biochemical features. In present study, haemato-biochemical alterations in ETEC affected diarrhoeic calves were studied.

Materials and Methods

The present study was undertaken in calves at Cattle and Buffalo Farm, I.V.R.I, Izatnagar. The E. coli isolated from the diarrhoeic calves were characterised by using primer sequences, F-5'-CCGATACGCTGCCAATCAGT-3', R-5'-CGCAGACCGTAGGCCAGAT-3' (Chen and Griffiths, 1998) amplifying 884 bp product from USP A target gene. Those animals found positive by ETEC PCR were used for studying haemato-biochemical profile.

Haematological and Serum Biochemical Profile:

Approximately 0.5 ml of whole blood was collected in EDTA vials for estimation of Haemoglobin (Hb), packed cell volume (PCV) and seven ml of blood was collected in glass test tubes and processed for separation of serum for biochemical analysis. Determination of Hb and PCV were done within two hours of collection. However serum samples were stored at -20°C for further analysis. Haemoglobin concentration (g/dl) in the whole blood was estimated

by cyanomethemoglobin method (Vankampen and zinglstra, 1961**).** Packed cell volume (PCV %) in whole blood was determined by capillary microhaematocrit method (Coles, 1980).

Serum samples stored in deep freeze at (–) 20 ^{0}C were used for estimation of the Blood urea nitrogen (BUN) by diacetylmonosamine method (Marsh *et a*l., 1965), serum creatinine by Jaffe's alkaline picrate method (Marsh *et a*l., 1965), serum alanine amino transferase (ALT) by Reitman and Frankel method (1957), serum aspartate amino transferase (AST) by Reitman and Frankel method (1957), serum total protein (TP) by modified Biuret (Varley *et al.*, 1980), albumin by Dumas method (Varley*et al.*, 1980), serum globulin was estimated by subtracting albumin from total protein, serum A/G ratio was estimated by dividing albumin value with globulin value. Sodium and potassium (Trinder, 1951; Miller, 1984), serum glucose – O'toludine method (Hultman, 1959) and serum chloride - thiocyanate method (Schoenfeld and Lewellen, 1964) were also estimated**.**

Result and Discussion

A total number of 150 calves were borned during the study period. Out of which 73 calves showed signs of diarrhoea. In which 65 were found to be infected with *E. coli* infection indicating 43.33% morbidity due to *E.coli* infection among diarrhoeic calves. 45 calves were found to be affected with ETEC from which 18 calves were used for haemato-biochemical analysis. Table 1.

The haemato-biochemical changes in ETEC affected diarrhoeic calves and control calves are shown in table. No significant change in haemoglobin values was observed in diarrhoeic calves but highly significant increases in the value of PCV were recorded in all the 18 clinical cases of calf diarrhoea (43.16±0.94) as compared to the healthy control calves (35.66± 0.49). Similarly Tenant *et al.* (1968) observed a higher value of PCV in scouring calves as compared to normal value (39.03±0.88%). Grove-White and White (1999) observed a significantly increased value of PCV in diarrhoeic calves 48.4 $\pm$10.3 % as compared to the non-diarrhoeic control calves 33.6 $\pm$ 4.3 %. Increase in PCV value in diarrhoeic calves was apparently due to haemo-concentration associated with dehydration and hypovolaemia.

A significant increase in TSP (7.82±0.13) and serum albumin (3.78±0.13) in diarrhoeic calves was observed apparently due to associated dehydration. Tenant *et al.,* (1972), Constable *et al.* (1996) and walker *et al.* (1998) observed the significant increase in PCV and TSP both, which indicates hypovolaemia, haemoconcentration and reduced glomercular filtration rate. A significant increase in A: G ratio (0.94±0.06) was observed in diarrhoeic calves suggesting either increase in albumin, it may be due to dehydration (Kaneko, 1989), or decrease in globulins. No significant changes in SGPT and SGOT activities were observed, indicating the absence of marked hepatic damage. These results are in consistent with the findings of Lewis *et al.,* (1975).

The marked reduction in the value of serum glucose in diarrhoeic calves (44.67±1.80 mg/dl) as compared to healthy control calves (62.47± mg/dl) was observed during the study, which is in close agreement with Lewis *et al.* (1975) observed hypoglycaemia with glucose concentration below 40 mg/dl of plasma in acute severe diarrhoea, Grove-White (1999) observed a reduced glucose level of 4.87(1.9) mmol/L in diarrhoeic calves as compared to the non-diarrhoeic control calves whose mean (SD) level of glucose was 5.8(0.88) mol/L. The factors responsible for development of hypoglycaemia in calves with diarrhoea are unknown. Morris *et al.* (1985) suggested that severe hypoglycaemia may occur as a result of reduced rate of conversion of lactic acid to glucose. Other factors

Table 1. Haemato- biochemical changes in ETEC affected diarrhoeic calves

Parameters	Healthy control calves (n = 6)	Diarrhoeic calves (n= 18)
Hb(g%)	12.35±0.02	12.36±0.09
PCV (%)	35.66± 0.49	43.16±0.94**
Total protein (g/dl)	7.14±0.08	7.82±0.13**
Albumin (g/dl)	3.15±0.04	3.78±0.13**
Globulin (g/dl)	3.99±0.11	4.04±0.15
A:G ratio	0.79±0.03	0.94±0.06*
Glucose (mg/dl)	76.33±2.45	40.30±1.93**
Creatinine (mg/dl)	0.66±0.04	1.66±0.05**
Urea nitrogen (mg/dl)	20.07±0.95	26.75±0.90**
Sodium (mmol/L)	140.91±1.51	110.24±2.33**
Potassium (mmol/L)	4.2±0.09	4.99±0.11**
Chloride (mmol/L)	101.91±1.58	94.95±1.61**
SGPT	39±1.56	39±1.2
SGOT	15±0.98	15±1.13

* P<0.05 -Significant, ** P<0.01—Highly Significant

involved in causing hypoglycaemia in diarrhoeic calves may include anorexia, decreased intestinal absorption of glucose (Bywater *et al.*1969; Bywater, 1977), a low level of glucose reserves at this age (Shelly, 1969) and alternation in tissue metabolism caused by decreased blood flow and oxygenation associated with hypovelemic shock (Tenant *et al.,* 1972) which has been invariably present in hypoglycaemic diarrhoeic calves.

A significant decrease in the level of serum sodium in diarrhoeic calves (115.50±1.27 mmol/L was recorded against the normal values of healthy control (127.20±1.28 mmol/L). Our findings are in agreement with the findings of Sridhar *et al.,* 1988; Maach *et al.,* 1992; Aly *et al.,* 1996 and Grove-White and White, 1999). Hyponatraemia occurs as a result of excessive secretion of the Na^+ ions by intestinal villus cells which are lost through the intestinal tract particularly in enterotoxigenic *E.coli* induced diarrhoea (Radostits *et al.,* 2000). Hirschhorn *et al.* (1991) observed that most of the diarrhoea causing microorganisms disrupts intestinal function and dehydrate the body either by increasing the chloride-secreting activity of the crypt cell or impair the absorption of sodium by the villus cells or both. Then the fluid that is normally returned to the blood across the intestinal wall is lost in the watery stool.

A significant increase in serum potassium value (4.99±0.11mmol/L) was observed in diarrhoeic calves in the present study. Similar findings are also reported by Sridhar *et al.* (1988) and Grove-White. (1999). Hyperkalaemia in experimentally induced diarrhoea has been observed by Roy *et al.* (1984), Groutides and Michell (1990a), Michell *et al.* (1992) Constable *et al.* (1996) and Walker *et al.* (1998). Hyperkalaemia may be due to increased K retention by kidney and also due to cellular damage (Fisher, 1971).

A reduced value of serum chloride was observed in diarrhoeic calves (97.40±0.68 mmol/L) as compared with the normal value of healthy control calves (101.96±0.51mmol/L. This is in agreement with the report of Hartmann *et al.,* 1983, Maach *et al.,* 1992 and Aly *et al.,* 1996. However, the present findings are contradictory to the reports of some previous studies in which hyperchloraemia have been observed in diarrhoeuc calves (Hervay Magdolna, 1976 and Sridhar *et al.,* 1988). Hypochloraemia is reported to occur as a result of prolonged increased loss of Cl^- ions in the intestinal tract during diarrhoea (McSherry and Grinyer, 1954; radiosits *et al.,* 2000), and failure of gastric H^+ and Cl^- ions to be reabsorbed by the villi of small intestine (Radostits *et al.,* 2000).

A moderate increase in serum urea nitrogen in diarrhoeic calves (26.37±0.87 mg/dl) was recorded as compared to the value of healthy control (19.52±1.01 mg/dl) and increased serum creatinine value was also recorded in diarrhoeic calves (1.45±0.05 mg/dl) against the value of healthy control calves (0.96±0.03 mg/dl). An increase in both the parameters was also observed by many workers in diarrhoeic calves (Groutide and Michell 1990a, Michell *et al.,* 1992, Constable *et al.,* 1996, Walker *et al.,* 1998 and Grove-White 1999). Tenant *et al.,* (1972) observed a significant increase in BUN of 50.1±18.5 mg/dl in calves with acute enteric infection Grove-White and White (1999) also recorded a significant increase in urea level as 13.9$\pm$7.1 mmol/L in diarrhoeic calves against the value of non-diarrhoeic control calves, 3.3$\pm$0.8 mmol/L. Similarly, a significant increase in creatinine level of 221 $\pm$129.5 mmol/L was also observed in diarrhoeic calves as compared to the value of non-diarrhoeic control calves, 101$\pm$13.7 mmol/L. The significant increase in serum urea nitrogen and creatinine in the present study is attributable to dehydration as a result of diarrhea. The hypovolemia to dehydration results in concentration of the plasma solutes with proportionate increases in both the parameters. However, such cases of pre-renal azotaemia need to be differentiated from other conditions including decreased peripheral vascular perfusion. Further, in cases of hypovolemia impaired excretion of urea and creatinine may occur secondary to reduced renal blood flow and GFR (glomerular filtration rate). Tenant *et al.* (1972) demonstrated varying degrees of renal in sufficiency in calves with severe diarrhoea. They believed that renal insufficiency was caused primarily by decreased blood flow (pre-renal azotemia) commonly observed by many workers in diarrhoeic calves (Groutides and Michell, 1990a and Bouda et al. (1997). Haemato- biochemical changes in ETEC affected diarrhoeic calves recorded in present study may be useful for treatment of calf diarrhea as it is the most common cause in neonatal calves.

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Electron microscopy based detection of PPR virus in goat and its confirmation by sandwich-ELISA and RT-PCR

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Abstract

In the present study four nasal swab samples from naturally infected goats suspected for Peste des petits ruminant were processed and examined by transmission electron microscopy (TEM). The detailed examination of the processed samples revealed the presence of pleomorphic virion typically of Paramyxoviridae family (mostly circular and elliptical in shape with diameter of intact particles ranging from 140 to 380 nm approximately with 6 to 14.5 nm thickness of the envelopes) in two out of four samples. Further the presence of virus in swab samples were confirmed by sandwich ELISA and RT-PCR, (using F1/F2 and NP3/NP4 sets of primers). Although both the samples positive in TEM were shown positive by sandwich ELISA and NP3/NP4 primer based RT-PCR but F1/F2 based RT-PCR failed to produce desirable amplicon of 372 bp in any of the swab samples shown positive by TEM, sandwich ELISA and NP3/NP4 primer based RT-PCR.

Keywords: Goat, Peste des petits ruminant (PPR), RT-PCR, Sandwich ELISA,Transmission electron microscopy (TEM),

Ever since the publication of first electron micrograph of poxvirus in 1938, transmissible electron microscopy (TEM) had made a major contribution to virology from discovery to diagnosis. However, with the passage of time the use of TEM for diagnosis had gradually decreased and replaced by more sensitive and specific immunological methods like PCR, ELISA, VNT, CFT etc. and same was the case with Peste des petits ruminant (PPR) Virus which causes an acute and highly fatal disease in small ruminants. The PPR virus belongs to genus *Morbillivirus* of family Paramyxoviridae (Gibbs *et al.,* 1979) and is related to Canine distemper, Rinderpest and Measles virus (Van mol *et al.,* 1995). Earlier electron micrograph of PPR virus particle by transmissible electron microscopy technique was studied by Bourdin and Laurent-Vautier, (1967). Whereas Durojaiye *et al.* (1985) were among the first to study the ultra structure of PPR virus by using TEM. Loney *et al.* (2009) demonstrated the ultra structure and genome packing of Paramyxovirus using cryo-electron tomography of Sendai virus a prototype of paramyxovirus. Zahur *et al.* (2009), in his studies in Pakisthan isolate PPR virus in vero cell line and identified it though cytopathic effect, Ic-ELISA and transmission electron microscopy.

Despite of these encouraging reports, TEM has not been yet used widely in diagnosis of PPR virus infection. This paper demonstrates the practical use of TEM for the detection and identification of PPR virus directly from clinical specimens.

Material and Methods

A total of four nasal swab samples from naturally infected goats showing symptoms and lesions suggestive of PPR were collected by visiting field outbreak in Kathua districts of Jammu and Kashmir State during April 2010 and stored at -20 ^{0}C till use.

Detection of Virus by TEM

Stored samples were thawed at room temperature by suspending in 0.5 ml of PBS (pH 7.4), samples were vortexed and squeezed along the wall of the tube to drain out all the contents of the swab, suspension thus obtained was then filtered through a millipore disposable syringe filter (0.22µm). The filtrate thus obtained was then centrifuged at 10,000 rpm for 15 minutes at 4-7^{0}C and about 100 µl of the supernatant was transferred to 200 µl micro centrifuge tube and the rest was stored at -20^{0}C for further processing.

Of the 100 µl, about 3µl of sample was pipetted out on a paraffin wax strip. Sample was then loaded in a carbon coated copper grid (300mm) by floating it on the wax strip for 1 minute. Negative staining using 2%

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urinyl acetate was performed as per method suggested by Brenner and Horne (1959). Specimens (Grid) was mounted in a dedicated holder and analyzed using a TEM (JEM 1011) with a BioTwin lens configuration and a Lab6-filament operating at an acceleration voltage of 80 kV under different magnifications.

Detection of virus by Sandwich ELISA

PPR sandwich-ELISA kit for PPRV antigen detection along with the user manual was obtained from Rinderpest Laboratory, Division of Virology, IVRI, Mukteswar. The test was performed as per the method suggested by Singh *et al*. (2004).

Detection of virus by RT-PCR

For RT-PCR, RNA from swab samples was isolated by GeNei™ TRIsoln as per procedure mentioned by the manufacturer. Purity of RNA was judged on the basis of optical density ratio at 260:280 nm. The samples with acceptable purity (*i.e.* ratio 1.7-2.0) were quantified by using the formula suggested by Sambrook *et al*. (1989) and Manchester, (1996).

RNA with acceptable purity was then amplified by using GeNei™ One Step AMV RT-PCR Kit. Mastermix was prepared as per manufacture instruction. PCR amplification was carried out by using F gene-specific primers F1 and F2 (Forsyth and Barett, 1995) and N gene specific primers NP3 and NP4 (Couacy Hymann *et al.* 2002), amplifying 372 bp and 351 bp products from the region between 777 and 1148 of F gene and 1232 to 1583 of N gene of PPR virus respectively. Reaction master mix I contain; RNasin, GeNei™ 2X RT PCR reaction mix, GeNei™ RT-PCR enzyme mix and primers with final concentration of 0.6 µM was prepared in separate 0.2 ml tube. Whereas master mix II containing template RNA was taken in separate 0.2 ml PCR tubes and heated at 65°C for 5 minutes in water bath to denature the template RNA and then chilled on ice before adding it to the Master Mix I. These PCR tubes were then placed in thermocycler and reverse transcription was done at 50°C for 30 min followed by 35 PCR cycles. The reaction conditions for F1/F2 primers and NP3/NP4 primers were kept similar to that described by Forsyth and Barett (1995) and Couacy- Hymann *et al.* (2002) for respective primers.

Ten microliters of the PCR products were resolved on a 2% agarose gel stained with 0.5 µl/ml ethidium bromide and electrophoresed at 75V for 30 min in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Alphaimager, Alphainnotech, Taiwan).

Statistical Analysis

The sensitivity, specificity and overall agreement between different tests used for detection of PPR were analyzed by epidemiological methods used by Samad *et al.* (1994). In addition, Kappa statistics (κ) were analyzed as per the method suggested by Thrusfield (1995).

Results and Discussion

The present study demonstrates the presence of PPR virus particle in suspected goat nasal swab sample by transmission electron microscopy. The detailed examination of samples by TEM under different magnification revealed the presence of pleomorphic virion typically of Paramyxoviridae family mostly circular and elliptical in shape with diameter of intact particles ranging from 140 to 380 nm approximately and the thickness of the envelopes were 6 to 14.5 nm in two out of four samples.

Our results are in line with results documented by Zahur *et al.* (2009), who in their studies in Pakistan isolated PPR virus in vero cell line and identified it though cytopathic effect, Ic-ELISA and TEM.

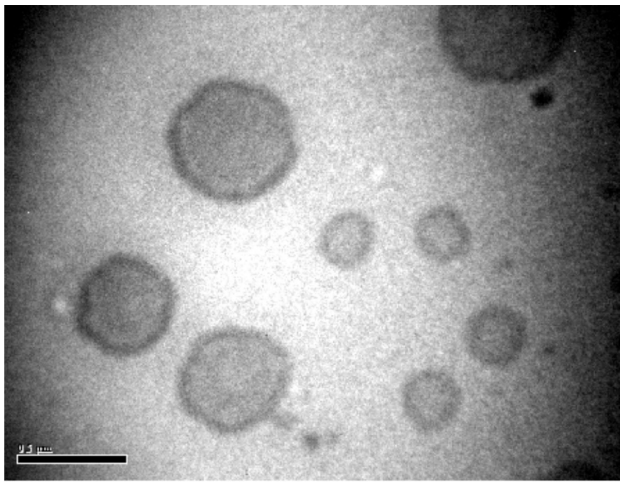

Fig.1: Electron Micrograph of PPR Virus by negative staining. Total magnification: X 66,300; Exposure time 3.18 sec.

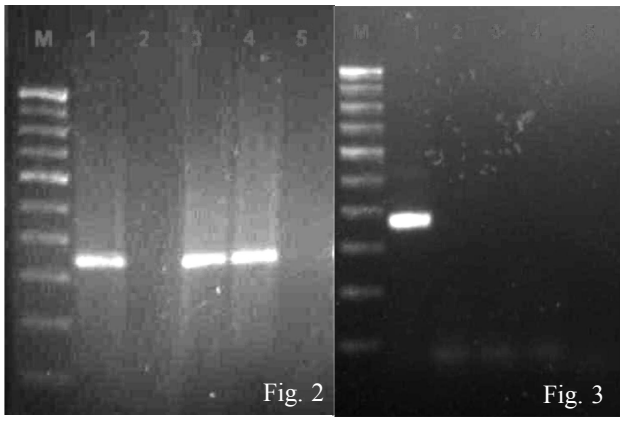

Fig. 2: Agrose gel electrophoresis of PPRV amplified by NP3/NP4 primer showing amplicon size of 351bp.
M- 100 bp marker, 1- positive control, 2-5 samples

Fig. 3 Agrose gel electrophoresis of PPRV amplified by F1/F2 primer showing amplicon size of 372bp.
M- 100 bp marker, 1- positive control, 2-5 samples

Structurally the viral nucleocapsid and particles (Fig. 1) were similar to those depicted by Durojaiye *et al.* (1985) by negative staining technique and reported that the virus particle was found to be pleomorphic with diameter of intact particles varying between 130-390 nm and thickness of viral envelope ranges between 8.5-14.5 nm approximately.

The present results are also in agreement with the results of Loney *et al.* (2009), who demonstrated the ultra structure and genome packing of Paramyxovirus using cryo-Electron tomography of Sendai virus a prototype of paramyxovirus and reported that the virons are highly visible in size, ranging approximately from 110-540 nm in diameter whereas envelope glycoproteins are densely packed on viron surface, while nucleocapsids are clearly resolved in the viron interior.

As far as we could determine it was the first time in India when TEM was used for detection of PPR virus particles in samples collected directly from naturally infected goats. All the studies documented earlier were either conducted on virus isolated from cell culture or from virus isolated from experimentally infected goats.

Result obtained by TEM was confirmed by sandwich ELISA and RT-PCR, (using F1/F2 and NP3/NP4 sets of primers). The comparison of the results revealed that the samples positive in TEM were shown positive by sandwich ELISA and NP3/NP4 primer based RT-PCR (Fig. 2) whereas F1/F2 based RT-PCR (Fig. 3) failed to produce desirable amplicon of 372 bp in any of the swab samples shown positive by TEM, sandwich ELISA and NP3/NP4 primer based RT-PCR.

When the results of TEM was compared with sandwich ELISA and NP3/NP4 primer based RT-PCR keeping them as gold standard, the sensitivity and specificity of TEM was observed 100% as all samples positive by NP3/NP4 primer based RT-PCR and sandwich ELISA were shown positive by TEM with overall agreement of 100% and kappa value of 1 suggesting almost perfect agreement between the different tests. Whereas on the other hand when results of TEM was compared with F1/F2 primer based RT-PCR (Gold Standard) the overall agreement between the two test was 50% with kappa value of 0 suggesting a poor agreement between the two tests.

Although these immunologic and molecular diagnostic tests have almost unlimited throughput, the high specificity of these assays may result in failure to identify etiologic agents. Our arguments was supported by the studies conducted and documented by Hammond *et al.* (1984) and Jiang *et al.* (1996) who observed that even when an immunologic and molecular diagnostic test was appropriate for the etiologic agent, the sensitivity may only equal that of electron microscopy. Further, mutations in the primers target region on virus genome may negate the effectiveness of primers and also the nucleic acid amplification techniques like PCR was not able to identify subviral components such as empty virions, which may be produced late in an infection. Our observations and arguments was supported by the studies conducted and documented by Ando *et al.* (1995), Vinje *et al.* (2000) and Green *et al.* (2002), who, suggest that the practical level of sensitivity of molecular and serological test does not always exceed that of electron microscopy.

The present study demonstrates the practical use of TEM in detection of PPR virus infection. The results suggested that it may be applied as a frontline method and can be coordinated to run in parallel with other diagnostic techniques for diagnosis of viral infection.

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In vitro antibacterial activity in various extracts of seeds of *Embelia ribes, Butea frondosa* and *Vernonia anthelmintica*

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Abstract

Antibacterial activity in various extracts of seeds of *Embelia ribes, Butea frondosa* and *Vernonia anthelmintica* and oil of *Vernonia anthelmintica* against common pathogenic bacteria *Staphylococcus aureus*, *E. coli*, *Pasteurella multocida*, *Salmonella enterica enterica* and *Shigella flexneri* was evaluated. Methanol, hexane and aqueous-methanol extracts of *E. ribes* and oil of *V. anthelmintica* revealed antibacterial activity against all the five bacteria. Methanol extract of *V. anthelmintica* had antibacterial activity against all except *E. coli*. Antibacterial activity in oil of *V. anthelmintica* was comparable to most of the tested standard antibiotics.

Keywords: Antibacterial activity, *Butea frondosa, Embelia ribes* and *Vernonia anthelmintica*

Antimicrobials are used in human and animal practice against diseases. Resistance against antimicrobials is extending, worldwide problem (Vaananen *et al*., 2006; WHO, 2000) which threatens successful treatment of several infections and risks the further spread of resistant organisms to other patients and to the community.

Plants produce a vast and diverse assortment of organic compounds. The substances like polyphenols, have antioxidant, anti-mutagenic, anti-carcinogenic, anti-inflammatory and antimicrobial effects that might potentially be beneficial in preventing diseases and protecting the stability of the genome (Ferguson, 2001).

The present paper reports antibacterial activity in various extracts of seeds of *Embelia ribes*, *Butea frondosa* and *Vernonia anthelmintica* and oil of *Vernonia anthelmintica* against five common pathogenic bacteria.

Materials and Methods

Seeds of *B. frondosa*, *E. ribes* and *V. anthelmintica* were collected locally and were identified through Medicinal Research and Development Centre of the University and Central Institute for Medicinal and Aromatic Plants, Nagla, Udham Singh Nagar. After shade drying and grinding, the powder was stored properly in brown coloured bottles at room temperature till further use.

Embelia methanol (EM), *Embelia* hexane (EH1 and EH2), *Embelia* aqueous methanol (EMA), *Butea* methanol (BM), *Butea* ether (BE), *Butea* aqueous methanol (BMA), *Vernonia* methanol (VM), *Vernonia* hexane (VH) and *Vernonia* chloroform (VC) extracts were prepared and vaccum evaporated at 42-45^{0}C and residues were kept in screw capped vials at 4^{0}C for further use. Dried *V. anthelmintica* seeds were hydro distilled using Clevenger apparatus and distilled oil was dried over anhydrous sodium sulfate.

The yield of EMA, EM, EH1 and EH2 extracts were 3.35, 5.06, 3.24 and 2.76%, of BMA, BM and BE extracts 16.66, 16.14 and 20.15% and of VM, VC and VH extracts 8.17, 7.38 and 10.29%, respectively. The yield of essential oil of *V. anthelmintica* was only 0.2%.

Standard pure cultures of *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 443), *Pasteurella multocida* (MTCC 1148), *Salmonella enterica enterica* (MTCC 3255) and *Shigella flexneri* (MTCC 1457) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India, as Microbial Type Culture Collection (MTCC) and maintained in the laboratory by regular sub culturing on to nutrient agar slant.

The antibacterial activity of all the extracts was screened by agar cup method (Cruickshank *et al.,* 1975). Dilutions of extracts were prepared in distilled water with tween-20 to obtain 100 mg/ml concentration of the extract residue. Subsequently 100 µl of each extract was poured into different wells of the plates. These plates were incubated at 37°C. After 18-24 hr, plates were examined for the formation of clear zone around the wells, if any.

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Minimum inhibitory concentration (MIC) of the extracts was determined by tube dilution method (Robert and Scott, 1966) with slight modifications. For it, serial dilutions of the extracts were prepared from 500 mg/ml dilution with the help of tween-20.

Confirmation of MIC was done by sub culturing method (Rathchandi *et al*., 2001) with some modifications. On the back side of the sterile petri plate, 11 divisions were marked. Content from a tube was streaked over a division of the nutrient agar petri plate and repeated it for all the 11 tubes over 11 divisions. The plate was incubated at 37^0C and examined after 18-24 hr. The lowest concentration of extract preventing the growth on the plate was taken as MIC for that extract. It also provided data regarding bacteriocidal and bacteriostatic property. Effectiveness of extracts was determined by means of sensitivity test, i.e. disc diffusion method (Blair *et al.,* 1974 and Singh *et al.,* 2005).

Antibacterial activity of extracts was compared with standard antibiotics (Vidyasagar *et al.,* 2002) and percent inhibition was calculated with respect to the clear zones of the standard antibiotics.

$$\% \text{ inhibition} = \frac{\text{AIC-AIT}_x}{\text{AIC}} \times 100$$

Where AIC = Area of inhibition of standard antibiotic, AIT_x = Area of inhibition of extract

Results

Methanol extract of *V. anthelmintica* showed antibacterial activity against *S. aureus*, *P. multocida*, *S. enterica enterica* and *S. flexneri* while its chloroform and hexane extracts didn't show any activity. Hexane, methanol and aqueous-methanol extracts of *E. ribes* and oil of *V. anthelmintica* revealed antibacterial activity against all the five bacteria tested. Extracts of *B. frondosa* showed no antibacterial activity.

MIC of methanol extract of *V. anthelmintica* was lowest (7.81 mg/ml) against *S. aureus* and highest for *S. enterica enterica* (62.5 mg/ml) with no activity against *E. coli* (Table 1). MIC of methanol extract of *E. ribes* was lowest for *S. aureus* and maximum for *P. multocida* and *S. enterica enterica*. Its aqueous-methanol extract revealed MIC 62.5 mg/ml against *S. aureus* and *S. flexneri* (Table 1). *V. anthelmintica* oil exhibited lowest MIC for *S. aureus* and highest for *E. coli* (Table 1).

Antibacterial activity of all the extracts and essential oil that were found positive for activity tested were also observed to be bactericidal in nature. This is because the growth was found completely inhibited on divisions having MIC of extract on agar plate and full growth was observed on divisions having concentration higher than MIC.

The inhibitory zones made by methanol extract of *V. anthelmintica* were almost similar for *S. aureus*, *S. enterica enterica* and *S. flexneri* while zone made for *P. multocida* was somewhat higher. Inhibitory zones formed by methanol extract of *E. ribes* were different for different bacteria tested and the value was highest (14.00±1.73) against *S. aureus* and lowest (6.67±.88) for *E. coli*. Aqueous methanol extract of this plant exhibited almost similar zone of inhibition against all the bacteria and the values ranged between 9 to 10 mm (Table 1). Zone of inhibitions noticed in hexane-II extract residue of this plant were similar for *S. aureus*, *S. enterica enterica* and *S. flexneri* whereas its activity was higher against *P. multocida* and lower against *E. coli*.

Inhibitory zones recorded in essential oil of *V. anthelmintica* were higher than those observed by the extracts and largest zone of this inhibition was for *S. aureus*, followed by *S. flexneri*. Inhibition zones against *P. multocida* and *S. enterica enterica* were almost similar and smallest for *E. coli* (Table 1). Ciprofloxacin revealed highest inhibitory zone against all the tested bacteria except *P. multocida* against which gentamicin revealed highest inhibitory zone (Table 1). Against *S. aureus* amikacin revealed least inhibitory zone (Table 1).

Comparative study between inhibitory zones formed by various extracts and oils and standard antibiotics against different bacteria showed that *Vernonia* oil was more effective than other extracts (Table 2).

Discussion

Various extracts of *V. anthelmintica* and *E. ribes* showed inhibitory activity against various bacterial cultures tested. None of the extracts of *B. frondosa* showed efficacy against any bacteria probably due to absence of saponins in all its extracts. Birk and Petri

Table 1. MIC of extract residues (mg/ml) and oil of *V. anthelmintica* (µl) and diameter of inhibitory zones made by extract residues and standard antibiotics (in mm)

Extract residue/ Antibiotic	*S. aureus*	*E. coli*	*P. multocida*	*S. enterica enterica*	*S. flexneri*
Vernonia Methanol	10.3±0.33(7.81)	Nil(0.0)	18.3±0.88(31.25)	11.0±0.58(62.50)	11.7±0.88(31.25)
Embelia Methanol	14.0±1.7(7.81)	6.7±0.88(62.50)	12.3±0.8(125.0)	8.3±0.88(125.0)	9.7±1.2(62.50)
Embelia Aqueous Methanol	9.0±0.58(62.50)	10.0±0.58(31.25)	10.0±0.58(31.25)	9.7±1.2(31.25)	9.0±1.5(62.50)
Embelia Hexane-I	9.7±1.2(15.63)	8.0±0.58(31.25)	13.3±0.88(15.63)	8.7±1.20(15.63)	8.7±0.88(15.63)
Embelia Hexane-II	9.3±0.88(31.25)	7.0±0.58(31.25)	12.7±0.88(62.50)	9.7±1.8(31.25)	9.0±0.58(125.0)
Vernonia Oil	30.0±0.58(0.10)	17.3±0.89(12.50)	21.0±0.58(1.56)	21.3±0.33(6.25)	24.7±0.33(6.25)
Amikacin	9.7±1.2	20.0±0.58	14.7±1.5	23.6±0.88	21.0±0.58
Ciprofloxacin	35.3±0.67	33.0±0.58	21.0±0.58	42.0±0.58	35.0±0.58
Ampicillin	19.3±0.33	20.0±0.58	21.0±0.0	25.0±0.58	21.3±0.88
Gentamicin	23.0±0.58	18.3±0.33	23.0±0.58	22.0±0.58	22.0±0.0
Tetracycline	12.3±0.88	24.3±1.2	11.0±0.58	12.0±0.58	14.0±1.2

Figures in parentheses indicate MIC

Table 2. Growth inhibition (%) of different bacterial strains for different extracts

Microorganisms	Standard antibiotics				
	Amikacin	Ciprofloxacin	Ampicillin	Gentamicin	Tetracycline
Methanol extract of *E. ribes*					
S. aureus	-44.83	70.75	46.55	55.07	16.22
E. coli	66.67	79.80	66.67	63.64	72.60
S. enterica enterica	64.79	80.16	66.67	62.12	30.56
S. flexneri	53.97	76.19	60.94	62.12	40.48
P. multocida	15.91	41.27	41.27	46.38	-12.12
Aqueous methanol extract of *E. ribes*					
S. aureus	6.90	74.53	53.45	60.87	27.03
E. coli	55.00	72.73	55.00	50.91	63.01
S. enterica enterica	59.15	76.98	61.33	56.06	19.44
S. flexneri	57.14	74.29	57.81	59.09	35.71
P. multocida	31.81	52.38	52.38	56.52	9.09
Hexane-I extract of *E. ribes*					
S. aureus	0.00	72.64	50.00	57.97	21.62
E. coli	60.00	75.76	60.00	56.36	67.12
S. enterica enterica	63.38	79.37	65.33	60.61	27.78
S. flexneri	58.73	75.24	59.38	60.61	38.10
P. multocida	9.09	36.51	36.51	42.03	-21.21
Hexane-II extract of *E. ribes*					
S. aureus	3.45	73.58	51.72	59.42	24.32
E. coli	65.00	78.79	65.00	61.82	71.23
S. enterica enterica	59.15	76.98	61.33	56.06	19.44
S. flexneri	57.14	74.29	57.81	59.09	35.71
P. multocida	13.64	39.68	39.68	44.93	-15.15
Methanol extract of *V. anthelmintica*					
S. aureus	-6.90	70.75	46.55	55.07	16.22
E. coli	100.00	100.00	100.00	100.00	100.00
S. enterica enterica	53.52	73.81	56.00	50.00	8.33
S. flexneri	44.44	66.67	45.31	46.97	16.67
P. multocida	-25.00	12.70	12.70	20.29	-66.67
***Vernonia* oil**					
S. aureus	-210.35	15.09	-55.17	-30.43	-143.24
E. coli	13.33	47.47	13.33	5.45	28.77
S. enterica enterica	11.27	50.00	16.00	4.55	-75.00
S. flexneri	-1.59	39.05	0.00	3.03	-52.38
P. multocida	-68.18	-17.46	-17.46	-7.25	-124.24

(1980) stated that saponins combine with sterols present in the bacterial cell membrane causing changes in cell

morphology leading to lysis. Sandabe (2002), using thin layer chromatography, detected two types of saponins in various plants. Ahmad and Beg (2001) observed that phenols and tannins are the most common active constituents responsible for antimicrobial activity. These findings correlate with the observations of Silva *et al*. (1996). *Embelia ribes* and *Butea monosperma* showed moderate antibacterial activity against multi-drug resistant *Salmonella typhi* in a trial conducted by Rani and Khullar (2004). Elkatib *et al*. (2004) reported *in vitro* antimicrobial activity of *Vernonia adoensis*, member of the family Asteraceae against different G+ve and G-ve bacteria. Methanol extract of *V. anthelmintica* showed similar results in the present investigation also. However, Mishra *et al* (2009) demonstrated the potent bactericidal action of a flavonoid fraction isolated from the ethyl acetate extract of the stem bark of *B. frondosa.*

Alcohol was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad *et al*., 1998). The results of the present study also corroborate with these observations, as the alcoholic extracts of the plants revealed better inhibitory zones as compared to other extracts.

Chitra *et al*. (2003) found embelin, a benzoquinone-derivative isolated from *E. ribes*, to have significant antibacterial activity mostly in the higher concentration of 100 µg. In present *in vitro* trial also, all *E. ribes* extracts showed antibacterial activity, which can be attributed probably to the presence of embelin in seeds of this plant. They also found that efficacy of embelin was more against *S. aureus* and *S. flexneri* in comparison to *S. enterica enterica*; which is in accordance to the findings of present study.

It was noticed that in most of the trials, diameter of inhibition zones was not proportional to the MIC, which might be due to the varying diffusibility and varying amount of extract in nutrient agar for different bacteria (Rathchandi *et al*., 1998).

The extracts had lesser activity than *V. anthelmintica* oil probably due to poor diffusing power of extracts as compared to the essential oil on the nutrient agar surface (Singh *et al*., 2005). The activity of essential oil was marked against *S. aureus* than other bacteria which is in agreement with the findings of Kumar *et al*. (1988) who mentioned that G +ve bacteria are more sensitive to oils than G -ve bacteria.

The activity of some of the plant extracts on different organisms explains their broad spectrum nature while some of the plant extracts found to have effect on limited organisms, which may be due to their narrow spectrum of activity. This difference in activity appears to be directly related to the qualitative and/or quantitative diversity of the compounds that are being accumulated by the plants which were investigated. Though, the MIC of the plant extracts are no match to those of the standard antibiotics, it is hoped that they might produce comparable effect on further purifications and/or isolation of the active constituents. Further, the active phytocompounds of these plants against multi drug-resistant bacteria has to be characterized and the efficacy of non-toxic extracts has to be evaluated *in vivo*. Study of the synergistic interaction of active phytocompounds with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms. In the case of test bacteria, the basis for their differences in susceptibility might be due to the differences in the cell wall composition of G +ve and G -ve bacteria (Grosvenor *et al*., 1995).

The extracts of *V. anthelmintica* and *E. ribes* showed inhibitory activity during *in vitro* antibacterial assays. Oil of *V. anthelmintica* exhibited highest antibacterial activity against all the bacteria tested. Oil had results comparable to standard antibiotics but all the extracts showed lower activity.

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Pharmacokinetics of Florfenicol following single dose administration in sheep

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Abstract

In the present study, pharmacokinetics of Florfenicol 30% injection (Florjat, M/s Montajat Pharmaceutical Ltd.) was established in the plasma of the healthy sheep. Florfenicol was administered at a single dose of 20 mg.kg^{-1} in sheep following intravenous (i.v.) and intramuscular (i.m.) routes of administration. The $^{1}Vd_{(area),}$ $^{2}Cl_{B}$ and ^{3}AUC of Florfenicol following i.v. administration were 8.26 L.kg^{-1}, 0.72 L.kg^{-1}.h^{-1} and 28.33 µg.mL^{-1}.h, respectively following i.v. administration and relative $Vd_{(area),}$ relative Cl_{B} and AUC following i.m. administration were 7.09 L.kg^{-1}, 0.91 L.kg^{-1}.h^{-1} and 24.80 µg.mL^{-1}.h, respectively, in sheep. Based on pharmacokinetic data, the dosage regimen of 8.48 and 13.24 mg.kg^{-1} at 12 h interval was calculated following i.v. and i.m. administration of Florfenicol, respectively.

[1] Apparent Volume of distribution, [2] Clearance, [3] Area Under the Curve

Keywords: Pharmacokinetics, Florfenicol, Sheep, Intravenous, Intramuscular

Florfenicol [d-threo-3-fluoro-2-dichloroacetamido-1- (4-methylsulfonyl- phenyl)-1- propanol] is a fluorinated structural analog of thiamphenicol and chloramphenicol (Keizer, 1974; White *et al*., 2000). Florfenicol exhibits a broad antibacterial spectrum and a strong antibacterial activity, similar to those of chloramphenicol. At the same time, its toxicological profile is more favourable compared to that of thiamphenicol, because of the absence of the aromatic nitro-group (Lobell *et al*., 1994; Jianzhong *et al*., 2004). Florfenicol has terminal fluorine instead of a primary hydroxyl group in its structure and this modification is considered to be less affected by chloramphenicol acetyltransferase inactivation (Syriopoulou *et al.,* 1981). The pharmacokinetics of Florfenicol has been studied in wide variety of animal species. However, disposition kinetics and bioavailability of Florfenicol in sheep has not been fully documented. Marked interspecies variation in the disposition kinetics of Florfenicol limits the extrapolation of data from other species to sheep and its pharmacokinetic properties need to be investigated in sheep to enable its rational clinical use. The purpose of the present study was to determine single dose pharmacokinetics and dosage regimen of Florfenicol following single dose i.v. and i.m. routes of administration.

Materials and Methods

The pharmacokinetic study was conducted in six non-lactating female sheep (2.0 - 2.5 years of age and weighing 35±5.0 kg). The animals were procured from the Livestock Production Management, Department of the college. These animals were housed in animal house of the department and kept on pre-experimental period of one month to acclimatize them to the new environment. The animals were provided *ad libitum* stall-feeding of green fodder supplemented with concentrate ration and partial grazing. The animals had free access to clean fresh drinking water. All the animals were dewormed with albendazole (5mg.kg^{-1} body weight) one month before the commencement of experiment.

For pharmacokinetic study, Florfenicol (Florjat) was injected as single dose 20 mg.kg^{-1} (De Craene *et al.,* 1997) following i.v. and i.m. administration in sheep. An intervening wash out period of two week was given before injecting the drug in the same animals for pharmacokinetic study following i.m. administration of Florfenicol. The blood samples were collected in heparinized tubes through an i.v. cannula placed in the contralateral jugular vein at 0, 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 36 h. Plasma was separated by centrifugation at 1500 x g for 15 min and stored at -30° C till analysis.

Drug extraction from plasma samples was carried out as per the method of Jianzhong Liu *et al*., (2003) with slight modifications. One ml of ethyl acetate was added to 0.5 ml of vortex mixed plasma sample for the precipitation of proteins. The mixture was vortexed for 10 seconds and centrifuged at 2,360g for 10 minutes. The supernatant was poured into clean tube and the sample was kept under nitrogen flow (60^{0}C) for evaporation of the organic material. The residue was

reconstituted in mobile phase (77% water and 23% acetonitrile) and filtered through 0.22 µm filter paper. An aliquot of 20 µl of the sample thus obtained was injected into HPLC system for analysis.

The estimation of Florfenicol concentration in the plasma was done by high performance liquid chromatography as per the method described by Jianzhong Liu *et al*., (2003) with slight modifications. Separation was achieved using C_{18} reverse phase column, particle size 5µm (4×150 mm, Merck) as a stationary phase. An isocratic mobile phase consisted of acetonitrile (23%) and water (77%). The flow rate of mobile phase was kept at 0.6 $ml.min^{-1}$ for elution of the compound to be detected at UV wavelength of 223 nm at 25^0C. Drug standards were prepared by dissolving 5 mg of pure Florfenicol compound (Sigma Aldrich Ltd.) in 5 ml of plasma. Further dilutions were made from this stock solution in plasma in the concentrations of 10.0, 5.0, 2.5, 1, 0.5, 0.25 and 0.1 $\mu g.mL^{-1}$. 20µl of these concentrations were injected into HPLC under the conditions mentioned above. A standard calibration curve was obtained by plotting concentrations against the peak areas obtained for Florfenicol. Percent recovery of Florfenicol from plasma was 84 % with standard deviation of 3.88. The pharmacokinetic analysis was done by computer software "PK Solution 2.0" (M/S Summit Research Services, Colorado, U.S.A.).

Results

The plasma drug concentration-time profile following single dose (20 $mg.kg^{-1}$) i.v. and i.m. administration of Florfenicol in sheep is depicted in Table 1. The concentration of Florfenicol ranged from 16.12±0.97 to 0.07±0.003$\mu g.mL^{-1}$, in the present study. Pharmacokinetic values describing the disposition kinetics of Florfenicol following single dose (20$mg.kg^{-1}$) i.v. and i.m. administration in sheep are presented in Table 2. A two compartment model adequately described plasma concentration time profile in sheep following single dose i.v. administration. The values of zero time intercept of distribution phase (A) and elimination phase (B) in the present study to be 6.25±0.69 $\mu g.mL^{-1}$ and 0.60±0.08 $\mu g.mL^{-1}$, respectively. The elimination rate constant (β) was 0.06 h^{-1} with an elimination half-life ($t_{1/2}$ β) of 7.94±0.44 h. The volume of distribution (Vd_{area}) and total body clearance (Cl_B) of Florfenicol were observed to be 8.26±0.79 $L.kg^{-1}$ and 0.72±0.05 $L.kg^{-1}.h^{-1}$, respectively. The hybrid rate constant of distribution phase (α) was 1.2±0.06 h^{-1} with distribution half-life ($t_{1/2}\alpha$) of 0.79±0.08 h. The mean area under curve (AUC) and

Table 1. Plasma concentration ($\mu g.mL^{-1}$) of florfenicol following single dose (20 $mg.kg^{-1}$) i.v. and i.m. administration in sheep (n=6)

Time (h)	i.v. route (Mean ± S.E.)	i.m. route (Mean ± S.E.)
0.08	16.12±0.97	0.85±0.02
0.16	10.54±0.77	0.93±0.06
0.25	8.50±0.67	1.29±0.22
0.5	6.89±0.84	1.82±0.13
1	6.66±0.73	2.71±0.06
2	4.08±0.46	2.3±0.07
4	1.68±0.17	1.75±0.18
8	0.65±0.06	1.20±0.04
12	0.21±0.02	0.75±0.03
24	0.07±0.003	0.16±0.02

Table 2. Pharmacokinetic parameters of florfenicol following single dose (20 $mg.kg^{-1}$) i.v. and i.m. administration in sheep (n=6)

Parameters	Units	i.v. route (Mean ± S.E.)	i.m. route (Mean ± S.E.)
A	$\mu g.mL^{-1}$	6.25±0.69	-
B/B'	$\mu g.mL^{-1}$	0.60±0.08	3.61±0.39
α/ K_a	h^{-1}	1.20±0.06	0.72±0.06
β/ K_e	h^{-1}	0.06±0	0.12±0
$t_{1/2}\alpha$/ $t_{1/2}k_a$	h	0.79±0.08	1.03±0.16
$t_{1/2}\beta$/ $t_{1/2}k_e$	h	7.94±0.44	5.40±0.40
AUC	$\mu g.mL^{-1}.h$	28.33±2.50	24.80±1.18
MRT	h	4.21±0.21	0.82±0.28
AUMC	$\mu g.mL^{-1}.h^2$	3562.72± 239.52	6142.52± 488.85
CL_B/(Cl/F)	$L.kg^{-1}.h^{-1}$	0.72±0.05	0.91±0.03
Vd_{area}/(Vd/F)	$L.kg^{-1}$	8.26±0.79	7.09±0.24
Vd_{ss}	$L.kg^{-1}$	3.05±0.31	6.69±0.17
C_{max}	$\mu g.ml^{-1}$	-	2.73±0.06
T_{max}	h	-	60±0.00
F	%	-	88.47±4.00

A=Zero time intercept of distribution slope in two compartmental model; **B/B'** =Zero time intercept of elimination phase following i.v. and i.m. route; **α/K_a** = absorption/distribution rate constant following i.v. and i.m. route; **β/K_e** = elimination rate constant following i.v. and i.m. route; **$t_{1/2}\alpha/t_{1/2}k_a$** = absorption/distribution half life following i.v. and i.m. route; **$t_{1/2}\beta/t_{1/2}k_e$** =Elimination half life following i.v. and i.m. route; **AUC** = Total area under the time concentration curve; **MRT** = Mean residence time; **AUMC** = Area under first moment curve; **Cl_B/ (Cl_B/F)** = clearance/relative clearance of drug from the body following i.v. and i.m. route; **$Vd_{(area)}$/ (Vd/F)** = Apparent/ relative volume of distribution following i.v. and i.m. route; **Vdss**= Volume of distribution at steady state; **C_{max}** = Peak plasma concentration; **T_{max}** = maximum time required to attain peak plasma concentration; **F**= Bioavailability.

mean residence time (MRT) calculated were 28.33±2.50 μg.mL^{-1}.h and 4.21±0.12 min, respectively. Area under the first moment of the concentration-time curve from zero to infinity with extrapolation of the terminal phase (AUMC) and volume of distribution at steady state (Vd_{ss}) concentration were 3562.72±239.52 μg.mL^{-1}.h^2 and 3.05±0.31L.kg^{-1}, respectively

The peak plasma concentration (2.72±0.06μg.mL^{-1}) of Florfenicol was attained at 1 h post i.m. administration. Thereafter, the plasma drug concentration decreased slowly to a minimum of 0.16±0.02 μg.mL^{-1} at 24 h. A one compartment model adequately described plasma concentration- time profile of Florfenicol in following single dose i.m. administration. Zero time intercept of the elimination phase (B') was 3.61±0.39 μg.mL^{-1}. The hybrid rate constant of absorption phase (K_a) was 0.72±0.06 h^{-1} with absorption half-life ($t_{1/2}$ k_a) of 1.03±0.16 h. The elimination rate constant (K_e) and plasma half-life ($t_{1/2}$ k_e) were calculated to be 0.12±0 h^{-1} and 5.40±0.40 h, respectively. AUC and MRT calculated were 24.80±1.18 μg.mL^{-1}.h and 0.82±0.28 min, respectively. The relative volume of distribution (Vd/F) and relative total body clearance (Cl/F) was determined as 7.09±0.24 L.kg^{-1} and 0.91±0.036 L.kg^{-1}.h^{-1}. AUMC and Vd_{ss} were 6142.52±488.85 μg.mL^{-1}.h^2 and 6.69±0.17 L.kg^{-1}, respectively. Bioavailability (F%) following intramuscular administration was 88.47±4.00 %.

Discussion

In the present study, the pharmacokinetics of Florfenicol was studied following single dose (20mg.kg^{-1}) i.v. and i.m. administration in sheep. The peak plasma concentration (2.72 μg.mL^{-1}) of Florfenicol was attained at 1 h post i.m. administration. Peak plasma concentration in the present study was higher as compared to that reported (1.04μg.mL^{-1}) (Ali *et al.,* 2003) in sheep following i.m. administration. This difference could be due to the fact that we measured Florfenicol concentration by HPLC while they measured the Florfenicol using a microbiological assay. It is known that the two methods may yield different result for the same species. (Atef *et al.,* 2001). The microbiological method measures only the biological active unbound compound found in Florfenicol. In the present study, Vd_{area} of Florfenicol was 8.26 L.kg^{-1} and 6.27 L.kg^{-1} in sheep following i.v. and i.m. administration, respectively. The volume of distribution in the present study was found higher to that reported (0.50 L.kg^{-1}) in sheep following i.v administration (Lane *et al.,* 2004). However, Vd_{ss} was 3.05 L.kg^{-1} and 6.69 L.kg^{-1} in sheep following i.v. and i.m. administration, respectively. Vd_{ss} in our study was found higher to that reported (0.55 L.kg^{-1}) in sheep following i.v administration (Lane *et al.,* 2004). This difference may be attributed to breed variation (lipid composition of body tissues) and the physiochemical properties of Florfenicol formulations. Longer elimination half life of Florfenicol following i.v. administration (476.34 min) than i.m. administration (324.56 min), it may be due to ion trapping resulting in redistribution. The findings of the present study can be correlated with that of Jianzhong *et al.,* (2004) who have reported elimination half-life of 10.34 h following i.m. administration in sheep. In the present study, the mean and relative total body clearance (Cl_B, Cl/F) of Florfenicol was 0.72 L.kg^{-1}.h^{-1} and 0.91 L.kg^{-1}.h^{-1} following i.v. and i.m. administration, respectively. Cl_B following i.v. administration in the present study was found to be higher as compared to that reported (0.36 and 0.26 L.kg^{-1}.h^{-1}) in sheep (Jianzhong *et al.,* 2004; Lane *et al.,* 2004) following i.v. administration. In the present study, MRT of Florfenicol was 4.21 h and 8.22 h following i.v. and i.m. administration, respectively. In our findings, MRT in sheep was found to be higher as compared to the findings (3.42 h, 1.44 h) of Ali *et al.,* (2003) and Lane *et al*., (2004) in sheep following i.v. administration. This difference may attributed to large Vd_{ss} in the present study as compared to that reported. In our study, AUC of Florfenicol were 28.33 μg.mL^{-1}.h and 24.80 μg.mL^{-1}.h following i.v. and i.m. administration, respectively. AUC in our study was smaller as compared to that reported (76.31 μg.mL^{-1}.h) in sheep (Jianzhong *et al.,* 2004) following i.v. administration. This difference may be attributed to slow distribution of Florfenicol. In the present study, F% of Florfenicol in sheep was 88.47 % following i.m. administration. In the present study, F% of Florfenicol is almost similar to that reported (89.04) by Jianzhong *et al.,* (2004) in sheep following i.m. administration.

Based on the pharmacokinetic data, an intravascular dosage regimen with a priming dose of 8.48 mg.kg^{-1} followed by a maintenance dose of 4.35 mg.kg^{-1} at every 12 h interval, while an intramuscular dosage regimen with a priming dose of 13.24 mg.kg^{-1} followed by a maintenance dose of 10.10 mg.kg^{-1} at every 12 h interval was calculated and recommended.

The minimum therapeutic concentration used to calculate the dosage regimen was 0.50µg.mL^{-1}.

It can be concluded from the findings of the present study that Florfenicol show a favourable pharmacokinetics in terms of longer elimination half life, slower clearance and good volume of distribution. The study also concluded that in pharmacokinetic study breed variation may also play an important role.

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Effect of area specific mineral mixture supplementation on clinico haematological and mineral profile of buffaloes suffering from fluorosis

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Abstract

Supplementation of area specific mineral mixture @ 50 gm per animal per day for 60 days to fluorotic buffaloes (group I) resulted in decline in plasma concentrations of Ca, Mg, Mn, Zn, Mo; while increase in Cu, Fe and F on day 60. On the other hand, supplementation of the same mineral mixture to non-fluorotic buffaloes (group II) resulted in increase in Ca, Cu, Fe, Mo, F, but decrease in Mg, Mn and Zn in blood on day 60. Day 30 values, however, showed variable trends in both group I and II. Peripheral blood picture in both groups revealed increase in hemoglobin, hematocrit and total erythrocyte count on both day 30 and 60. Results of the present study showed favourable response of mineral mixture supplementation in fluorotic buffaloes on blood mineral profile. Moreover, improvement in blood picture, increase in milk production and clinical recovery of lameness problem further indicated beneficial effects of area specific mineral mixture supplementation in fluorotic buffaloes.

Keywords: Blood, Fluoride-toxicity, Haematology, Minerals, Mineral- mixture

Ground water in many parts of the country contains excess fluoride (F) which is responsible for chronic toxicity in both man and animals, commonly referred as fluorosis. In cattle and buffaloes dental and bony lesions develops besides causing significant loss of production and reproduction efficiency (Shupe *et al*., 1992; Swarup and Dwivedi, 2002). Fluoride can react with almost all other elements and organic compounds except oxygen and nitrogen to form fluorides (Banks and Goldwhite, 1966). Replacement of hydroxyl groups of calcium hydroxyapatite and incorporation of inorganic fluoride as calcium fluoroapatite is thought to be major mechanism of its toxic action and development of bony lesions in fluorosis. There are several research reports documenting alteration in mineral profile in blood and other tissues during fluoride toxicity (Kanwar and Singh, 1981; Ranjan *et al*., 2008). Also, supplementation of several individual minerals like boron, zinc, calcium etc. has been reported to alleviate experimentally induced fluorosis in laboratory and domestic elements (Wheeler and Fell, 1983; Khandare *et al*., 2005; Bharti *et al*., 2007). However, there seems no report available on effects of mineral mixture supplementation in buffaloes naturally suffering from fluoride toxicity. The present study was conducted to evaluate the effects of specifically formulated mineral mixture in dairy buffaloes suffering from fluorosis.

Materials and Methods

Several villages of district Bhatinda are endemic for fluorosis (Ayoob and Gupta, 2006). Fluoride concentrations in drinking water of different villages were estimated and water buffaloes in the study area were examined for various signs and lesions of fluorosis. Villages in which water fluoride concentrations were >1.00 ppm, along with high incidence of fluorotic lesions like dental mottling, periodontitis, knee posture to cross legged posture and lameness in buffaloes, were considered endemic for fluorosis. On the other hand, villages in which water fluoride concentration were d”1.00 ppm along with absence of fluorotic signs and lesions in water buffaloes were considered free from fluorosis.

A total of twenty lactating fluorotic buffaloes (Group I) were selected randomly from fluoride endemic villages. Another twenty healthy lactating buffaloes (Group II) were selected from fluorosis free villages to constitute non-fluorotic control. Each selected animal was given area specific mineral mixture @ 50g/day for 60 days. Composition of the area specific mineral mixture recommended for South-western zone (Malwa zone) of Punjab on the basis of plasma mineral status of dairy animals, recorded in earlier studies is given in table 1.

Blood samples from each animal were collected on day 0^{th}, 30^{th} and 60^{th} of treatment. Concentration of calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and molybdenum (Mo) were estimated using Atomic Absorption Spectrophotometer (Perklin Elmer A Analyst 700, USA) after wet acid digestion of blood plasma samples. Before

Ca and Mg estimation, digested samples were diluted (in 1:50) with 0.1 per cent lanthanum III chloride to prevent the interference of phosphorus.

Plasma inorganic phosphorus (Pi) was estimated by method of Tausky and Shorr (1953). Fluoride levels in both plasma and water samples were estimated using digital ion-analyzer (Orion 4 Star Series, USA) equipped with fluoride and iodide specific electrodes (Ahmed *et al*., 2004). Haematological parameters were estimated using standard laboratory methods (Feldman *et al.,* 2000). The data obtained were analyzed statistically using computer software SPSS for Windows (version 16.0; Microsoft).

Results and Discussion

Representative water samples from the study and concentration. All water samples collected from villages of Maur block contained F above the permissible level of 1.00 ppm, with the highest mean F concentration (1.75 ± 0.26 ppm) among different blocks. On the other hand, F concentration in Sangat block was within the safe limit with lowest mean value (0.91 ± 0.18 ppm). Lactating buffaloes in Maur block also showed poor health status along with high incidence of fluorotic lesions like dental mottling, bony outgrowth, lameness, reproductive problems and low milk yield. Buffaloes in Sangat block, in general, had better health status, greater average milk production and absence of dental and bony fluorotic lesions.

Changes in blood mineral profile following area specific mineral mixture supplementation are given in table 2. Blood Ca level showed a decreasing trend in group I, while an increasing trend was observed in group II. It could be, therefore, inferred that mineral mixture supplementation was effective in raising plasma Ca levels in buffaloes free from fluorosis (Group II) but was not equally effective in improving the plasma calcium status in fluorotic buffaloes (Group I). Lower plasma Ca level in group I might be due to competitive inhibition of Ca absorption in intestine due to presence of excess fluoride in drinking water (Stamp *et al.*, 1988). It may also be attributed to replacement of F by Ca in bones, resulting in to increase in bone F deposition. Contrary to this finding, Singh (2006) observed an increase in the plasma Ca level of fluorotic animals from both endemic and non endemic areas following calcium chloride supplementation. Variation in response may be due to complex interaction between different minerals. There are over 70 known mineral interrelationships in which an additional dietary quantity of one mineral element influences absorption and utilization of another mineral element (Jacobson *et al*., 1971).

Table 1. Composition of area specific mineral mixture for South-Western zone (Malwa zone)

Ingredients	Qnty.(in Kg)
Dicalcium phosphate	50
Magnesium oxide	6.66
Potassium Iodate	0.085
Copper Sulphate	0.549
Manganese Sulphate	0.462
Cobalt Sulphate	0.363
Zinc Sulphate	0.996
Limestone Powder	41.21

The Mg concentration decreased significantly in group I on day 30, but it increased, albeit non-significantly, on day 60. In group II animals Mg concentration increased on day 30; but on day 60 mild decrease in level was observed. In a previous study by Mircha (2009), almost similar trend of change in plasma Mg concentration was observed after feeding 50g mineral mixture to a group of dairy animals; it slightly decreased on day 45 after an initial increase on day 21. Dhiman and Sasidharan (1999) also observed a decrease in blood Mg concentration following administration of calcium chloride. However, contrary to this finding, Sharma *et al.* (2002) recorded an increase in plasma Mg concentration in animals fed with mineral mixture containing higher than usual amounts of Ca, Mg and P.

No significant variation in plasma Pi was observed in both group I and II on different observation period. A trend of decrease in Pi level was observed in both group I and II, except on day 60 in fluorotic buffaloes. Similar observations were recorded by Mircha (2009) in dairy animals following mineral mixture supplementation.

In comparison to day 0 values, Mn level in both groups were lower on day 60, although the difference was significant only in group I. The decline in plasma Mn concentration in present study could also be related to increased Mn deposition in bones in fluorotic animals.

Significant decrease in Zn concentration was observed on day 60 in both groups. Higher plasma Zn level before the treatment could be ascribed to release

of Zn from liver and kidneys in the presence of toxic levels of fluoride, as suggested by Singh (1984).

The mean plasma Cu levels in both the groups increased non- significantly on day 60, though higher increase was recorded in group II (19.2%) in comparison to group I (6.66%). Lower increase in plasma Cu concentration in fluorotic animals may be due to the antagonistic relationship between Cu and F. Severe Cu deficiency in rats have been observed during experimental fluoride toxicity.

Mean plasma Mo level in group I decreased significantly on day 60, while significant increase was recorded in group II. Molybdenum has been considered as possible alleviator of fluorosis (Yao and Chang, 1993). The decline in plasma Mo concentration in fluorotic buffaloes could be ascribed to competitive antagonism between molybdenum and fluoride.

The Fe levels in both the groups increased on day 60th. However, higher increase in Fe level was observed in group II. It seems that mineral mixture supplementation was more effective in healthy animals in comparison to fluorotic animals. This was also supported by greater increase in haemoglobin concentration and haematocrit values in healthy buffaloes.

The initial increase in F levels in both the groups after the treatment was suggestive of increased mobilization of fluoride from the bone into the blood and increased F excretion through urine (Reddy *et al.*, 1985).

The various haematological indices viz. Hb,

Table 3. Changes in haematological parameters in buffaloes supplemented with area specific mineral mixture

Parameter	Group	Day of observation		
		0 day	30 day	60day
Haemoglobin (g/dl)	I	9.39±0.39^{a}	10.15±0.47^{ab}	11.23±0.31^{b}
	II	9.4±0.46^{a}	10.28±0.54^{ab}	11.46±0.45^{b}
Packed cell volume (%)	I	24.92±.98^{a}	25.77±1.21^{a}	25.36±0.61^{a}
	II	25.14±1.16^{a}	26.92±1.25^{a}	26.85±1.05^{a}
Total erythrocytic count (x10^{6}/µl)	I	6.11±0.22^{a}	5.96±0.29^{a}	6.24±0.23^{a}
	II	6.08±0.28^{a}	6.58±0.27^{a}	6.87±0.31^{a}

Values bearing different superscripts in a row differs significantly ($p<0.05$)
Group I: Fluorotic; Group II: Control

Table 2. Blood plasma mineral profile of buffaloes supplemented with area specific mineral mixture

Minerals	Group	Day of observation		
		0 day	30 day	60day
Ca (ppm)	I	115.82±4.44^{a}	109.16±4.2^{a}	107.57±3.88^{a}
	II	102.79±4.83 ^{a}	104.84±5.10 ^{a}	111.34±7.36 ^{a}
Mg (ppm)	I	31.14±1.20^{b}	24.47±1.98^{a}	26.92±2.28^{ab}
	II	25.96±1.72 ^{a}	26.65±1.32 ^{a}	22.54±2.19 ^{a}
Mn (ppm)	I	0.34±0.10^{b}	0.13±0.03^{a}	0.17±0.02 ^{a}
	II	0.18±0.04 ^{a}	0.20±0.03 ^{a}	0.11±0.02 ^{a}
Cu (ppm)	I	0.75±0.10^{b}	0.47±0.10 ^{a}	0.80±0.10^{b}
	II	0.52±0.10^{ab}	0.39±0.05 ^{a}	0.62±0.11^{b}
Zn (ppm)	I	1.85±0.24^{b}	0.49±0.10 ^{a}	0.49±0.12 ^{a}
	II	1.92±0.32^{b}	0.41±0.10 ^{a}	0.48±0.10 ^{a}
Fe (ppm)	I	8.08±1.05^{b}	2.25±0.34 ^{a}	9.96±2.21^{b}
	II	3.12±0.38 ^{a}	2.78±0.31 ^{a}	7.99±1.36^{b}
Mo (ppm)	I	0.09±0.01^{b}	0.03±0.01 ^{a}	0.01±0.002 ^{a}
	II	0.04±0.004 ^{a}	0.02±0.004 ^{a}	0.09±0.03^{b}
F (ppm)	I	0.21±.01^{a}	0.53±0.20 ^{a}	0.25±0.01 ^{a}
	II	0.17±0.01 ^{a}	0.33±0.03 ^{a}	0.24±0.01^{a}
Pi (mg/dl)	I	4.86±0.35^{a}	4.76±0.20 ^{a}	4.91±0.19 ^{a}
	II	5.40±0.33 ^{a}	5.15±0.25 ^{a}	5.09±0.21 ^{a}

Values bearing different superscripts in a row differs significantly ($p<0.05$), Group I: Fluorotic; Group II: Control

TEC and PCV recorded before and after mineral mixture supplementation in the dairy animals are presented in Table 3. There was significant increase in Hb concentration in both the groups on day 30, and it further increased on day 60. Likewise, PCV and TEC also increased, but values did not show any statistical significance.

From the results of the present study, it can be concluded that supplementation of area specific mineral mixture had a positive response on haematological parameters, blood mineral profile, production and reproductive performances of fluorotic buffaloes. However, the response did not show any consistent pattern and linear relationship for various elements.

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Short Communication

Assessment of reference values of cardiac biochemical parameters in Labrador retrievers

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Abstract

Reference ranges for some clinical cardiac biochemical parameters in the Labrador retriever were calculated. The effect of body weight and sex on the values was determined. Creatine kinase (Ck), lactate dehydrogenase (LDH), serum cholesterol and triglycerides were found to have non-significant effect of body weight and sex. However, reference ranges were found comparatively narrower than those given for general dog population, which is helpful in interpreting the data more clearly.

Keywords: Biochemical, Cholesterol, CK, Labrador retriever, LDH, Triglycerides.

The blood is an important medium in assessing the health status of animals. Both the physiological and pathological conditions of animals can be assessed by the evaluation of hematological and biochemical analyses of the blood (Coles, 1986; Bush, 1991). Factors such as nutrition, age, sex, breed and climate were known to affect biochemical and haematological parameters of clinically healthy dog (Coles, 1986; Awah and Nottidge, 1998). Though ample work has been done on establishing the base line values of biochemical and haematological parameters of dogs (Oduye, 1978; Awah and Nottidge, 1998), earlier workers have either concentrated on the local dogs or combined different breeds of dogs together, thus, making it difficult to determine the sole effect of breed on these parameters. This study was carried out to investigate the effect of breed on some cardiac biochemical parameters of clinically healthy Labrador retriever dog which is the most widely used breed for hunting, tracking, detection, disabled assistance and carting in India.

Materials and Methods

The study was conducted on 24 clinically healthy Labrador retriever dogs of both the sexes with body weight ranging from 18-30 kg. The study was conducted in two phases viz., screening and quantitative measurements of cardiac biochemical parameters. All the animals were subjected to screening initially to ascertain that they were clinically healthy. Criteria to accept a dog as clinically healthy were: Cardiovascular physical examination (auscultation), electrocardiographic examination, thoracic radiography, echocardiography and blood examination. None of the dog had any heart murmur or a gallop rhythm. Radiographic examination was done to rule out any thoracic (pleural effusions) or cardiac problem (pericardial effusions). Electrocardiography and two-dimensional echocardiography was done to rule out any anatomical or functional disturbance. All dogs were free from circulating microfilaria examined by modified Knott's method.

Measurement of cardiac biochemical parameters

Five millilitres of blood was collected from the cephalic vein or recurrent tarsal vein of the animals by aseptic technique using sterilized syringes. Harvested serum was then analyzed for the following biochemical parameters:

Serum Triglyceride - by using Kit (Triglyceride test kit, Span Diagnostic Ltd). Serum Cholesterol – by using Kit (Cholesterol test kit, Span Diagnostic Ltd). Serum activity of Creatine kinase – by using kit [Ck (NAC act.) kit, Coral Clinical Systems]. Serum activity of Lactate dehydrogenase – by using kit [LDH (P-L) kit, Coral Clinical Systems].

Statistical analysis

Statistical analysis was done by using statistic software SPSS 15.0. Significance control of the difference among different parameters of female and male dogs was analysed by independent sample *t*-test. In order to analyze the data with respect to body weight, animals were grouped into 3 classes according to their body weight.

Group Number	Body weight
1	18-22 Kg
2	22-26 Kg
3	26-30 Kg

Results and Discussion

Analysis after grouping the animals with respect

to sex revealed that Mean±SE of males and females did not show significant variation. For general dog breed population similar findings were reported in previous studies for Ck (Ariyibi *et al*., 2002), LDH, Triglycerides and cholesterol (Kaneko *et al*., 2008).

Non-significant variation in the values of biochemical parameters were observed in different body weight groups. All these parameters were found to be well in the range specified for general dog breed population. However, the maximum values in the present study for Ck and LDH were much lower than their corresponding values for general dog population. Non-significant effect of gender was observed on both the parameters. Similar findings had also been reported for Indian Spitz dog (Saxena, 2008). There are different mean values reported in previous studies for total Ck in general dog population [36.3±29 (Yasuda *et al*., 1982); 115.6±91.5 (Watanabe *et al*. (1982); 22.0 (Heffron *et al*. (1976)]. These values are much higher compared to those observed in the present study. Similarly for LDH, much higher values (342.0±163.0) have been reported for the general dog population (Zanatta *et al*., 2003). The possible reason for such differences might be due to the incorporation of different breeds in previous studies.

Although, the cholesterol values of Labrador retriever dogs are comparable to the one reported by Rovira *et al*. (2007) and Tvedten (1981), the maximum value of this investigation for cholesterol was much lower than their corresponding values for resting dogs. This variation may be attributed to the breed difference as in their study general dog breed population was considered. Non-significant difference was seen with respect to gender in accordance to those reported in German shepherd (Omer, 2009). However, Pasquini *et al*. (2008) claimed that gender has to be taken into consideration while interpreting the cholesterolemia in dogs. Lower triglycerides range than that obtained in this investigation was reported by Rovira *et al*. (2007) and Tvedten (1981) for resting dogs and a reference value for unspecified breed, respectively. Pasquini *et al*. (2008) and Omer (2009) reported that gender does not prove to be an important factor in the evaluation of triglycerides values in canines and is in accordance to the present study.

The data of the present study may be used as indicator to diagnose different cardiac ailments along with other diagnostic modalities. Though, deviation in the values of above parameters especially Ck and LDH may also occur in conditions unrelated to the cardiovascular system as in the present study the total Ck and LDH were measured and not the one specific to heart. There is increase in Ck level in some of the cardiac problems like myocardial diseases secondary to Parvovirus infection (Carpenter, *et al*., 1980), Dirofilaria infection (Kitagawa *et al*., 1991), endocarditis (Calvert, 1982), myocarditis (Ahmad *et al*., 1982) and chronic mitral valve disease (Bakirel and Gunes, 2009) while decrease in Ck activity occurs in myocardial infarction (Devries *et al*., 1986). However, there are some conditions like cardiac hypertrophy (Ingwall, 1984) and cardiac dilatation (Sottiaux, 1981)

Table 1. Reference ranges and Mean±SE of biochemical parameters in animals.

Parameter	LDH (µ/L)	CK (µ/L)	Chol (mmol/L)	Tg (mmol/L)
Values	96.0-203.0(150.83±6.41)	2.15-19.1(10.83±1.21)	3.4-6.6(4.51±0.18)	0.22-0.49(0.35±0.02)

On analysis, all these biochemical parameters were found to be well in the normal range described for that parameter in dogs.

Table 2. Mean±SE of biochemical parameters of male and female animals.

Parameter	LDH (µ/L)	CK (µ/L)	Chol (mmol/L)	Tg (mmol/L)
Male	143.92±9.47	10.08±1.99	4.40±0.25	0.33±0.03
Female	157.75±8.57	11.58±1.44	4.61±0.27	0.36±0.03

Table 3. Mean±SE of biochemical parameters of animals after they were grouped with respect to body weight.

Parameters	LDH (µ/L)	Ck (µ/L)	Chol. (mmol/L)	Tg (mmol/L)
Group 1	150.38±11.78	10.48±2.31	4.71±0.34	0.35±0.03
Group 2	142.50±11.70	9.76±1.95	4.16±0.23	0.31±0.03
Group 3	159.63±10.43	12.25±2.21	4.65±0.36	0.38±0.03

wherein no change in the activity of Ck occurs. Increased LDH activity has been reported in chronic mitral valve disease in dogs (Bakirel and Gunes, 2009), myocardial infarction (Jaffe *et al*., 1996) and cardiac hypertrophy (Bishop and Altschuld, 1970). Excess triglycerides in the form of low-density lipids, and cholesterol cause clogging of blood vessels leading to narrowing of their lumen. Thus, increases afterload on the heart leading to congestive cardiomyopathy, secondarily.

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Haemato biochemical changes in experimentally induced lead toxicity in rats

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Abstract

An experiment was conducted to study the haemato-biochemical changes in experimentally induced by lead acetate toxicity in 48 Wistar rats of either sex, divided uniformly into four different groups. The rats of group I received only deionized water as control while, group II, III and IV were given lead acetate @ 250 ppm, 500 ppm and 1000 ppm, in drinking deionised water respectively for 8 weeks. In group II, III and IV dose dependant significant ($P<0.05$) reductions in TEC, Hb, PCV and TLC were observed. Non significant increase was observed in neutrophil count in treatment groups, whereas the lymphocyte count decreased significantly ($P<0.05$) in group III and IV. Absolute monocyte, basophil and eosinophil count did not vary significantly among different groups. A dose dependant significant ($P<0.05$) increase in AST, ALT values while total protein and albumin levels were decreased in group II, III and IV. Average serum globulin values were non significantly lower in all treatment groups when compared with levels of control group of study.

Keywords: Biochemical, Haematological, Lead acetate Toxicity, Wistar rats.

In recent years, lead has become a regulatory concern and subject of much interest among pharmacologist, environmental scientist and clinicians because of its widespread distribution in environment due to its continuous emission from industrial sources, automobile exhaust and its pharmacological behavior to remain bound to mammalian tissues for a long duration (Freeman, 1970). Many reports are available regarding lead toxicity and its deleterious effects in various species of animals and there has been lot of work carried out on pharmacokinetics and genotoxicity but very few researchers tried to correlate haemato-biochemical alterations of lead acetate at different dose levels in laboratory animals especially in rats as they considered as a suitable animal model.

Materials and Methods

The present study was carried out on 24 male and 24 female rats randomly divided into 4 groups with six male and six female in each group. Animals of group II, III and IV were given lead acetate @ 250 ppm, 500 ppm and 1000 ppm in deionised drinking water, respectively while group I received only deionized water for 8 weeks. Blood samples were collected from rats of each group at the time of sacrifice in vials containing EDTA @ 2mg/ml of blood as anticoagulant. Blood smears were also prepared for differential leukocyte count was evaluated by the formula as described by Schalm *et al.* (1975). For biochemical studies, blood was collected without the addition of anticoagulant in test tubes that were allowed to stand at room temperature, (IFCC method) by using standard kits obtained from Span Diagnostic Ltd, Surat, Gujarat. The data were analyzed using one way ANOVA by SPSS (Ver. 16.00).

Results and Discussion

The result of haemato-biochemical changes has been presented in (Table-1). Reduction in Hb, TEC, PCV, MCV, MCH and MCHC was observed following exposure of lead acetate in rats of group II, III and IV which revealed microcytic hypochromic anemia. This haematological alteration might be due to effect of lead on activity of aminolevulinic acid dehydratase (ALAD), key enzyme of heme synthesis. Moreover lead also inhibit the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in haemoglobin production and shortened life span of erythrocytes (Klassen, 2001). Progressive destruction of RBCs due to binding of lead with RBCs, leading to increase fragility and destruction; could be another reason for decrease haematological values (Rous, 2000). Similarly significant decrease in Hb, PCV, MCH, MCV and MCHC were observed following exposure of rats to lead acetate. (Helmy *et al.,*2000). Analysis total leucocytes count and differential leucocyte count revealed leucopenia and lymphopenia in higher dose group. This might due to direct toxic action of lead on leucopoiesis in lymphoid organs. Decrease in TLC is directly related with either their decreased production

Table 1. Haemato-biochemical values (mean± SE) in rats of different groups. (n=6)

Parameters	Week PE	Group I	Group II	Group III	Group IV
Hb (g/dl)	**8th**	13.20±0.27^{a}	12.35±0.20^{b}	11.46±0.22^{c}	10.25±0.16^{d}
TEC (10^6/mm^3)	**8th**	7.02±0.06^{a}	6.65±0.05^{b}	6.40±0.04^{c}	5.95±0.05^{d}
PCV (%)	**8th**	42.85±0.81^{a}	40.30±0.62^{b}	37.65±0.67^{c}	34.00±0.48^{d}
MCV (fl)	**8th**	60.99±1.14^{a}	60.55±0.74^{a}	58.74±0.86^{b}	57.07±0.74^{b}
MCH (pg)	**8th**	18.78±0.37^{a}	18.55±0.25^{a}	17.89±0.29^{b}	17.20±0.24^{b}
MCHC (%)	**8th**	30.80±0.04^{a}	30.64±0.04^{b}	30.44±0.05^{c}	30.14±0.04^{d}
TLC(10^3 / mm^3)	**8th**	8.04±0.24^{a}	7.57±0.19^{ab}	7.23±0.20^{b}	6.49±0.22^{c}
Neutrophil (%)	**8th**	20.66±1.14^{a}	20.83±0.83^{a}	20.16±0.54^{a}	22.00±0.25^{a}
Lymphocyte (%)	**8th**	72.16±0.60^{a}	72.00±0.51^{a}	70.50±0.56^{b}	68.66±0.33^{b}
Monocytes (%)	**8th**	5.16±0.30^{a}	5.16±0.30^{a}	5.30±0.36^{a}	5.50±0.22^{a}
Eosinophils (%)	**8th**	1.00±0.25^{a}	1.16±0.30^{a}	1.00±0.21^{a}	1.33±0.21^{a}
Basophils (%)	**8th**	0.83±0.30^{a}	0.83±0.30^{a}	1.00±0.21^{a}	1.00±0.30^{a}
TSP (g/dl)	**8th**	6.08±0.07^{a}	5.69±0.13^{b}	5.23±0.10^{c}	4.62±0.19^{d}
Alb (g/dl)	**8th**	3.64±0.03^{a}	3.18±0.16^{b}	2.75±0.16^{c}	2.14±0.22^{d}
Glb (g/dl)	**8th**	2.44±0.10^{a}	2.42±0.09^{a}	2.40±0.10^{a}	2.39±0.10^{a}
AST (IU/ml)	**8th**	1.35±1.08^{a}	1.49±1.13^{b}	1.64±2.33^{c}	1.89±3.93^{d}
ALT (IU/ml)	**8th**	56.47±1.07^{a}	68.49±0.91^{b}	81.84±1.34^{c}	98.32±1.81^{d}

Means within row with different superscripts differ significantly (P< 0.05)

from the germinal center of lymphoid organs or increased lysis due to presence of lead in the body (Avdheshkumar *et al.,* 1998).

In the present study, increased in AST and ALT was observed in group III and IV, which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by lead acetate. These findings are in accordance with Shalan *et al.* (2005). Decrease in total protein and albumin in group III and IV revealed compared to alteration in protein patterns which might be due to binding of lead to albumin. (Stone and Soares, 1976). Non significantly lower globulin concentration was observed in group IV followed by group III, II and I rats. In conclusion, lead exposure at the levels of 250 ppm, 500 ppm and 1000 ppm in drinking deionized water leads to toxicity in Wistar rats.

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Evaluation of trace mineral status in arsenic toxicity and ameliorative potential of *Ocimum sanctum* in rats

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Abstract

The present study was conducted to determine the toxic effects caused by arsenic in brain tissue as well as prophylactic efficiency of Ocimum sanctum. Trace element concentration was assessed in treated and untreated animals. Significantly increased arsenic and zinc and reduced copper and cobalt levels were observed in brain tissue in positive control. The effect of therapeutic efficacy of *Ocimum sanctum* on arsenic induced brain toxicity was studied. It was concluded that treatment of HAEO 100 & 200 was found effective to reduce the toxic effect of arsenic in different parts of brain and the drug has chelating effect on arsenic.

Keywords: Sodium arsenite, Rats, Brain, Toxicity, *Ocimum sanctum,* Trace elements.

Acute and chronic arsenic exposure has detrimental effects on the central nervous system and mental health. Several studies have reported on arsenic-induced brain damage (Samuel *et al*., 2005). Arsenic crosses the blood–brain barrier and has a wide range of effects on brain white matter (Rosado *et al*., 2007). Epidemiological and experimental studies indicate that cerebral and cerebellar cortexes may be affected with arsenic (Piao *et al.,* 2005). Many medicinal plants sources of natural antioxidants are reported to be useful against arsenic-induced injury of brain (Kapoor *et al*., 2009). Thus the present study was intended to determine the ameliorative effect of *Ocimum sanctum* in arsenic toxicated brain tissue.

Materials and Methods

Seventy five Wistar rats (n=75) of 8-12 weeks of age and 100-120 gm body weight were procured from Sanjay saha 24 Paragana (N) West-Bengal, maintained in polypropylene cages with natural light and dark cycles in the Department of Veterinary Biochemistry and supplied ad lib feed and water. Deworming was done by using albendazole @ 7.5 mg/kg body weight orally to individual rats. Rats were divided randomly in to five groups of 15 rats in each to study the correlation of arsenic and trace elements like zinc copper and cobalt by estimating the residual effect of these elements in brain tissues (Cerebrum, cerebellum, medulla oblongata and spinal cord) arsenic and trace elements burden in tissues and ameliorative potential of Tulsi (*O. sanctum*), during oral exposure of arsenic in rats through drinking water. Group I rats were kept as healthy control received distilled water ad lib for a period of 45 days whereas group II received arsenic as sodium arsenate salt @ 20 ppm in drinking water daily. Group III received 50mg per kg body weight of *Ocimum sanctum* leaves extract ($HAEO_{50}$)once in day along with 20 ppm sodium arsenate daily, group IV animals received 100mg leaves extract of *Ocimum sanctum* /kg body weight ($HAEO_{100}$)once in day and @ 20ppm sodium arsenate daily and group V received 200 mg/kg body weight *Ocimum sanctum* ($HAEO_{200}$) leaf extract along with 20 ppm sodium arsenate respectively in drinking water for a period of 45 days. All experimental animals were under the described treatments for continuous 45 days. Part of tissue samples were digested immediately after collection by adding concentrated

Nitric acid and 30% Hydrogen peroxide (3:1). Digestion was carried out and dilution to known concentration was finally made with triple distilled water. Samples were finally stored at -20^0C till analysis with the help of atomic absorption spectrophotometer by standard methods (APHA, 1992).

Results and Discussion

Arsenic concentration increased significantly ($P<0.05$) in cerebral cortex and cerebellum on 45th day in positive control animals (group II). Co-therapy with HAEO in group III, IV and V showed decreased arsenic concentration due to chelating action of HAEO. Arsenic concentration in medulla oblongata in group II was found increased non significantly and maximum value 0.48±0.19ppm achieved in compare to 0.17±0.13ppm in group I on 45th day (Table.1).

Treatment with HAEO was found effective in

Table 1. Effect of arsenic (20mg/kg-1 b.wt) exposure and HAEO on arsenic and other trace elements (ppm) of experimental rats

Tissues	Elements	Groups				
		I	II	III	IV	V
Cerebral cortex	Arsenic	0.14±0.05	3.84±0.06*	0.81±0.04	0.24±0.03	0.17±0.04
	Zinc	14.96±0.51	17.50±0.31	16.07±0.33	15.23±0.42	15.31±0.44
	Copper	17.52±0.66	15.31±0.87	16.87±0.51	17.31±0.33	17.68±0.59
	Cobalt	0.52±0.06	0.43±0.06	0.46±0.04	0.48±0.006	0.52±0.003
Cerebellum	Arsenic	0.27±0.25	2.62±1.30*	0.96±1.22	0.53±1.16	0.30±1.32
	Zinc	16.34±0.46	18.47±0.31	17.82±0.59	15.80±0.31	16.28±0.33
	Copper	18.63±0.51	17.15±0.26	17.33±0.41	18.22±0.60	18.43±0.62
	Cobalt	0.37±0.06	0.36±0.04	0.41±0.02	0.41±0.05	0.42±0.02
Medulla oblongata	Arsenic	0.17±0.13	0.48±0.19	0.32±0.11	0.20±0.14	0.18±0.16
	Zinc	12.70±0.40	15.62±0.38	12.86±0.35	12.71±0.45	12.65±0.29
	Copper	14.77±0.41	14.04±0.32	14.40±0.33	14.59±0.43	14.76±0.41
	Cobalt	0.25±0.03	0.25±0.06	0.24±0.02	0.24±0.02	0.25±0.06
Spinal cord	Arsenic	0.12±0.03	0.23±0.03	0.13±0.04	0.07±0.05	0.05±0.06
	Zinc	11.69±0.44	12.82±0.47	12.51±0.37	12.55±0.36	12.44±0.28
	Copper	12.41±0.22	12.41±0.22	11.82±0.33	12.21±0.41	12.65±0.28
	Cobalt	0.26±0.005	0.25±0.006	0.23±0.004	0.26±0.004	0.27±0.003

Values are mean ± SE; n = 15; * Significant ($P \leq 0.05$) compared to Negative control (Group I).

group III, IV and V but at the end of study arsenic values completely restored in group IV and V and only slight reduction in arsenic concentration was observed in group III as compared to group II.

Non significant increased arsenic concentration was also observed in spinal cord in positive control animals. Complete restoration was observed in group IV and V who received $HAEO_{100}$ and $HAEO_{200}$ along with arsenic exposure. Arsenic crosses the blood–brain barrier and has a wide range of effects on brain white matter (Rosado loc cit). Kamaluddin and Misbahuddin, (2006), also observed increase arsenic concentration in brain in arsenic intoxication.

Arsenic exposure result in non significant increase in zinc concentration was observed in studied brain tissues. On 45th day in positive control animals in cortex, 17.50±0.31ppm zinc concentration as compared to group I value (14.96±0.41ppm), in cerebellum 18.47±0.31 as compared to 16.34±0.46 ppm in group I, in medulla oblongata, 15.62±0.38ppm as compared to 12.70±0.40ppm and in spinal cord it was 12.82±0.47 ppm as compared to 11.69±0.44ppm. On percent basis maximum increase was observed in medulla oblongata (22%0 followed by cerebral cortex (17%), cerebellum (13%) and lowest in spinal cord (9%). Zinc induces increase in metallothionein (MT) which is a sulphhydryl rich metal binding protein and decreases the toxicity of arsenic (Austin *et al.,* 2004). In the present study, the interaction between arsenic and zinc was evident as zinc level was decreased in blood but increase in cerebral cortex, cerebrum, medulla oblongata and spinal cord following arsenic administration. Milton *et al.* (2004) also reported the negative correlation between the arsenic and zinc. Zinc concentration gradually reduced as a result in HAEO treatment in group III, IV and V, Complete restoration was observed in tissues. However maximum restoration was observed in group IV and V. Complete restoration in all HEAO treatment groups was recorded in spinal cord and medulla oblongata.

Slight reduction in copper concentration was observed as a result of arsenic exposure. On percent basis maximum declined values was observed in spinal cord (15%), followed by cerebral cortex (12%), cerebellum (8%) and 5% decrease in medulla oblongata. Peraza *et al.* (1998) also reported decrease copper in arsenic intoxication. HAEO treatment responded well in all studied brain tissues and complete restoration was observed with all studied doses of *Ocimum sanctum*.

Non significant decrease in cobalt concentration was observed in positive control animals as compared to negative control animals. On 45th day 18%, 3%, 0% and 4% declined values of cobalt was observed in cerebral cortex, cerebellum, medulla oblongata and spinal cord respectively. Co administration of HAEO was found effective in arsenic toxicity but HAEO100 and HAEO200 showed complete restoration to normal values in brain parts. However it was difficult to explain the precise mechanism of ocimum sanctum.

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Electrocardiographic study in dogs at different age groups

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Abstract

The present study was conducted to evaluate the effect of age on different electrocardiographic waves in dogs. A total of 18 dogs were included in the study. Electrocardiographic patterns in dogs at different age groups showed significant differences ($p<0.05$) between the groups with respect to heart rate. The heart rate was highest in young dogs compared to mature and old dogs which correlated well with the increase in duration of P wave, QRS complex, PR and QT intervals with advancement of age. There was an increase in P and R wave amplitudes with advancement of age which might be due to increase in heart size with the advancement of age.

Keywords: Age group, Dog, Electrocardiography.

Electrocardiography is a non-invasive and relatively inexpensive technique and has now generally been accepted as an important diagnostic tool in detecting cardiac abnormalities in dogs (Tilley, 1985). It detects not only the disturbances of cardiac rate and rhythm but also chamber enlargement, myocardial disease, ischemia, pericardial disease, certain electrolyte imbalances and some drug toxicities (Gugjoo *et al*., 2013). Though the normal electrocardiogram was established during late 1940's, many scientists have reported variations in canine ECG pattern because of wide variation in age and body conformation of dogs (Gugjoo *et al*., 2013; Hanton and Rabemampenia, 2005). Hence, in present study an attempt was been made to study the ECG pattern in different age group of dogs.

Materials and methods

Eighteen apparently healthy dogs (Non-descript -6, Spitz-8; German shepherd-4) of either sex were included in the study. Animals were divided into three different body weight groups, each group consisting of 6 dogs. These groups were: dogs below 6m of age, dogs aged between 7m to 5 yr. and dogs above 5 yr of age.

Electrocardiogram was recorded in standard bipolar leads (Lead II) at paper speed of 50mm per second using cardiart, 12 lead electrocardiographs (BPL India Ltd.) as per the procedure described by Tilley (1992). The cardiart ECG recording paper (BPL India Ltd.), a thermo sensitive paper of 50 mm width with a recording width of 40 mm was used. The heart rate was calculated as per the procedure by Tilley (1992).

Results and discussion

Significant differences ($P<0.05$) were observed between different age groups of dogs with respect to many electrocardiographic parameters. The mean electrocardiographic values of different groups are given in table.

The ECG pattern in different age groups of dogs has been depicted in figs. 1-3.

The mean heart rate was highest in dogs aged below 6 months (138±12 beats per minute) as compared to those aged 7 months to 5 years (120±9 beats per minute) and above 5 years (104±10 beats per minute). Similar observations were also reported by Ettinger and suter (1970), Tilley (1992) and Sanjaya kumar (2003). The faster heart rate in young animals could be partly due to their small size (higher metabolic rate per unit of body weight) and other fact being that vagal inhibition

Table 1. Mean electrocardiographic values in different age group of dogs

Parameters	< 6 m	7 m-5 yr	➢	5 yr
P wave	Duration(sec)	0.035± 0.013[a]	0.038± 0.005 [a]	0.040± 0.004 [b]
	Amplitude(mV)	0.32± 0.083 [a]	0.387± 0.089 [a]	0.40± 0.041 [b]
R wave	Amplitude(mV)	1.14± 0.45 [a]	1.24± 0.41 [a]	1.60± 0.33 [b]
QRS complex	Duration(sec)	0.040± 0.000	0.040± 0.000	0.043± 0.080
T wave	Amplitude (mV)	0.28±0.14 [a]	0.39±0.13 [a]	0.44±0.14 [b]
PR interval	Interval(sec)	0.05± 0.008 [a]	0.06± 0.010 [b]	0.06± 0.009 [b]
QT segment	Interval(sec)	0.160± 0.016 [a]	0.203± 0.019 [b]	0.225± 0.041[c]
Heart rate	Beats/min	138± 12.0 [a]	120± 9.00 [b]	104± 10.0 [b]

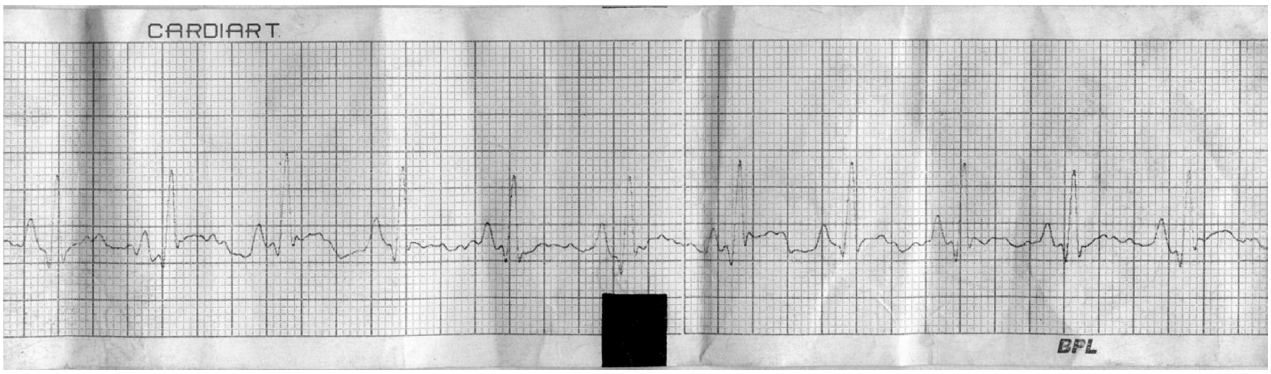

Fig.1 Group I

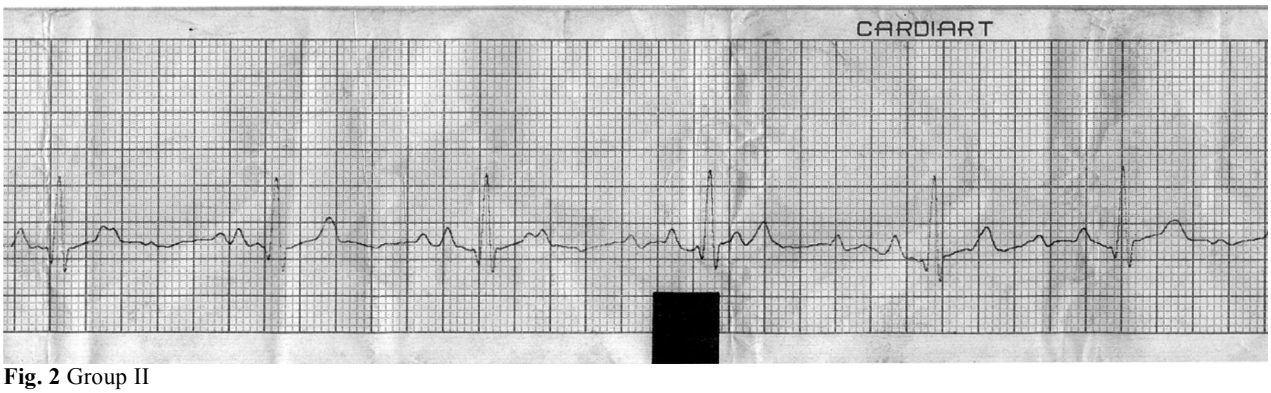

Fig. 2 Group II

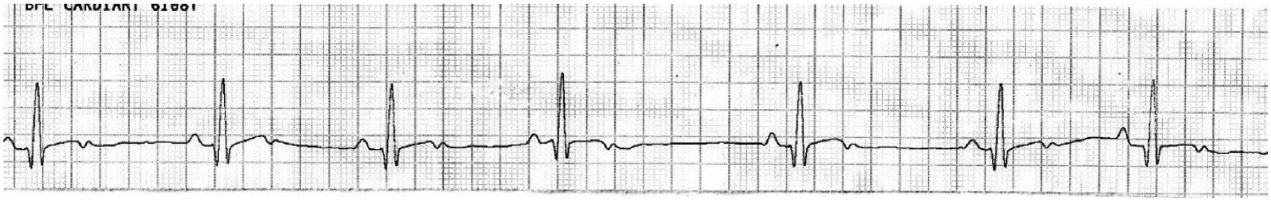

Fig.3 Group III

is less developed in young animals (Dukes, 1990; Sanjaya kumar, 2003).

The P wave amplitude was observed significantly higher in dogs as the age advanced. It was lower in dogs below 6 months of age (0.32mv) and higher in dogs above 5 years (0.4mv). The increase in P wave amplitude with age might be due to increase in heart weight that take place with advanced age (Kirk *et al*., 1975 and Changkija, 2007). P wave duration also increased significantly with age. This might be due to the decrease in heart rate with age.

Significant (P<0.05) increase was seen in the amplitude of R wave as the age advanced. It was lower in dogs below 6 months (1.14mv) and higher in dogs above 5 year (2.23mv). Increased R wave amplitude could be possibly owing to the development of left ventricle. Significant (P<0.05) difference was observed between different age groups of dogs with respect to duration of QRS complex. The increase in the duration of QRS complex with age agrees with results obtained by Suganuma, (1972) and Shimizu *et al*., (1986). The relationship between QRS complex and age appears to relate to increase in heart rate with age (Edwards, 1987).

Significant (P<0.05) difference was observed in T wave amplitude with respect to age groups. Dogs of older age had higher amplitude than lower age group dogs (Bernal *et al*., 1995). This increase in T wave amplitude corresponds to increase in R wave amplitude.

Significant (P<0.05) difference was observed in the duration of PR interval. The mean duration of

PQ interval was shorter in dogs below 6 months (0.05sec) and longer in dogs with age groups between 7m and 5 yr (0.06sec) and above 5 years (0.06sec). This may be one of the contributing factors for the slowing of the heart rate as the age advances as indicated by Razakhani *et al*., (1990). Tilley was of the opinion that PQ interval varies with heart rate, higher the heart rate, shorter the conduction time through the atria and the AV node to the bundle of HIS.

The mean duration of QT segment was shorter in dogs aged below 6 m (0.160sec) and longer in dogs aged above 5 yr (0.225sec). This increase in Duration with age could be due to the decrease in heart rate.

Q wave and S wave (elevation and depression) were considered as variable parameters as there is no consistency in occurrence of these.

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Efficacy of Imidocarb dipropionate in managing of *Ehrlichia canis* infections in dogs

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Abstract

Ehrlichiosis was confirmed in eight referred dogs by microscopic examination of peripheral blood smear stained with Giemsa stain. The dogs were earlier treated with antibiotic and had the history of severe tick infestation. The clinical examination of the animals revealed increased body temperature, pallor of visible mucous membrane, and heavy tick infestation. The animals were dull and depressed with poor skin condition. The complete blood count (CBC) of eight naturally affected dogs revealed mild leucopenia. There was neutropenia in two dogs and also neutrophilia in five affected dogs. The haemoglobin % was slightly (n=3) to severely (n=1) decreased in some cases and was within normal range in other 4 affected dogs. The dogs were successfully treated with imidocarb dipropionate @ 6.6 mg/kg, S/C.

Keywords: *E. canis*, Haematology, Imidocarb, Microscopy

Ehrlichiosis has been a subject of increasing interest from veterinary and public health perspectives over the last few decades (Paddock and Childs, 2003; Skotarczak, 2003). *Ehrlichia canis* is an intracellular rickettsial organism with cosmopolitan distribution, and is the primary etiological agent of canine monocytic ehrlichiosis (CME). It is biologically transmitted to dogs by Brown dog tick, *Rhipicephalus sanguineus*. Dogs reportedly undergo an acute phase of the diseases after an approximately 8-10 days prepatent period. During this period, pyrexia and leucopenia are common clinical observations. Affected hosts recover 20-30 days post-infection, commonly followed by a subclinical to severe chronic phase that is thought to be a carrier state during which tick vectors could still acquire and disseminate the pathogens to other hosts.

The quickest diagnostic method of *E.canis* is finding the morulae within the circulating monocytes via microscopic examination of peripheral blood smear stained with Wright's or Giemsa stain. The present study deals with haematological alterations associated with canine ehrlichiosis and its successful management with imidocarb dipropionate.

Materials and methods

Eight dogs of different breeds were presented to Teaching Veterinary Clinical Complex, OUAT Bhubaneswar with the history of inappetance, high fever, vomiting and presence of ticks in the kennel premises. The dogs were previously treated with paracetamol, antibiotics, antiemetic, antacids and ectoparasiticide.

Blood samples were collected from recurrent tarsal vein from 8 dogs using EDTA as anticoagulant for haematological studies. The wet blood smear was examined for presence of trypanosomes or microfilaria. Blood smears were stained with Giemsa stain.

The dogs were treated with imidocarb dipropionate (6.6 mg/kg, S/C), ceftriazone with tazobactum (25 mg/kg, I/V for five days), paracetamol (500 mg, PO for two days) and advised to disinfect the kennel premises.

Results

The clinical examination of the animals revealed increased body temperature, pallor of visible mucous membrane with heavy tick infestation. The animals were dull and depressed with poor skin condition. Examination of wet blood smear did not reveal any moving parasites.

The microscopic examination of the blood smears revealed the presence of morulae of *E. canis* within the monocytes (fig-1). Based on the above microscopic findings the cases were diagnosed as *E. canis* infection. The complete blood count (CBC) of eight naturally affected dogs revealed mild leucopenia. There was neutropenia in two dogs and also neutrophilia in five affected dogs. The haemoglobin perecntage was slightly (n=3) to severely (n=1) decreased in some cases and at normal range in other four affected dogs. Thrombocytopenia was found in five dogs out of eight. Significant clinical improvement was observed within two days after initiation of treatment with normal

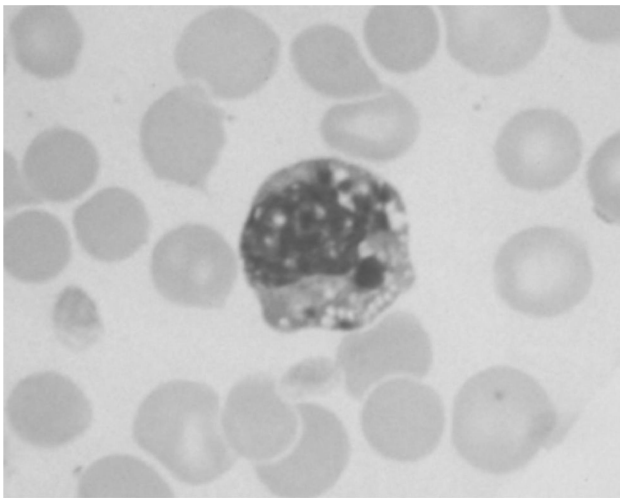

Fig. 1: Morula of *Ehrlichia canis* in monoyte of dog

temperature and appetite.

Discussion

Ticks are one of the important biological vectors for transmission of various bacterial, viral, protozoan and *rickettsial* diseases in dogs. In our study, ticks were found in the body coat of infected dogs. After microscopic examination of peripheral blood smear stained with Giemsa stain, the morulae of *E.canis* was found in circulating monocytes which confirms the diagnosis of canine monocytic ehrlichiosis.

Mild leucopenia has been recorded in canine *Ehrlichiosis* (Waddle and Littman, 1988). Neutropenia is attributed to the excessive destruction against Rickettsial infection (Ettinger and Feldman, 2000). There is decrease haemoglobin level in infected dogs probably due to localization of organism in blood. In the presented case after haematological analysis there is mild leucopaenia, neutropenia in three cases and neutrophilia in other four cases. Neutrophilia observed in some cases might be due to secondary bacterial infection. Hence the dogs were treated with Ceftriazone with Tazobactum along with Imidocarb dipropionate.

Imidocarb dipropionate have been suggested to induce satisfactory therapeutic response against canine ehrlichiosis (Price and Dolan, 1980). The mode of action of Imidocarb dipropionate is uncertain and it has been suggested that it acts against *Trypanosoma brucei* by interfering poly amine production and against *Babesia ovis* by blocking the entrance of inositol into erythrocytes (Bacchi *et al.,* 1981; Mc Hardy, 1986). It was concluded that canine ehrlichiosis can be successfully treated with imidocarb dipropionate.

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Effect of Captopril on renovascular hypertension in chronic renal failure

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Abstract

Present study recorded 50% prevalence of renovascular hypertension in chronic renal failure dogs. Captopril (ACE inhibitor) was used for the therapeutic management of six dogs suffering from chronic renal failure with hypertension, with dose of 1 mg/kg b.wt. po,bid. ACE inhibitors treated patients showed, significant and progressive decrease in hypertension during four days of treatment with concurrent non significant increase in serum creatinine.

Keywords: ACE inhibitors, Arterial hypertension, Chronic renal failure, Creatinine, Dogs

Renovascular hypertension or renal hypertension is a secondary hypertension whose cause is identifiable and syndrome consists of high blood pressure caused by the kidneys hormonal response to narrowing of the arteries supplying the kidneys (Sparkes *et al.,* 1999). Due to low local blood flow, the kidneys mistakenly increase blood pressure of the entire circulatory system by more renin secretion that causes the angiotensinogen conversion to angiotensin I. Angiotensin I then proceeds to converted to Angiotensin II via Angiotensin Converting Enzyme (ACE). Angiotensin II causes vasoconstriction and aldosterone release leading to water and sodium retention and potassium depletion (Stepien and Rapoport, 1999). The increased blood volume and vessel constriction contributes to increased blood pressure which can lead to hypertension. As a result, an ACE inhibitor generally induces little change in GFR in patients who have normal renal function. Present study reports renovascular hypertension in CRF affected dogs and its management by captopril (angiotensin converting enzyme inhibitor) with alteration in serum creatinine level during period of therapy.

Materials and Methods

A total of 100 dogs presented to Teaching Veterinary Clinical Complex, DUVASU, Mathura, suspected to be suffering from renal disease on the bases of history and clinical examination were screened. Serum creatinine level (> 2.0 mg/dl), duration of illness (> 15 days), non-regenerative anemia and high level of protienuria (qualitatively) was used as inclusive criteria for dogs for chronic renal failure to be selected for the study**.** A total 12 clinical cases of dogs were diagnosed to be suffering from chronic renal failure (CRF) irrespective of age, sex and breed with mean age of 84.58 $\pm$21.86 months.

Oscillometric blood pressure measurement technique was used to measure blood pressure placing the cuff on the antebrachium (Stepien and Rapoport, 1999). For end organ effects of hypertension kidney, eyes, brain and heart were examined clinically. Based on history, clinical examination, laboratory examination and blood pressure measurement, renovascular hypertension was diagnosed in 6 clinical cases out of 12. Therapeutic management of dogs with renal hypertension was done with ACE inhibitor (Captopril @ 1 mg/kg b.wt, po, bid) and its effect on serum creatinine level was observed after 5 days of treatment.

Results and Discussion

Arterial hypertension with mean systolic pressure 220.5$\pm$5.08 and diastolic pressure 94.50$\pm$1.89 mm hg was observed in 6 dogs out of 12 dogs suffering from chronic renal failure, showing 50 % prevalence (Tab-1). End organ effects of hypertension was noticed in kidney in form of enhanced rate of decline of renal function, proteinuria in all the six cases as described by Brown, (2001), eyes in the form of retinal detachment and retinal hemorrhage, retinal edema in two cases , which was consistent with Davidson, (2001), heart in the form of secondary murmurs/gallop rhythms. After four days of captopril therapy it reduce to 182.83$\pm$3.56 (systolic) and 77.33$\pm$1.86 mm Hg (diastolic). Comparable normal values generated using direct and indirect methods in dogs are available (Stepien and Rapoport, 1999) for comparison. Some authors have suggested that blood pressures greater than two standard deviations from the mean normal values should be

Table 1. Values of blood pressure before and during captopril therapy

Days	B.P.(mmHg)	
	S (Systolic)	D (Diastolic)
0Day(Before treatment)	220.5+5.08	94.50+1.89
First day	209.17+6.29	91.83+1.76
Second day	202.33±5.64	79.33±1.78
Third day	193.67±3.43	86.17±1.42
Fourth day	182.83±3.56*	77.33±1.86*

* Values differ significantly ($p<0.05$)

considered diagnostic for hypertension (Sparkes *et al.,*1999), but relatively small increases in renal blood pressure may have detrimental effects on end organ function (e.g., progressive glomerular disease). Current recommendations for dogs suggest that systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg measured by any method are reasonable values at which concern is warranted (Cowgill, 2001). HCP, (2002) suggested that blood pressure more than 180/120 always have high risk and require treatment first followed by diagnostic work-up for underlying disease.

Findings of present study were in accordance with Steele *et al.* (2001) who reported that ACE inhibitors decrease the systemic hypertension that is frequently observed dogs with CRF. Grauer *et al.,* (2000) also suggested that angiotensin-converting enzyme inhibitors are useful for therapy of both renovascular hypertension and renal protein loss. The effect of angiotensin-converting enzyme (ACE) inhibitors on renal function in the hypertensive patient is related both to the glomerular actions of angiotensin II and to the mechanism of autoregulation of the glomerular filtration rate (GFR). Angiotensin II constricts both the afferent and efferent arterioles, but preferentially increases efferent resistance (Arima *et al.,* 1997).

Results of captopril treatment showed non-significant increase (from5.43±0.514 mg/dl to 6.64±0.580 mg/dl) in serum creatinine in all the cases treated with ACE inhibitor. The decline in GFR induced by an ACE inhibitor typically occurs within the first few days after the onset of therapy, since angiotensin II levels are rapidly reduced. As a result, the plasma creatinine concentration should be re-measured three to five days after the institution of therapy in patients with known renal artery stenosis and in those at high risk for the disease. The rise in the plasma creatinine concentration is usually mild and acceptable as seen in present study, but large decline in renal function occurs in less than 5 to 10 percent of cases Steele *et al.* (2001). These patients generally require either institution of other antihypertensive agents, like calcium channel blocker, which do not interfere with autoregulation (Arima *et al.,* 1997).

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Clinico biochemical and pathological alterations in experimentally induced arsenicosis in goats

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Abstract

The Jamunapari crossbred goats intoxicated with sodium arsenite @2mg/kg body weight, orally, showed clinical signs of diarrhea and dehydration with death in two of six animals. The blood arsenic level was 2.28±0.47mg/L. The biochemical analysis revealed increased blood glucose (69.34±3.4mg/dl) and total serum protein (6.78±0.38mg/dl) concentration. The pathological study showed severe congestion of abomasal mucosa, fatty degeneration of hepatocytes along with atrophy and vacuolar degeneration of epithelial cells of crypts of liberkuhn.

Keywords: Arsenic, Goats, Clinical signs, Biochemical, Pathology.

Arsenic poisoining is less common in animals. It occurs in two forms. The enteric form is characterized by gastroenteritis whereas nervous form is manifested with incoordination, restlessness and convulsions (Radostits et al 2007). The present puts on record the clinic biochemical and pathological alterations caused by experimentally induced arsenicosis in Jamunapari goats.

Twelve Jamunapari cross goats of Goat farm, College of Veterinary Science & A.H. Anjora, Durg (C.G) were selected for the study. Animals were divided into two groups having six animals in each. Group I animals were kept as healthy control and group II animals were intoxicated with sodium arsenite @2mg/kg body weight orally for the period of one month. All animals were observed for clinical signs and blood samples were collected before and after the trial. Biochemical parameters viz. blood glucose and total serum protein were determined by semi automated biochemistry analyser (Robonik, 13000 using commercial available kits. Arsenic level in blood samples was determined by atomic absorption spectrophotometry (APHA, 1992). Tissues form dead animals were collected and processed for pathological study. Experimental study was undertaken after approval from Institutional Ethics Committee.

Clinical symptoms started on day 4 of experimentation which included anorexia, depression, weakness, diarrhea with blood and dehydration. Two goats of group II animals died on 7th day of experimentation. Similar effects were described by Radostits et al., 2007. The arsenic concentration in blood at the end of the experiment was 2.28±0.47mg/L in intoxicated goats as compared to 0.67±0.33mg/L in healthy control animals. The mean total blood glucose increased significantly ($P<0.01$) to a level of 69±3.4mg/dl from the normal value of 53±1.68mg/dl. This hyperglycemia might be due to gluconeogenesis because of stress of poisoning as also observed by Ghafghazi et al., 1980. The level of serum protein increased form normal value 6.04±0.27g/dl to 6.78±0.38g/dl probably due to haemoconcentration on account of diarrhea.

Pathological study revealed fatty degeneration in hepatocytes because of inflammation caused by the intoxicant. Atrophy and vacuolar degeneration of epithelium of deeper parts of crypts of liberkhun was also observed. Intestine showed infiltration of lymphocytes in the lamina propria. In addition severe congestion of mucosa was evident. Similar post mortem findings were described by Sandhu and Barar (2000) in animals suffering from arsenate poisoning.

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Effect of oral zinc supplementation on plasma zinc status in zinc deficient buffaloe

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Abstract

To assess the effect of Zn propionate, Zn amino acid and Zn methionine on plasma Zn status of buffaloes, a study was conducted on 15 buffaloes with normal plasma Zn level (Group A) and 15 with low plasma Zn levels (Group B). The animals of both the groups were further divided into 3 groups each and were administered 360 mg of elemental zinc daily for 30 days in form of Zn propionate, Zn amino acid and Zn methionine. Blood samples were collected on day 0, 15, 30 and 60 of the start of the supplementation. In Zn deficient animals, an increase in mean plasma Zn concentration was recorded at day 30 and this increase was sustained till day 60 of the start of the supplementation. On the other hand, the animals of Group A showed a non-significant decline in mean plasma Zn levels at day 15 and 30 followed by a non-significant increase at day 60.

Keywords: Buffaloes, Mineral, Plasma, Zinc amino acid, Zinc methionine, Zinc propionate.

Minerals, both macro and microminerals, are essential for better productivity of dairy animals. Low levels of many of the essential minerals have been found in the soil and crops of the Indian state of Punjab (Nayyar *et al.,* 1990) affecting the livestock dependent on it. Zinc deficiency has been recorded among 42.4% dairy animals in three districts of the state (Chhabra, 2006). Various zinc supplementation products have been used in the past by different workers with varying outcome (Spears, 1989 and Pechin *et al*., 2001).Keeping these variations in mind, different sources of zinc were used in Zn deficient animals and those with normal plasma Zn levels to assess the effect of these products in the present scenario.

Material and Methods

For this study, a total of 15 buffaloes with low plasma zinc levels (<12.2 μmol/l) were divided into three equal groups (Group B) of 5 animals each for oral zinc supplementation with zinc propionate, zinc amino acid and zinc methionine. An equal number of buffaloes with normal plasma zinc levels (Group A) were included as control animals for each type of zinc supplement. Each group was given oral supplement equivalent to 360 mg of elemental zinc, daily, for 30 days. All the animals were maintained under same feed and fodder regimen and management conditions throughout the experiment period. Blood samples from all the animals were collected on 0, 15, 30 and 60 days of the start of supplementation. Plasma was separated by centrifugation and was stored at - 10°C for subsequent analysis.

Concentration of zinc was measured by Atomic Absorption Spectrophotometer (SpectrAA 20 plus, Varian, Melbourne, Australia). Statistical analysis of the data was done by method described by Singh *et al.* (1989).

Results and Discussion

In animals of group B after the zinc supplementation with zinc propionate, the mean plasma zinc level increased non-significantly from 8.8 ± 1.33 to 12.95 ± 0.78 μmol/l by day 30 of the study which was above the critical level of 12.2 μmol/l (Radostits *et al* 2000) and this normal level was maintained by the 60th day (Table 1).

Oral supplementation with zinc amino acid resulted in non-significant variations in mean plasma zinc levels of animals of both groups (Group A and B). The initial decline was followed by increase in the values. Mean plasma zinc levels of the deficient buffaloes (Group B) increased and were in normal range by day 30 of the study as was the case in zinc propionate supplementation (Table 1). Similarly the animals of control group A showed a non-significant decline in the mean plasma zinc levels by the day 30 of the therapy. However, by the day 60 of the start of therapy, this value increased (Table 1).

After oral supplementation with zinc methionine, mean plasma zinc levels varied similarly as in case of zinc amino acid supplementation. In the deficient animals of Group B, an initial non-significant decline in the mean plasma zinc at 15th day was followed

Table 1. Effect of zinc supplementation with different zinc sources on the plasma zinc levels of deficient and normal buffaloes (Mean ± SE).

Zinc supplement	Groups	Plasma zinc (μmol/l)			
		0 day	15 day	30 day	60 day
Zincpropionate[1]	A	16.45 ± 4.97	13.39 ± 2.68	16.03 ± 2.8	18.7 ± 2.42
	B	8.8 ± 1.33	9.1 ± 1.50	12.95 ± 0.78	12.25 ± 0.94
Zincamino acid [2]	A	25.05 ± 6.51	14.73 ± 1.44	14.15 ± 1.44	35.19 ± 5.90
	B	9.2 ± 0.87	8.8 ± 0.81	12.65 ± 0.97	13.45 ± 1.89
Zinc methionine[3]	A	27.33 ± 8.12	19.13 ± 1.99	19.13 ± 3.98	24.67 ± 2.10
	B	8.33 ± 0.88	8.2 ± 0.75	12.1 ± 1.61	13.43 ± 1.66

*$P \leq 0.05$; Group A – Buffaloes with normal plasma zinc levels; Group B – Buffaloes with deficient plasma zinc levels.
1 - Kemin Nutritional Technologies Pvt. Ltd., Chennai, INDIA.; 2 - Availa Zinc; Avitech Animal Health Pvt. Ltd., Gurgaon, Haryana, INDIA.; 3 - Vetcare India Ltd. Bangalore, INDIA.

by increase at 30th and 60th day of the study, respectively (Table 1). Spears (1989) recorded non-significant increase in the mean plasma zinc concentration after supplementation with zinc methionine. Pechin *et al* (2001) observed a significant increase in the plasma zinc concentration after 6 months of oral supplementation of Zn in Hereford cattle. On the contrary, Kegley and Spears (1994) recorded no effect on the plasma Zn levels of the lambs after zinc oxide and zinc methionine supplementation. Kegley and Silzell (1999) also reported no variations in the serum zinc levels of steers after 28 days of daily oral supplementation of 360 mg Zn.

On the other hand, in the animals with normal plasma zinc (Group A), the initial decline in the plasma zinc concentration was more prominent as compared to the Zn deficient animals and it was observed till the day 30 but then, again there was an increase in the value at 60th day of the study as shown in Table 1. These findings can be explained by the findings that enhancement of absorption of zinc during its depletion and inhibition during zinc overload occurs rapidly within a week of change in the zinc supply (Underwood and Suttle 1999).

All the above observations revealed that in the zinc deficient animals, there was an increase in mean plasma zinc concentration at 30th day of the therapy after oral supplementation of zinc by various sources. Thereafter, these increased levels were sustained till the observation period of 60 days. On the other hand, in animals of Group A, there were non-significant variations in the mean plasma zinc concentration after oral supplementation of zinc by various sources that could be due to the fact that zinc was removed from blood rapidly by tissues, and that tissues saturated with zinc (muscle) translocated zinc to unsaturated tissues (livers, pancreas and kidneys) (Pekas 1968). Underwood and Suttle (1999) observed that the absorptive efficiency of zinc varied according to the need and if given in excess of need, it would under estimate the potential of dietary zinc sources. This led to the inference that zinc was absorbed according to the need and the absorptive efficiencies.

Five of the zinc deficient animals manifested clinical signs of alopecia and poor body condition. Alopecia had earlier been reported in zinc deficient ruminants by McDowell (2003). Other zinc deficient animals did not show any clinical abnormality. Oral supplementation of zinc for 30 days improved the body and coat condition by the day 60 of the initiation of the treatment.

It is concluded that oral zinc supplementation by all the three types of Zn sources resulted in non-significant increase of Zn plasma levels up to day 60 along with clinical improvement in Zn deficient animals.

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Comparative efficacy of indirect tests in diagnosis of bovine sub clinical mastitis

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Abstract

The present study was conducted on total 614 quarter milk samples collected from 162 apparently healthy lactating cows to determine the comparative efficacy of California mastitis test (CMT) and White side test (WST) in the detection of Subclinical mastitis (SCM) in dairy cows taking somatic cell count (SCC) as standard test. On comparison with SCC, the sensitivity of CMT and WST were 90.63 and 86.72 %, respectively. CMT revealed 93.83% specificity as compared to 89.50% of WST. The percent positive and negative predictive values for CMT were calculated to be 79.45 and 97.43%, respectively, while for WST these were 68.52 and 96.24%, respectively. The overall agreement of CMT (93.16%) was found to be higher than that of WST (88.92%).

Keywords: Comparative, Diagnosis, Efficacy, Sub clinical Mastitis and Tests

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large numbers of leukocytes (Radostits *et al.,* 2007). It results in losses due to reduced milk production, treatment costs, increased labour, milk withheld following treatment, death and premature culling (Kaneene and Hurd, 1990; Miller *et al.,* 1993). Annual losses in the dairy industry due to mastitis is 526 millions dollars in India, in which subclinical mastitis are responsible for approximately 70% of these losses (Varshney and Naresh, 2004).

Diagnosis of clinical mastitis is based on the physical changes in milk as watery, bloody appearance, clots and pus in the milk while the diagnosis of subclinical mastitis is more problematic since the milk appears normally but usually has an elevated somatic cell count (SCC). Subclinical mastitis (SCM) is 3-40 times more common than the clinical mastitis and causes the greatest overall losses in most dairy herds and constitutes a reservoir of organisms that can affect other animal within the herd due to its contagious nature (Schultz *et al.,* 1978).

For detecting SCM in cows, viz. SCC, California mastitis test (CMT) and White Side test (WST), are preferred to be screening test for SCM as these can be used easily and yielding rapid as well as satisfied results (Lesile *et al.,* 2002). CMT and WST are based upon the amount of cellular nuclear protein (DNA) present in the milk sample and SCC has been widely implemented as a screening test to identify intramammary infection in lactating cows (Sharma *et al.,* 2008).

Hence, the present work was undertaken to compare efficacy of CMT and WST in the detection of SCM in dairy cows taking SCC as standard test.

Materials and Methods

The present study was conducted on total 614 quarter milk samples collected from 162 apparently healthy lactating cows, belonging to the Instructional Dairy Farm, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand state, India. After proper cleaning of teat surface with 70% ethanol, about 15 ml of milk sample from all four quarters viz. left fore (LF), left hind (LH), right fore (RF) and right hind (RH) were collected aseptically after discarding few streams in separate sterile polyethylene screw capped, wide mouth vials as described by Buswell (1995) with some modifications. All the milk samples were tested by CMT and WST immediately after collection and were kept in an ice box and carried to the laboratory, where samples were kept at 4^0C in refrigerator for further determination of SCC. The SCC was done by standard microscopic counting method as per the guidelines of Indian Standard Institute (1960) while CMT was conducted as per the method described by Schalm *et al.* (1971). For WST, a glass plate was used that has been etched with vertical and horizontal lines to provide 1.5 inch square.Total 4-5 drops of cold milk were placed in the center of the square and 2 drops of 4% NaOH was added. An applicator stick or toothpick was used to stir the mixture

Table 1. Results of CMT and WST taking SCC as Gold Standard test

Test	SampleTested	SamplePositive	TruePositive	FalsePositive	FalseNegative	TrueNegative
CMT	614	146	116	30	12	456
WST	614	162	111	51	17	435

Table 2. Comparative efficacy of CMT and WST

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OA (%)
CMT	90.63	93.83	79.45	97.43	93.16
WST	86.72	89.50	68.52	96.24	88.921

PPV = Positive predictive value; NPV = Negative predictive value; OA =Overall agreement

vigorously while spreading it over a circular spot of 3 cm in a diameter. The Whiteside test was recorded as positive (+) or negative (-).

The following test characteristics were determined using SCC result as gold standard test. Sensitivity = TP/ (TP + FN) x 100; Specificity = (TN/ TN+FP) x 100; Positive predictive value (PPV) = (TP/ TP+FP) x 100; Negative predictive value (NPV) = (TN/ FN+TN) x 100) Overall agreement (OA) = {(TP + TN)/ N)} x 100; Where: TP-true positive, FP-false positive, TN-true negative, FN-false negative, N-total sample tested.

Results and Discussion

Of 614 milk samples tested by SCC, 128 samples were indicative of subclinical mastitis while 486 samples were found to be negative for SCM. The milk samples found positive by CMT and WST were 146 and 162, respectively. The samples tested to be positive for SCM by SCC were taken as true positive (TP) and the samples negative for SCM by SCC were taken as true negative (TN) for determining the comparative efficacy of CMT and WST in the detection of SCM (Table 1).

On comparison with SCC, the sensitivity of CMT and WST were found to be 90.63 and 86.72 %, respectively. CMT had 93.83% specificity while WST revealed 89.50% specificity. The percent positive and negative predictive value for CMT were calculated to be 79.45 and 97.43%, respectively, while for WST these were 68.52 and 96.24%, respectively. The overall agreement of CMT (93.16%) was recorded to be higher than that of WST (88.92%) (Table 2).

Kathariya *et al.* (2009) also compared efficacy of CMT and WST using SCC as the standard test and found efficacy of CMT as 89.10% and WST as 81.82%, which are in close agreement to the values observed in the present study. Though the reports suggest lower efficacy of CMT and WST, in comparison to the observations in the present study, the superiority of CMT over WST in diagnosis of subclinical mastitis has been reported by other workers as well (Sharma *et al.,* 2010; Sharma *et al.,* 2008; Vihan and Sahani, 1987).

Saravanan *et al*. (2008) recorded specificity of WST as 81.55% and found CMT to be less specific (79.41%), which is contrary to the findings of present study. The sensitivity 73.98% and 58.16%; the positive predictive value 73.85% and 71.25%; the negative predictive value 79.51% and 71.26%; and overall agreement 77.02% and 71.25%; for CMT and WST, respectively, were observed in their study. Though the efficacies reported in the discussed study were lower, relative order of efficacies were in agreement with that recorded in the present study.

The present study suggests that CMT is a reliable diagnostic test and could be used as a regular SCM screening test under field condition.

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Diagnostic potential of vertebral heart scale in dilated cardiomyopathy in dogs

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Abstract

Present study reports the potential of vertebral heart scale (VHS) in diagnosis of dilated cardiomyopathy (DCM) in dogs. A total of 10 dogs suffering from DCM were included in the study. The radiographs were taken in both right lateral and dorso-ventral view. The VHS (mean ±SE) was found to be higher (10.3 ±0.47v) in dogs with DCM.

Keywords: Cardiomyopathy, Dogs, Vertebral heart Scale.

Dilated cardiomyopathy is a disease of myocardium of heart and characterized by dilation of all the cardiac chambers, but in particular left ventricular chamber leading systolic and sometimes diastolic dysfunctions (Tidholm and Jonsson, 2005). Dilated cardiomyopathy (DCM) is one of the most common acquired heart diseases in the dogs of medium to large size breed. The diagnosis of DCM can be attempted by using a combination of history, physical examination and diagnostic imaging, usually thoracic radiography. Determination of heart size is important in evaluating patients with heart disease and an enlarged cardiac silhouette in radiography may be a reliable index of pathological cardiac changes (Buchanan and Bucheler, 1995). Vertebral heart scale (VHS) has been investigated in geriatric dogs (Buchanan and Bucheler, 1995), normal and obese cats (Litster and Buchanan, 2000) for assessment of heart diseases.

Materials and Methods

Radiography: Both right lateral (RL) and dorso-ventral (DV) views of thoracic cavity were taken in full respiration state. A high kV range of 70-90 with low mAs up to 20 mAs were used in the study.

Vertibral heart scale (VHS): In lateral views, the caliper was repositioned over thoracic vertebrae, beginning with the cranial edge of T4 in each lateral views (Buchanan and Bucheler, 1995). In DV radiographs, the sum of the heart axes was measured against the thoracic vertebrae in the RL view. The distance of the caudal calliper point was estimated to the nearest 0.1 vertebra. Long and short axes dimensions were then summed to obtain a value that indicated heart size relative to vertebral length or VHS.

Cardiothoracic ratios (CTR): The CTR was calculated by dividing the largest width of the heart by that of the chest on the films in lateral (CTRL) and transverse (CTRT) plane (Danzer, 1919).

Results and Discussion

The x-ray was taken in RL and DV view. The VHS (mean ±SE) was found to be higher in dogs with DCM. The cardio-thoracic ratio is presented in Table-1 and Fig. 1.

Lee (1975) advocated both RL and DV views for a full radiographic examination in cases of cardiac diseases, as one view may give a misleading impression. A DV view, as opposed to ventro-dorsal, is preferred as this helps ensure the heart is in normal position and prevents superimposition of the scapula. The mean VHS in present study was (10.3 ±0.47v) with range of 9.0v to 13.0 v. The 3 dogs were having VSH (13.0v, 12.0v and 12.0v) which is significantly higher than the mean VHS (9.7v) suggested from earlier study (Buchanan and Bucheler, 1995). While the other 7 dogs were having normal VHS values, however on echocardiographic examinations they were also found to be cardiomyopathic (data not presented). Similarly, in another study, the average VHS value in normal canine were 9.7 (range: 8.5-10.6) (Buchanan and Bucheler, 1995). Lamb *et al.* (2000) evaluated the influence of VHS in radiographic diagnosis of cardiac diseases in dogs and reported that dogs with cardiac disease had a higher mean VHS than normal dogs. Lamb *et al*. (2001) on the other hand studied the breed specific ranges for the VHS in dogs and found significant differences between mean values of the scale for the different breeds. A VHS over 10.7 on the lateral radiograph was suggested as moderately accurate sign of cardiac diseases (Lamb *et al.* (2000).

In DCM, the enlarged caudal border of heart may overlay the diaphragmatic shadow. Generalized

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Table 1. VHS and Cardiothoracic ratio in cases of canine dilated cardiomyopathy

Parameters	Dilated cardiomyopathy Mean± SE	Range
VHS	10.3 ±0.47v	9.0v -13v
CTRT	0.69 ± 0.03	0.56 -0.79
CTRL	0.605±0.02	0.50 – 0.70

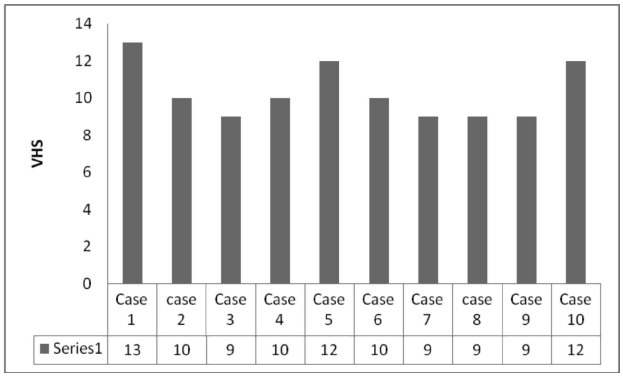

Fig. 1: The case wise VHS in cases of canine dilated cardiomyopathy.

cardiomegaly, enlarged pulmonary vessels, interstitial or alveolar pattern, and enlarged vena cava were the main radiographic findings of DCM (Gugliemini, 2003). Lamb *et al.* (2000) concluded that little was to be gained by measuring VHS when evaluating radiographs of dogs with suspected cardiac disease. However, with cardiac diseases that does not necessarily result in cardiomegaly such as aortic stenosis, dysrythmias and chemodectoma, and HCM so the VHS created a bias against the method. These findings are in close agreement of present study.

The CTR finding of present study are within the range 0.75±0.06 and 0.7651±0.013 (Nabi, 2010) for normal canine population. However, in cases of normal heart weight, a larger CTR may indicate cardiac dilatation due to congestive heart failure or circulatory blood volume overload (Poggi and Maggiore, 1980). Cardiothoracic ratio is a fundamental indicator of cardiac anatomy and function for evaluating cardiac hypertrophy; dilatation and circulatory blood volume overload (Ernst *et al*., 2001). CTR depends on heart weight, and was larger in most cases of heart diseases, suggesting that persistent cardiac overload was a predisposing factor for heart attacks, which is in accord with its general acceptance as a risk factor in clinical evaluation (Hemingway *et al.,* 1998).

In nut shell it can be concluded that radiographic assessment of canine cardiac disease could be useful as adjunct tool to echocardiography and cardiac specific markers. Alone the VSH and CTR could not provide substantial information even the dogs is suffering from DCM. Possibly, this could be due to breed to breed variation in VHS and CTR, and error in CTR that may arise while radiographs are not taken at full inspiration state as expiration could falsely decrease the CTR.

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Evaluation of recombinant LigB antigen in Enzyme linked immunosorbent assay for serodiagnosis of canine leptospirosis

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Abstract

The present study compared the efficacy of Lig B recombinant antigen based Enzyme-linked immunosorbent assay (ELISA) with Microscopic Agglutination Test (MAT) for the diagnosis of canine leptospirosis. The Lig B genes were amplified, cloned and expressed. The recombinant Lig B protein was purified and standardized for ELISA. The sensitivity and specificity of the test were recorded to be 100% and 98.7% respectively No cross-reactions were observed with serum samples from animals showing serum antibodies to other pathogens.

Keywords: Dog, ELISA, Lig B, MAT

Leptospirosis is a disease of worldwide distribution that affects domestic animals, wild life and humans. The standard reference test for leptospirosis, the microscopic agglutination test (MAT), is laborious and time-consuming (Champagne *et al*., 1991). Though there are many other tests for diagnosis of leptospirosis, there is a need for developing a rapid, sensitive, and appropriate diagnostic test that could be used under routine laboratory conditions to detect antibodies against leptospirosis in dogs. Recombinant protein based ELISA is a suitable and safe procedure for the examination of large number of sera as it involves an immunodominant antigen and lacks non-specific moieties present in whole-cell preparations. LigB is a major outer membrane protein, which was found to be conserved among the pathogenic serovars of *Leptopsira* (Koizumi and Watanable, 2004).

The present study explored the utilization of conserved domains of recombinant LigB protein as an antigen in ELISA for bulk screening of anti-leptospiral antibodies in dog. Microscopic agglutination test was carried out according to Faine (1982), using reference strains of *Leptospira interrogans* serovars: Canicola, Pomona, Autumnalis, Australis, Pyrogenes, Patoc, Javanica, Hebdomadis, Icterohaemorrhagiae, Grippotyphosa and Hardjo. A total of 300 canine serum samples, obtained from various parts of the country, were screened for leptospirosis.

The Lig B genes were amplified using specific primer and the polymerase chain reaction product (PCR) was cloned. The gene insert from positive recombinant clones were inserted into expression vector and transformed in *Escherichia coli* DH5α cells. The recombinant Lig B protein was expressed and purified and the optimum concentration of the purified antigen for ELISA was determined by checkerboard titration (Surya *et al*., 2009). ELISA was carried out as per standard procedures of Engvall and Perlmann (1971) with some modifications. The relative sensitivity, specificity, and accuracy of the ELISA were evaluated in comparison with MAT.

Out of 300 canine serum samples subjected to MAT, 143 were positive (47.7%). The sensitivity, specificity, and accuracy of rLIgB ELISA in relation to standard MAT is shown in Table 1.

rLigB ELISA with the recombinant LigB antigen had a sensitivity of 100% relative to MAT that showed MAT titres $\geq$100 when different serovars were tested. ELISA had a specificity of 98.7% relative to MAT when 155 MAT-negative sera were used. ELISA had a specificity of 100%, when tested with serum samples from dog suffering from other clinical conditions that were found to be negative in MAT. The test was found to be statistically significant ($p<0.01$) and had a kappa value of 0.99, suggesting perfect agreement. The advantages of using the recombinant LigB antigen include: high sensitivity and specificity, reproducibility of 100% when performed under the conditions described and faster and easier to perform particularly for testing large numbers of sample. The test is also safe since it eliminates the preparation of whole cell antigenic extracts of leptospires. In addition, the antigen used in the test is stable and the results can be interpreted objectively. The conserved nature and high level of expression of LigB among pathogenic *Leptospira* species (Matsunaga *et al*., 2003) suggest

Table 1. Comparison of IgG-ELISA with MAT for the detection of leptospiral antibodies in canine serum samples

		MAT (No of sera samples)		
		Positive	**Negative**	**Total**
ELISA	**Positive**	143(a)	2(b)	145
	Negative	(c)	155(d)	155
	Total	143(a+c)	157(b+d)	300

Sensitivity=100%; Specificity=98.7%; Accuracy=99.3%, X^2=292.04 ($p \leq 0.01$); κ=0.99

that rLigB ELISA may exhibit similar performance regardless of the locally predominant serovar. Thus, the use of the recombinant LigB antigen in ELISA has the potential to become a useful tool for serodiagnosis of canine leptospiral infection.

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Blood biochemical profile of Khillar breed of cattle in Karnataka

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Abstract

The present work was taken up to study the blood profile of some biochemical parameters in Khillar breed of cattle in Karnataka. Blood samples were collected and analyzed for biochemical profile. On comparing the mean values of the different biocehemical parameters significant difference was noticed only in mean SGPT values between 0-5 years age group and the other two groups (6-10 years and above 10 years). In any of the parameters means were not significantly different between male and female group of animals. These values can be taken as baseline data/reference values for the Khillar breed of cattle.

Keywords : Blood parameters, Cattle, Khillar breed, Reference value.

Karnataka is a state with a rich diversity of livestock with varieties of breeds. Among cattle, few indigenous breeds like Amrithmahal, Hallikar and Khillar are popular in the world for their draught power. Khillar breed of cattle is one, which is being regularly used in farm practice. In spite of recent developments in the preservation and conservation of local germ plasms, not much is known about the Khillar breed as regards to the biochemical profile. Keeping these in points view, the present research study was undertaken with the objective of determining the reference values for some of the blood biochemical parameters in Khillar breed of cattle.

Materials and Methods

This study was undertaken at Khillar Cattle Breeding Farm, Bankapur, Karnataka. A total of 97 apparently healthy animals of different age groups and sex formed the study group. The animals were in the range of 3 – 13 years of age, which were grouped based on the age into 0-5 years, 6-10 years and above 10 years and based on the sex into male and female groups. Within male and female groups, animals were further grouped into three groups each, based on the age of the animal into Group I (0 – 5 years), Group II (6 – 10 years) and Group III (> 10 years).

About 10 ml of blood was collected from the jugular vein and serum was separated by using standard procedures (Sharma *et al*, 2006), which was used for further analysis of biochemical parameters such as SGOT, SGPT, BUN, CRE, P, Ca, ALP, Glu, T.Pro and Alb. Biochemical analysis was carried out using ERBA Chem-5 plus V_2 semi automatic biochemical analyzer of Transasia Biomedicals Ltd., and standard biochemical kits. Results were tabulated and analyzed by using students 't' test and Analysis of variance, according to the procedure of Snedecor and Cochran (1968).

Results and Discussion:

Mean and standard error (SE) values of all the parameters of 97 animals are tabulated in Table I. The means of SGOT, SGPT, BUN, CRE, P, Ca, ALP, Glu, T.Pro and Alb of all 97 animals were 56.20 IU/L, 79.22 IU/L, 19.38 mg/dl, 0.54 mg/dl, 5.53 mg/dl, 8.79 mg/dl, 102.7 IU/L, 42.48 mg/dl 6.19 % and 3.09 mg/dl respectively.

The mean and SE values of all the parameters in 0 – 5 years, 6 – 10 years and above 10 years age groups are tabulated in Table 2. The comparative analysis of means of all the parameters between 0 – 5 years, 6 – 10 years and above 10 years age groups, revealed no significant difference, except mean SGPT values between 0-5 years and the other two groups (6 – 10 years and above 10 years of age) at $P < 0.05$.

The mean and SE values of the biochemical parameters of male and female groups, are tabulated in Table 3 and no significant difference was observed in means of all the parameters between the male and female

Table I. Mean ± SE of biochemical parameters of all the 97 animals

Sl.No.	Parameters	Mean ± SE
1	SGOT(IU/L)	56.20 ± 3.95
2	SGPT(IU/L)	79.22 ± 5.07
3	BUN(mg/dl)	19.38 ± 2.58
4	CRE(mg/dl)	0.54 ± 0.078
5	P(mg/dl)	5.53 ± 0.79
6	Ca(mg/dl)	8.79 ± 0.184
7	ALP(IU/L)	102.7 ± 5.58
8	Glu(mg/dl)	42.48 ± 2.38
9	T.Pro (%)	6.19 ± 0.21
10	Alb(mg/dl)	3.09 ± 0.182

group of animals (P > 0.05).

Based on the age, the male and female group of animals were further grouped into three groups each, Group I (0 – 5 years), Group II (6 – 10 years) and Group III (above 10 years) and mean and SE values of all the parameters are tabulated in Table IV and V. Within the male group of animals, comparison of mean values of all the parameters between Group I (0 – 5 years), Group II (6 – 10 years) and Group III (> 10 years), did not reveal any significant difference except SGPT values, in which mean SGPT values of Group I (0 – 5

Table III. Mean ± SE of biochemical parameters in male and female groups.

Sl.No.	Parameters	Male (n=22)	Female (n=75)
1	SGOT(IU/L)	46.28 ± 4.44	58.99 ± 5.60
2	SGPT(IU/L)	63.85 ± 5.85	69.51 ± 7.77
3	BUN(mg/dl)	16.49 ± 1.89	17.76 ± 1.20
4	CRE(mg/dl)	0.85 ± 0.15	0.34 ± 0.05
5	P(mg/dl)	6.30 ± 0.44	4.89 ± 0.29
6	Ca(mg/dl)	8.13 ± 0.33	10.12 ± 0.476
7	ALP(IU/L)	110.0 ± 1.450	116.6 ± 11.27
8	Glu(mg/dl)	45.45 ± 1.75	50.47 ± 4.96
9	T.Pro (%)	6.38 ± 0.24	5.07 ± 0.30
10	Alb(mg/dl)	2.7 ± 0.09	4.41 ± 1.55

Table 2. Mean ± SE of biochemical parameters in different age groups.

Sl.No.	Parameters	Group I (n=40)	Group II (n=40)	Group III (n=17)
1	SGOT(IU/L)	44.36 ± 5.68	59.19 ± 5.48	56.74 ± 7.79
2	SGPT(IU/L)	49.79 [a] ± 4.37	72.48 [b] ± 5.28	70.72 [b] ± 14.56
3	BUN(mg/dl)	15.46 ± 1.39	15.53 ± 0.17	15.61 ± 0.20
4	CRE(mg/dl)	0.77 ± 0.191	0.53 ± 0.17	0.61 ± 0.20
5	P(mg/dl)	6.21 ± 0.52	5.59 ± 0.55	4.87 ± 0.41
6	Ca(mg/dl)	8.319 ± 0.40	8.82 ± 0.57	9.85 ± 0.91
7	ALP(IU/L)	108.6 ± 7.09	117.4 ± 2.83	114.5 ± 1.90
8	Glu(mg/dl)	40.96 ± 2.52	54.88 ± 3.49	48.32 ± 4.02
9	T.Pro (%)	6.59 ± 0.39	5.36 ± 0.27	5.31 ± 0.26
10	Alb(mg/dl)	3.53 ± 0.83	2.81 ± 0.14	3.02 ± 0.25

b – significant at P < 0.05

Table 4. Mean ± SE of biochemical parameters in different age groups of male Khillar animals.

Sl.No.	Parameters	Group I (n=14)	Group II (n=6)	Group III (n=6)
1	SGOT(IU/L)	41.96 ± 7.32	53.68 ± 8.65	45.97 ± 0.01
2	SGPT(IU/L)	49.79 [a] ± 5.49	71.31 [b] ± 11.74	50.23 [a] ± 2.07
3	BUN(mg/dl)	15.47 ± 2.96	15.42 ± 3.07	18.03 ± 2.3
4	CRE(mg/dl)	0.89 ± 0.260	0.69 ± 2.24	1.11 ± 0.101
5	P(mg/dl)	6.71 ± 0.80	6.21 ± 0.77	5.66 ± 0.77
6	Ca(mg/dl)	7.97 ± 0.40	8.24 ± 0.74	8.97 ± 1.30
7	ALP(IU/L)	109.5 ± 9.01	121.1 ± 5.19	89.94 ± 6.64
8	Glu(mg/dl)	42.13 ± 2.36	49.32 ± 2.28	47.21 ± 4.01
9	T.Pro (%)	7.11 ± 0.23	5.764 ± 1.19	5.77 ± 0.36
10	Alb(mg/dl)	2.68 ± 0.10	2.69 ± 0.17	3.56 ± 0.05

b – significant at P < 0.05.

Table 5. Mean ± SE of biochemical parameters in different age groups of female Khillar animals

Sl.No.	Parameters	Group I (n=30)	Group II (n=34)	Group III (n=11)
1	SGOT(IU/L)	48.36 ± 10.40	64.69 ± 6.74	63.92 ± 11.76
2	SGPT(IU/L)	50.50[a] ± 8.62	73.66[b] ± 0.60	84.38[b] ± 1.83
3	BUN(mg/dl)	15.44 ± 0.97	15.53 ± 0.46	22.38 ± 0.79
4	CRE(mg/dl)	0.50 ± 0.14	0.25 ± 0.03	0.29 ± 0.03
5	P(mg/dl)	5.55 ± 0.52	4.76 ± 0.59	4.35 ± 0.11
6	Ca(mg/dl)	9.93 ± 0.82	9.98 ± 0.39	10.45 ± 1.34
7	ALP(IU/L)	106.4 ± 1.68	112.5 ± 1.73	130.9 ± 3.2
8	Glu(mg/dl)	48.21 ± 7.13	64.15 ± 5.26	49.06 ± 6.91
9	T.Pro (%)	5.54 ± 0.85	4.68 ± 0.31	5.01 ± 0.27
10	Alb(mg/dl)	2.54 ± 4.63	3.05 ± 0.27	2.66 ± 0.22

Table 6. Mean ± SE of biochemical parameters in different age groups within male and female Khillar animals.

Sl.No.	Parameters	MALE			FEMALE		
		Group I (n=14)	Group II (n=6)	Group III (n=6)	Group I (n=30)	Group II (n=34)	Group III (n=11)
1	SGOT(IU/L)	41.96 ± 7.32	53.68 ± 8.65	45.97 ± 0.01	48.36 ± 10.40	64.69 ± 6.74	63.92 ± 11.76
2	SGPT(IU/L)	49.79^{a} ± 5.49	71.31^{x} ± 11.74	50.23 ± 2.07	50.50^{y} ± 8.62	73.66 ± 0.60	84.38^{y} ± 1.83
3	BUN(mg/dl)	15.47 ± 2.96	15.42 ± 3.07	18.03 ± 2.3	15.44 ± 0.97	15.53 ± 0.46	22.38 ± 0.79
4	CRE(mg/dl)	0.89 ± 0.260	0.69 ± 2.24	1.11 ± 0.101	0.50 ± 0.14	0.25 ± 0.03	0.29 ± 0.03
5	P(mg/dl)	6.71 ± 0.80	6.21 ± 0.77	5.66 ± 0.77	5.55 ± 0.52	4.76 ± 0.59	4.35 ± 0.11
6	Ca(mg/dl)	7.97 ± 0.40	8.24 ± 0.74	8.97 ± 1.30	9.93 ± 0.82	9.98 ± 0.39	10.45 ± 1.34
7	ALP(IU/L)	109.5 ± 9.01	121.1 ± 5.19	89.94^{a} ± 6.64	106.4 ± 1.68	112.5 ±1.73	130.9^{b} ± 3.2
8	Glu(mg/dl)	42.13 ± 2.36	49.32 ± 2.28	47.21 ± 4.01	48.21 ± 7.13	64.15 ± 5.26	49.06 ± 6.91
9	T.Pro (%)	7.11 ± 0.23	5.764 ± 1.19	5.77 ± 0.36	5.54 ± 0.85	4.68 ± 0.31	5.01 ± 0.27
10	Alb(mg/dl)	2.68 ± 0.10	2.69 ± 0.17	3.56 ± 0.05	2.54 ± 4.63	3.05 ± 0.27	2.66 ± 0.22

b, y - significant at $P < 0.05$.

years) and Group II (6 – 10 years) and Group II (6 – 10 years) and Group III (> 10 years), differed significantly ($P > 0.05$). Within the female group, in any of the parameters no significant difference was noticed except mean SGPT values which differed significantly between Group I (0 – 5 years) and other two groups i.e., Group II (6 – 10 years) and Group III (above 10 years), at $P > 0.05$.

The means of all the biochemical parameters of each age group of males were also compared with all the three age groups of female by using Two way ANOVA test and presented in Table 4.

Critical analysis of the data between Group I of male and all the three age groups of female revealed no significant difference in any of the parameters, except between Group I of male and Group III of female in mean SGPT values at $P < 0.05$.

On comparing group II of male with all the three age groups of female, there was no significant difference in any of the parameters, excepting SGPT mean values between group II of male and group I and III of female ($P < 0.05$)

Comparison between Group III of male with all the three age groups of female revealed significant difference only in mean ALP values ($P < 0.05$) between Group III of male and Group III of female.

The findings of this study may serve as reference values in which alterations due to metabolic disorders, nutrient deficiency, physiological and health status could be compared for diagnostic and therapeutic purpose in the Khillar cattle breed, which are unique cattle breed adapted to existing climatic, nutritional, environmental and pathological environment of Northern Karnataka and parts of Maharastra.

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Comparative efficacy of Ivermectin, Eprinomectin and herbal paste of *Annona squamosa* leaves on Sarcoptic mange in dogs

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Abstract

The present study was conducted to reveal the comparative efficacy of Ivermectin, Eprinomectic and *Annona squamosa* leaves on sarcoptic mange in dogs. Biochemical and hematological parameters were assessed and efficacy was observed on the basis of normalcy of values. Decreased Hb, PCV, TEC whereas increased TLC was observed in affected individuals. Biochemical findings indicated decreased blood glucose and protein profile. Ivermectin and *Annona squamosa* was found effective but Eprinomectin is proved to be the most effective drug in the treatment of Sarcoptic mange.

Keyword: Dogs, Sarcoptic mange, *Annona squamosa,* Eprinomectin.

Sarcoptic mange commonly known as canine scabies is an extremely pruritic and contagious skin condition caused by parasitic Sarcoptic mange causes intensely itchy skin in dogs, cats and other mammals and is extremely contagious to other pet and its zoonotic potential cannot be ignored. Sarcoptic mange in dog is caused by mites, accounts for the fact that the disease is known as both sarcoptic mange and scabies. The disease spreads form dog to dog and also to human by contact (Dominguez et al., 1978). There are many medications which provide effective treatment option in dogs but have toxicity resistance, residual effects and reoccurrence after recovery. Hence further study is required to find out a suitable and effective therapy against sarcoptic mange in dogs.

Materials and Methods

For the study 6 healthy and 18 naturally infected dogs with sarcoptic mange were selected and randomly divided into four groups viz A, B, C, and D. The dogs of different group received the treatment as given in Table 1. The therapeutic efficacies of the different treatment regimen were evaluated on the basis of clinical improvement, negative skin scraping, and restoration of the hemato-biochemical parameters on 0, 7th 14th 21st and 28th day post treatment.

Fresh part of leaves of *Annana squamosa* wer prepared and applied twice a week for 4 weeks. The dogs were carefully watched so that it should not lick or eat the paste and after 10 minutes the paste was washed away.

Results and Discussion

Therapeutic efficacy of different drugs was evaluated on the basis of clinical symptoms and hemato-biochemical changes in dogs of different groups. There was appreciable improvement of all the three treated groups on 14th day of treatment. However, improvement was remarkable in the dogs of group B where eprinomectin was used. No mites were observed in the skin scraping of all treated dogs on 21st day post treatment. The lesions healed completely and the skin became glossy and regained its normal texture and colour. Reinfection was not observed for 3 months post treatment period.

The hemoglobin value increased slowly in all the treated groups and maximum restoration was observed in group B dogs followed by dogs of group A and C. The hemoglobin value increased gradually in treatment group and reached almost up to normal value on 28th day. The findings were similar to the findings of Dadhich and Khanna (2008). Maximum restoration was observed in group B animals might be due to good miticidal affect of eprinomectin against canine sarcoptic mange.

The mean packed cell volume in different treatment groups was almost similar to the findings of Chandy *et al.*, (2000). The mean PCV value of affected dogs was significantly low as compared to PCV value of healthy group before treatment. However there was gradual restoration of PCV values towards treatment.

Significant increase in the mean values of TEC recorded after therapy in all the groups of dogs. The findings simulating the findings of Dadhich and Khanna (2008). Significant increase in the mean TEC values in group B dogs could be due to early response to the therapy given to the dogs of group B.

Table 1. Showing treatment regimen for the dogs of different groups.

S.No	Groups	No. of animals	Treatment Regimen
1	A	6	Inj. Ivermectin @200µg/Kg B.wt. s/c weekly interval for 4 Weeks
2	B	6	Eprinomectin (0.5%) pour on @1ml/10Kg B.wt. weekly interval for 4 weeks.
3	C	6	Application of paste of *Annona squamosa* leaves twice a week for 4 weeks
4	D	6	Healthy control.

The mean TLC values of different groups were significantly higher than the mean TLC value of healthy control group. The present finding simulates the findings of Chandy *et al.*, (2000).

The mean values of neutrophil, lymphocyte, eosinophil of affected groups (0 day) were significantly higher than healthy control group. Neutrophilia might be due to mobilization of marginal and bone marrow granulocyte pool (Jani, *et al.*, 2003). Chandy *et al.*, (2000) also observed that the dogs affected with sarcoptic mange had significantly higher leukocytes, neutrophil, lymphocyte and eosinophil after the treatment. The mean value of monocyte and basophil remained unaltered. There was no significant difference between the mean values of monocyte and basophil of treated groups and healthy group.

Differential leukocyte count revealed significant leukocytosis, neutrophilia and higher eosinophil values in the affected dogs before treatment. The present findings simulated with the findings of Aujla *et al.*, (2000).

The mean blood glucose values of the affected groups were slightly lower than the healthy group. Similar findings were reported by Dadhich and Khanna (2008). The slight difference in blood glucose could be due to decreased food intake.

The mean value of total protein of affected groups (0 day) was slower than the mean value of total protein of healthy group. The decrease in the mean total protein value can be attributed to the seepage of protein through exudation. Significant difference was observed in the mean values of serum albumin and globulin of affected group as compared to healthy group. The present findings were in accordance with the findings of Chandy, et al., (2000). Thus form the present study it was confirmed that eprinomectin showed better efficacy against sarcoptic mange in dogs than ivermectin and *Annona squamosa* on the basis of clinical and hemato-biochemical studies.

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Clinical Article

Phalaris toxicity in a mare

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Abstract

A two and a half years old Kathiawari mare was presented to Teaching Veterinary Hospital, GADVASU, Ludhiana with mild nervous signs and a missary of being fed with *Phalories spp*. grass. The animal was treated with intravenous fluid (Ringers Lactate Solution and Normal Saline Solution), Flunixin meglumine @ 1 mg/ kg body weight intravenously. However, analgesics failed to respond and the animal collapsed after showing nervous signs suggesting a possibility of *Phalaris* toxicity. From the present report, it can be concluded that Phalaris spp grasses may be toxic to horses, hence should not be fed for long.

Keywords: *Phalaris*, Mare, Toxicity.

Grasses of genus *Phalaris* (Canarygrass) grow on every continent except Antarctica. In India, *Phalaris* spp (mainly Reed Canarygrass *Phalaris arundinacea*) grows mainly as a weed in wheat crop, but sometime it may occupy the entire field due to its rampant growth. It is a perennial grass with poor nutritional value, but can grow on wet, poorly drained soils and can survive prolonged flooding (Cheek 1995). In Australia, *P aquatica* is used as a fodder and thought to be safe for horses. However, many species of genus *Phalaris* are toxic including bulbous canarygrass or hardinggrass (*Phalaris aquatica*, previously known as *Phalaris tuberose*; it is supposed to be the most toxic), timothy canarygrass (*Phalaris angusta*), reed canarygrass (*Phalaris arundinacea*), littleseed canarygrass or annual canarygrass (*Phalaris minor*), and Carolina canarygrass or May grass (*Phalaris caroliniana*) (Binder *et al*., 2010). There are several reports documenting acute or chronic *Phalaris* toxicity in ruminants (Bourke *et al*., 2005; Odriozola *et al*., 1991). However, there is paucity of reports on *Phalaris* toxicity in horses. The present report describes a case of *Phalaris* toxicity in a mare.

A two and a half years old Kathiawari mare was presented to Teaching Veterinary Hospital, GADVASU, Ludhiana with mild nervous signs including excitation and colic like symptoms that was first noticed 48 hours back. The mare passed semi-loose faeces once about 12 hours before the presentation. The clinical examination revealed heart rate 90 beats per minute, respiration rate 56 per minute and cyanosis of visible mucous membrane. The respiration was abdominal; though auscultation of lung revealed normal lung sounds. When asked about the feeding history owner revealed that the mare was being fed with *Phalaris* spp grass, which is locally known as "Gulli danda". The blood samples were collected for haematological and biochemical examination. The lateral radiograph of chest revealed no visible abnormality. The blood picture revealed Hb 20.7 g per cent, TLC 11910/ cu mm; TEC 13.29 X 10^6/ cu mm, PCV 51.7 per cent, Neutrophil 60 per cent and lymphocyte 40 per cent). The biochemical parameters (Creatinine 1.2 mg/ dl, BUN 23 mg/ dl and GGT 30.5 Units/ L) were within the normal range. The animal was treated with intravenous fluid (Ringers Lactate Solution and Normal Saline Solution), Flunixin meglumine @ 1 mg/ kg body weight intravenously, Xylazine hydrochloride @ 0.5 mg/ kg body weight intravenously and Amoxicillin 2.5 gm intravenously. However, the condition failed to respond and the animal started showing nervous sings including generalized tremors, hyperexcitability, marked incoordination, hypermetria, salivation and tachypnea. Rectal temperature reached 103.5^0 F and heart rate 100 beats per minute. The animal collapsed within two hours after appearance of these nervous signs.

Symptoms including hyperexcitability, incoordination generalized tremors, , hypermetria and tachypnea are commonly observed in cattle in acute natural (Odriozola *et al*. 1991, Riet-Correa 1993) and experimental (Sousa and Irigoyen 1999) *Phalaris* toxicity cases. However, chronic *Phalaris* poisoning in cattle, sheep, horses and pigs is characterized by posterior paresis and paralysis, wherein accumulation of brownish pigment in neurons occur in the brain and spinal cord (Botha and Penrith 2008). In the present case, there was no history of impaction and the haematobiochemical parameters were almost normal, showing changes related to dehydration only. Moreover,

analgesics failed to respond and the animal collapsed after showing nervous signs suggesting a possibility of *Phalaris* toxicity. Similar clinical signs of sudden death after showing nervous signs were reported in horses suspected for blue canary grass (*Phalaris coerulescens*) poisoning (Colegate *et al*., 1999).

Grasses of genus Phalaris contains several toxic alkaloids and chemicals including tetrahydro-β-carbolines, oxindoles and furanobisindole (Phalarine) (Colegate *et al*., 1999). *Phalaris* poisoning comprises two major syndromes, a sudden death syndrome and a nervous syndrome (Bourke *et al*., 1990) In sudden death syndrome, sudden collapse and cardiac arrest occurs. The cause of cardiac arrest remains unknown but does not seem to be associated with tryptamine alkaloids (Kennedy *et al*., 1986; Bourke *et al*., 1988). The neurologic syndrome is caused by dimethylated indole amines (tryptamines). Bourke *et al*. (1990) suggested that the nervous syndrome is due to direct action of *Phalaris* alkaloids on serotonergic receptors in specific brain and spinal cord nuclei. Characteristic indole-like pigments are found in the brain and spinal cord in animals with *Phalaris* staggers which is supposed to be post-effect metabolites of tryptamine alkaloids present in this plant (Bourke *et al*., 1990). From the present report, it can be concluded that Phalaris spp grasses may be toxic to horses, hence should not be fed for long.

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Hypoparathyroidism in a leopard- A case study

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Abstract

A case of hypoparathyroidism was reported in a Leopard that had low Serum PTH, serum calcium and high serum phosphorus level. Improvement in affected leopard was observed when treated with calcium gluconate.

Keywords: Calcium gluconate, Hypoparathyroidism, Leopard.

The parathyroid gland appears to synthesize excessive quantities of PTH the amount of PTH available for secretion being controlled by Ca^{2+} dependant degradation. Therefore the secretory product of the parathyroid gland consist of intact hormone and fragments, principally carboxyl terminal fragments (Morrissey *et al*., 1980). Circulating PTH is heterogenous composed principally of intact hormone and middle and carboxyl terminal fragments of PTH. The ratio of large carboxyl terminal fragments to intact PTH in the circulation has been shown to vary depending on the plasma calcium concentration, the proportion of the intact hormone increasing in hypocalcemia (D'Amour *et al*., 1992).The principal function of PTH is to maintain plasma calcium concentration within a narrow range through action on bone and kidney.

A 3 year old leopard was presented to the Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, Nagpur veterinary college, Nagpur by the Maharajbag Zoo. The chief complaint was unable to rise with hind limbs and not taking food. The water intake was normal. On physical examination the leopard was quite responsive. The body condition was slightly thin and there was no evidence of clinical dehydration. The temperature of leopard was normal. Complete blood count, serum biochemistry, fecal sample examination was carried out. (Table 1).

Serum biochemistry reveled low serum Calcium (6.04 mg/dl) and high serum Phosphorus (6.82 mg/dl). Fecal sample was negative for parasitic ova. On fourth day serum sample reveled low PTH concentration (PTH 1.0 PG/ml). Leopard was treated for hypocalcemia with Calcium Gluconate (10%) 10 mg/kg slow IV. Animal revealed positive clinical response. On day 6 of post treatment serum Ca and P returned to their normal level i.e. 11.21 mg/dl and 4.12 mg/dl respectively.

Primary hypothyroidism is rare disease in feline (Bassett, 1998). Definitive diagnosis is based on demonstrating inappropriately low serum intact PTH concentration. Parathyroid hormone having three main action stimulation of osteoclastic bone resorption of calcium and phosphorus; stimulation of increase calcium and decrease phosphorus; reabsorption of parent Vit. D which act to increase calcium absorption. Hypocalcemia and hyperphosphatemia can occur due to

Table 1. Hemato-biochemical parameters of 3 year old leopard

Parameter	Result	Parameter	Result
Hb (gm/dl)	9.6	Serum Urea (mg/dl)	72
PCV (%)	30.9	Serum Creatinine (mg/dl)	1.27
MCV(%)	47	Serum Sodium (mmol/dl)	134
MCH (pg)	14.6	Serum Potassium (mmol/dl)	4
MCHC (gm/dl)	30.9	SGOT (IU/L)	44
TEC (million/cmm)	6.55	SGPT (IU/L)	58
Platelets (lac/cmm)	4.5	Total Bilirubin (mg/dl)	0.68
TLC (10^3/cmm)	8.5	Total Protein (gms/dl)	7.97
DLC		Albumin (gms/dl)	2.84
Polymorph (%)	57	Globulin (gms/dl)	5.13
Lymphocyte (%)	34	CPK (IU/L)	192
Eosinophil (%)	4	Calcium (mg/dl)	6.04
Monocyte (%)	5	Phosphorus (mg/dl)	6.82

hypoparathyroidism. The evaluated phosphorus was attributed to hypoparathyroidism because phosphorus excretion by the kidney must have been withheld.

Parathoromne estimation revealed 1.0PG/ml confirmed hypoparathyroidism. The leopard became normal on administration of Calcium gluconate IV.

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Therapeutic management of acute urea poisoning in a bullock- A case report

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ABSTRACT

A 5 year old crossbred Jersey bullock was presented to Teaching Veterinary Clinical Complex with history of restlessness, frothy discharge from mouth and nose, forced and rapid breathing, frequent kicking at abdomen, violent struggling and bellowing, twitching of ears and facial muscles, grinding of the teeth, colic and bloat after accidental ingestion of toxic dose of urea fertilizer. Examination of rumen liquor revealed watery, dark brown coloured rumen fluid with ammoniacal odour and alkaline pH. The case was diagnosed as acute urea poisoning on the basis of history, clinical findings and rumen liquor examination. The animal was treated successfully with acidifier agents, supportive therapy and cud transplantation.

Keywords: Bullock, Urea fertilizer, Urea poisoning

Dietary urea has been used as an effective and inexpensive source of non protein nitrogen (NPN) in ruminants. The process of microbial digestion of NPN by ruminal fauna helps in its utilization (Taylor-Edwards *et al.,* 2009). The rumen microorganisms hydrolyze urea into ammonium (NH_4^+) and ammonia (NH_3) to synthesize protein. At pH 7.0, approximately 99% of NPN is converted to ammonium and 1% as ammonia, and the ammonia level gets higher with increase in pH towards alkaline (Visek, 1984). The excess amount of lipid-soluble ammonia is absorbed into the blood stream. Normally, the liver can detoxify ammonia into urea by Krebs & Henseleit cycle in the hepatocytes, which is 40 times less toxic than ammonia and finally excreted by the kidneys (Visek, 1968). But urea poisoning occurs when the concentration of ammonia in the blood is beyond the capacity of the hepatocytes for detoxification, and when the urea level exceeds the kidney threshold for elimination (Davidovich *et al.,* 1977; Visek, 1984). Urea poisoning may occur periodically in ruminants that consume large quantities of urea when they are not adapted to it, and when feeds are inappropriately mixed with urea, or in a low energy, low protein and high roughage diets.

The onset of clinical picture may start in a matter of minutes to hours after consumption of urea and in most cases it is acute and can cause heavy mortality (Ortolani *et al.,* 2000). The present report describes a clinical case of urea poisoning and its therapeutic management.

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A 5 year old crossbred Jersey bullock, 300kg body wt. was presented in Teaching Veterinary Clinical Complex, OUAT, Bhubaneswar, with history of ingestion of toxic dose of urea fertilizer. On physical examination, the bullock was dull, weak and depressed possessing clinical signs like restlessness, frothy discharge from mouth and nose, forced and rapid breathing, frequent kicking at abdomen, violent struggling and bellowing, twitching of ears and facial muscles, grinding of the teeth, colic and bloat. Clinical examination revealed high rise of rectal temperature (104^0F), increased respiration rate (38/min), strong jugular pulse (90/min), tympanic sound on percussion of left para-lumbar fossa and reduced ruminal motility (2 times/5 min). The rumen fluid was dark brown in colour with watery consistency, ammoniacal odour and alkaline pH (pH - 8). From the above history, clinical findings and rumen fluid examination the case was diagnosed as acute urea poisoning.

Stomach tube was passed into the rumen to relieve gases from the rumen. Gastric lavage was done with 30L of cold water and then, 2L of vinegar (5% acetic acid) was administered into the rumen through the stomach tube followed by 500ml of 5% vinegar orally next day. The animal was given chlorpheneramine maleate (chloril) 10ml I/M once, hepatostimulant (Tribivet) 10 ml I/M on alternate days (3 injections), inj. Oxytetracycline 20 ml I/V and 10 ml intraruminal for 3 days, 2 bolus of bio-boost orally twice daily for two days and 3L of parenteral Ringers lactate for two days. On the third day, 5 L of fresh cud was transplanted through stomach tube and the owner was advised to feed good quality of soft palatable hay. Three days

following the cud transplantation, the animal recovered and the ruminal pH became 6.5, pulse (78/min) and respiration (18/min).

Acute urea poisoning generally occurs in ruminants due to accidental consumption of large quantities of urea (Antonelli *et al.,* 2004; Radostits *et al.*, 2006; Antonelli *et al.,* 2007). It can also occur in cattle due to ingestion of toxic levels of urea from concentrate feed, due to an error in urea addition or due to uneven mixing. The most useful diagnostic indicators of urea poisoning are the history of access to urea and the clinical signs shown by the affected animals. Laboratory tests of blood samples are not very helpful at the early stage, and no specific changes are seen at post-mortem examination. The clinical signs most often observed are restlessness, dullness, weakness, muscle tremors, profuse salivation, rumen atony, bloat, dyspnoea, in- coordination, vocalization, tonic-clonic convulsion etc. (Kitamura *et al.,* 2003; Radostits *et al.,* 2006). Vinegar was given as an acidifier agent for correction of ruminal pH and cold water can dilute the excess urea. Oxytetracycline was given to prevent further production of ammonia. Ringers lactate was used to avoid toxaemia and hepatoprotective agent helped in hepatic detoxification.

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Management of mange infection in New Zealand white rabbit

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Abstract

Ten rabbits with history of generalized dermatitis and cutaneous encrustation were brought to clinic for the treatment. Clinical and skin scrapping examination confirmed *Sarcoptic scabie* and *Psoroptes cuniculi* infections. All rabbits were treated with ivermectin @0.2 mg/kg b.wt. Subcutaneously and cured successfully.

Keywords: Mange, Pruritis, Sarcoptes and Psoroptes.

Mange infestation is a common problem for the fur bearing pets and rabbit is highly susceptible to the mange infestation. Siegenmund (1979) and Quesenbery (2000) also reported that the ear mites and mange mites infestations are major skin diseases in young and adult rabbits. Among the various parasitic infestations, mange infestation is of high incidence in rabbit (Rajeshwari *et al.*, 2001). Mange infestation is highly contagious and pruritic in nature and having zoonotic importance. Kumar *et al.* (2002) observed that mange is a most obstinate, persistent and contagious disease with zoonotic importance.

Ten New Zealand white rabbits between 1 to 2 years of age brought to Teaching Veterinary Clinical Complex, B.V.C., Patna for treatment were studied. The rabbits were suffering from severe dermatitis. Skin was wrinkled and dry crusts were noted on the face, upper part of the neck, around the eyes and on nostrils. Ear was severely affected and appeared as a cotton made artificial ear due to heavy crust formation and dermatitis. The lesions were also observed on both fore limbs and the toes of the fore and hind limbs. The lesion was pruritic in nature and on itching and scratching there was shedding of the fur and it was also severely tagged. The rabbits looked ugly in appearance and were dull, depressed and anorectic showing weakness and weight loss. The skin scrapings were positive for the presence of mange. Out of 10 rabbits, 2 were suffering from sarcoptic mange (*Sarcoptic scabei*) and 8 were suffering from *Psoroptes cuniculi*.

The rabbits were treated with the 4 doses of subcutaneous injection of Ivermectin (Neomec) @ 0.2 mg/kg body weight on 0 day, 7th day, 14th day and on 21st day. Amitraz (12.5%) was used as topical application on single occasion @ 3ml/lit. of water. Cypermethrin was also used for the cleaning of the cage and premises of the rabbit. A Probiotic (Proviboost) @ 3 drops orally twice a day for seven days. A skin lotion Kiskin was used topically on the lesion. After each injection of Ivermectin, deep skin scraping examination has been performed to detect the presence of mange infestation.

Generalized infection to the whole body appeared mainly due to contact during itching.

Fig. 1: Showing crust on ear pinna.

Fig. 2: Showing alopecia.

According to Soulsby (1982), sarcoptic mange in rabbits first appear on the head and ear and then becomes generalized being associated with intense pruritis with the loss of hair and infection is spread mainly due to contact by wandering, nymphs and fertilized young females. Weekly injection of Ivermectin for 3 weeks has given successful results in 8 rabbits, but 2 rabbits having severe lesion on their face, neck, ear, around the eyes could not completely recover and some lesions were left behind on their ear pinna and face. Hence, all rabbits were given 4th dose of Ivermectin on 21st day. After that, all rabbits completely recovered with disappearance of itching and crust formation. Gradually crusts started to disappear and after 4th week crusts completely disappeared and patches of alopecia were only left behind. From 5th week onwards hair begins to grow on the patches of hair loss. Weekly skin scraping examination indicated the reduction of concentration and severity of the infection of mange. The observations are in accordance with the findings of Queensberry (2000) and Kumar *et al.* (2002). Rabbits restored their appetite. After 2-3 months, the fur of the rabbit again grew on the patches and the rabbit looked normal.

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Polioencephalomalacia in a goat

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Abstract

A case of thiamine responsive polioencephalomalacia (PEM) in a 9 month old non-descriptive female goat showing signs of profuse salivation, in-coordination with occasional aim less movement, circling and falling down. The clinical examination revealed congested conjunctivae mucus membrane, swollen and bulging tendency of eye ball, profuse salivation with facial tremors and typical star gazing posture. The animal was treated with parenteral injection of thiamine (Inj Neurobion forte) preparation showed a good response within 12 hours after initial treatment. The immediate and dramatic response to thiamine treatment coupled with the reports of general hematology and other biochemical test used routinely in laboratory can differentiate it from other neurological conditions beside specific laboratory diagnosis of PEM.

Keywords: Goat, Polioencephalomalacia, Thiminine

Thiamine responsive polioencephalomalacia (PEM) is a non-infectious neurological disease of ruminants and characterized by neurological manifestation (Debasis and Ghosh, 2009; Radostits *et al.,* 2007). PEM was previously described as thiamine deficiency disease; however, more recent evidence indicates that the disease actually results from a disturbance in thiamine metabolism and absorption (Rachid *et al.,* 2011). The probable mechanisms include increased numbers of thiaminase producing bacteria in the rumen, production or ingestion of thiamine analogs and impaired absorption of thiamine (Radostits *et al.,* 2007; Rachid *et al.,* 2011). Animals which are kept on high-concentrate diets are at more risk, as the low pH of rumen favors the growth of thiaminase producing bacteria (Mouli and Basu, 2004; Radostits *et al.,* 2007). Several cases have been associated with PEM which includes sulfur intoxication, lead poisoning, and use of amprolium, thiobendazole and levamisole hydrochloride for medication (Pander, 2000; Radostits *et al.,* 2007). The present communication reports a case of thiamine responsive PEM in a goat its diagnosis and therapeutic management.

A non-descriptive female goat of 9 months, weight around 8 kg was presented to Referral Veterinary Polyclinic, IVRI, Izatnagar, with a history of sudden onset of anorexia, profuse salivation, in-coordination with occasional aim less movement, circling and falling down. The clinical examination revealed congested conjunctive mucus membrane, swollen and bulging tendency of eye ball, profuse salivation with facial tremors and typical star gazing posture. Although there was ruminal atony, but temperature, pulse and respiration were all in normal ranges with slight variation along with normal micturition and defecation. Blood, urine and stool samples were collected for further investigation. Blood sample was subjected to routine hematological (Hb, PCV, TEC, TLC, DLC and Haemprotozoans) and biochemical tests like blood glucose, BUN and serum creatinine. Whereas urine samples was screened for presence of ketone bodies by Rothera's test. All blood parameters and biochemical tests were in normal range with negative reports of blood smears and Rothera's test. Fecal examination showed no evidence of ova or oocysts of endoparasites.

Keeping in mind the clinical signs, blood picture and the other biochemical tests the case was presumed to be the cases of thiamine associated PEM and accordingly high doses of neurotropic vitamin-B injection (Inj. Neurobion forte) was given @ 5 ml iv in 250 ml of DNS for 3 days. There was sudden improvement in the case after 12hrs of treatment with normalcy towards gait and posture, and animal became normal after three days of treatment. The findings are in line with the finding of others in responsiveness toward thiamine treatment (Debasis and Ghosh, 2009; Mouli and Basu, 2004). Laboratory tests used to confirm PEM in ruminants are based upon blood chemistry to determine the depressed level of blood thiamine and RBC's transketolase activity and rising of pyruvate and lactate levels (Ramos *et al.,* 2006) whereas rumen and fecal samples can be tested for thiamine activity; however, these tests are not routinely done or available in laboratory (Margo *et al.,* 2002). But Signs of progressive CNS involvement coupled with the sudden responsiveness towards thiamine therapy as in the present case can be used as clinical diagnostic technique for thiamine responsive PEM in animals.

Since early treatment is necessary for ruminants with PEM as the prognosis is considered favorable if the treatment is started early in the disease. The present reports with rapid response to thiamine treatment offer clinicians a clinical method to diagnose PEM in field condition where specific laboratory tests are not available or feasible to perform.

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Editorial board and Indian Society for Veterinary Medicine wish "A Very Happy and Prosperous Happy New Year- 2014"

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Unilateral facial paralysis in cattle - A case report

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Abstract

Unilateral facial paresis or paralysis is uncommon in cattle and occurs due to trauma, inflammation, granulomas, tumors etc. Clinical signs of facial paralysis vary with the location, severity and chronicity of the lesion. In the present case one and half year old heifer was showed clinical signs such as relaxed third eye lid, no blinking of eyelids and slight drooping of ear. Based on the clinical signs it was diagnosed as dysfunction of auriculopalpebral branch of facial nerve which was successfully treated.

Keywords: Cattle, Facial, Paralysis, Unilateral.

Asymmetry of facial expression is a common occurrence with unilateral lesions of the facial nucleus or nerve in most species (Radostits *et al*., 1994). This condition is less commonly seen in cattle (Frank, 1976).The clinical signs are usually associated with trauma, inflammation, granulomas, tumors etc. Affected animals drool and have a dull facial expression. The nucleus of the facial nerve is located in the rostral medulla oblongata of the brain stem. Clinical signs of facial paralysis or paresis vary with the location, severity and chronicity of the lesion (Maenhout *et al.,*1984; Mouli, 2001; Krishnappa *et al.,* 2002). If a unilateral lesion is located in the facial nucleus or proximal portion of the facial nerve, paresis or paralysis of the eyelids, ears, lips, and nostrils on that side are seen. A lesion of the auriculopalpebral branch of the facial nerve, near the zygomatic arch, results in paresis or paralysis of the eyelids and ear only.

Case History and clinical observation

A one and a half year old, heifer was presented to veterinary hospital Nanjangud Mysore district Karnataka, with complaint of absence of blinking of right eye and relaxed third eye lid for four days.

On clinical examination, both upper and lower eyelids were found to have lost their reflexes, drooping and relaxed third eyelid due to retracted eyeball was evident and also slight drooping of ear on the affected side was noticed (Fig.1). Relaxed third eyelid partially covered the cornea. However, cornea was normal and vision of the animal was not significantly affected. Based on the clinical findings animal was diagnosed as facial nerve paresis affecting only auriculopalpebral branch of it, since lips and other muscles of facial expression were normal.

Treatment

Since, there is no definitive treatment for such conditions, symptomatic treatment was given. The relaxed third eyelid which was covering 2/3 of eye ball and intervening in the vision of animal, surgically excised to restore the vision. Animal was physically restrained on lateral recumbency. Then retrobulbar nerve block was done by Peterson technique. A 10 cm long spinal needle was inserted just above zygomatic arch 1.53 behind lateral canthus of eye and 10 ml of local anesthetic (2% lignocaine) was injected. Auriculopalpebral nerve was also blocked by injecting local anesthetic on mid way between lateral canthus of eye and base of ear. By securing third eyelid with small curved artery forceps third eyelid was excised including cartilage of third eyelid. Bleeding was arrested by applying cotton swab soaked in adrenaline. Animal was given neurotonic (Neurobion forte^{R}) daily for 7 days to restore the normal function of damaged nerve and post operative antibiotic Amoxycilline+Cloxacilline 2gm (Intamox^{R}) daily for 5 days. Owner was advised to wrap moist cloth on the affected eye to avoid injury to cornea and advised to massage for 15 min around eye and ear twice daily to stimulate the nerve. Animal started blinking eyelids slightly after 5days and after 15 days there was evident eyelid movement.

Discussion

The facial nerve, exits the brain stem near the vestibulocochlear nerve, passes through the petrous temporal bone, and then exits the skull through the stylomastoid foramen and splits into auricular, palpebral, and buccal branches. Clinical signs of facial paralysis vary with the location, severity, and chronicity of the lesion (Maenhout *et al.* 1984*)*. If a unilateral lesion

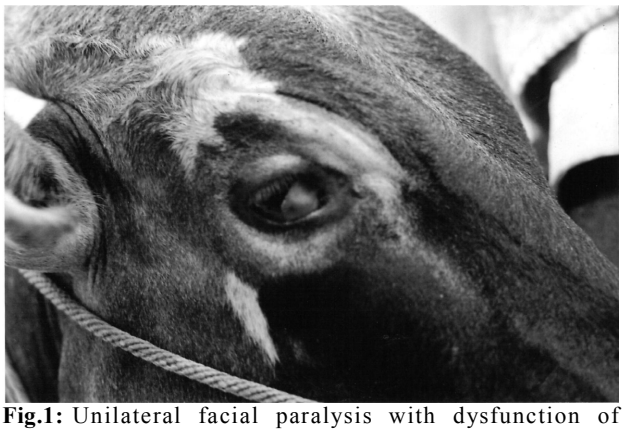

Fig.1: Unilateral facial paralysis with dysfunction of auriculopalpebral nerve

is located in the facial nucleus or proximal portion of the facial nerve, paresis or paralysis of the eyelids, ears, lips, and nostrils on that side are seen (Krisnappa *et al*., 2002). A lesion of the auriculopalpebral branch of the facial nerve, near the zygomatic arch, results in paresis or paralysis of the eyelids and ear only. A lesion of the palpebral branch of the facial nerve, crossing the zygomatic arch, results in paresis or paralysis of the eyelids only. With an inability to blink, corneal irritation may result in excessive tear production and may result in corneal ulceration. According to available literature and observed clinical symptoms the present case may be consequences of facial nerve paralysis involving auriculopalpebral branch.

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Parafilariosis in a non-descript bullock

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Abstract

A clinical case of dermal affection with haemorrhagic cutaneous nodules and dried clotted blood spots spread over hair coat in a non-descript bullock is reported. The exudates smear revealed microfilariae of *Parafilaria* spp. A clinical case of dermal Parafilariosis and its successful treatment with ivermectin is discussed.

Keywords: Bullock, Ivermectin, *Parafilaria* spp

The Parafilarial infection is a vector-borne disease of bovines and is characterized externally by focal cutaneous hemorrhagic nodules that ooze out marking dry clotted spots (Bleeding spots) on hair coat. Bovine parafilariosis caused by *Parafilaria bovicola* is prevalent in tropical region generally after rainy season through early summer, where the vector, face flies of genus *Musca* spp. are abundant. The draught animals like bullock appears to be more susceptible to Parafilariois. The haemorrhagic nodules are commonly found on the neck, shoulder, wither, thoracic area, lateral abdomen and back, which burst out during morning hours with oozing blood and serosanguinous exudates leading to the formation of bleeding sores (Bhatia *et al*, 2006). The present paper reports a case of haemorrhagic dermatitis due to Parafilarioiss and its therapeutic management in a non-descript bullock.

An eight year old male non-descript bullock was referred to the Department of Veterinary Medicine with history of subcutaneous nodules and bleeding spots particularly on shoulders, withers, lateral thorax and abdomen. There was no appreciable change in feed and water intake. Case was earlier treated with antihistaminic and corticosteroid considering allergic skin disease. Clinical examination of the bullock revealed presence of swollen nodules on shoulder, neck, dorsal aspect of thorax, and lateral abdomen with clotted bloody exudates dried on hair coat. The rectal temperature, respiration and heart rates were within the normal range.

Smears were prepared from bleeding points, oozing nodules (Soulsby, 1982) and were then stained with Giemsa stain. The blood was also collected in an anticoagulant vial. Haematological analysis indicated low levels of Haemoglobin (8.2gm%), Packed cell volume (24%) and Eosinophilia (8%). The stained smears from exudates of bleeding points and of squeezed haemorrhagic nodules revealed microfialriae of *Parafilaria* spp. (Soulsby, 1982) on intensive screening. On the basis of typical signs and presence of microfilaria(e) in the smear, the case was diagnosed as Parafilariosis.

Subcutaneous bruising with dried tarry coloured spots on hair-coat superior parts of the body viz., neck, shoulder, wither and lateral abdomen are characteristic clinical findings of Parafilariosis (Sharma *et al*, 2010). Bovine Parafilariosis is of economic importance particularly in beef breeds and can be easily recognized in light coloured animals. The present non-descript bullock had whitish-gray coat and was reared in a fly dominant area.

The dermal lesions were cleansed and dressed with antiseptic-fly repellent (Gamma benzene hexachloride 0.1% W/W, Proflavin hemisulphide 0.1% W/W and Cetrimide 0.45% W/W) cream twice a day. The bullock was further treated with two injections of Ivermectin @200µg/kg BW SC given three days apart. Supportive therapy was comprised of anti-inflammatory, antihistaminic and multivitamin preparations. Following ivermectin therapy, there was marked improvement with regression of haemorrhagic nodules and disappearance of bleeding points within a weeks time. Ivermectin is a novel endectocidal drug and therapeutic efficacy of ivermectin against Parafilriosis has been well documented by (Soll *et al*, 1991). The prepatent period of *Parafilaria* spp. is about 240 days and the female parasite producing subcutaneous nodules die off after ovipoisition and the animals are newly infected every year. The host become infected with third stage larvae,

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when the fly subsequently feed on it. The control programme for Parafilariosis, therefore, should aimed to control fly population and existing cutaneous infection (Radostitis *et al*, 2007). The present clinical case of Parafilariosis, therefore, showed remarkable response to combined therapy of topical antiseptic-fly repellent creams and subcutaneous ivermectin therapy. In present case, subsequent to Ivermectin therapy, disappearance of bleeding points and healing of subcutaneous nodules was observed. The smears prepared from skin lesions post-thearapy did not reveal any microfilaria. The present findings, therefore, indicated that ivermectin has substantial effect on *Parafilaria* spp. Too and is in agreement with the observations of Swan et al. (1983), Merker (1985) and Jana and Jhosh (2009) who successfully treated cattle and buffaloes infected with *Parafialra bovicola* with ivermectin therapy.

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Organophosphate poisoning in peacock

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Abstract

Poisoning in birds is common due to ingestion of pesticide treated grains and tte present report presents a review of 3 cases of organophosphate poisoning in peacock

Keywords: Organophosphate, Peacock, Poisoing

Farmers often use insecticide covered wheat flour or jiggery as bait in their farm to get rid of pig, blue bull and other animals that damages the crop. Poisoning in birds however, is common due to ingestion of pesticide treated grains. Organophosphate compounds though short lived in environment; there is wide variation in their toxic dose. Birds appear to be more sensitive and more than 100 avian species have been reported to be poisoned of which forate has the lowest LD 50 in birds. The death is rapid with neurological signs. These toxic compounds are phosphomidon, monocrotophos, fenthion, parathion, diazinon and carbamate carbofuran and are capable of disrupting the avian nervous system by inhibiting cholinesterase (Quick, 1982). More than10 to 1000 times the minimum toxic dose of pesticide was commonly detected in the stomach contents of wild birds which died (Kwon *et al.*, 2004).

The cases of suspected Organophosphate poisoning in peacocks due to ingestion of pesticide treated grains were brought to the notice of forest department by the farmers and were subsequently presented for the treatment. Post mortem details were made available of the one died earlier of four peacocks affected.

Three peacock weighing around 3.5 kilogram when presented were exhibiting clinical signs of cyanosis of mucus membrane, frothing at beak, ataxia, and were unable to bear weight when presented. Further they were dull, unable to get up with flaccid wide-open wings. One had diarrhea; another had severe respiratory distress, gasping, rapid and irregular heart rate and white frothing at beak. All these signs are seen in the organophosphate poisoning. (Clarke *et al.*, 1981). The peacock which died also showed convulsions besides these clinical signs before death. Post mortem report of dead bird had mentioned cyanotic mucus membrane and petechial hemorrhage on liver, heart and kidney. History, clinical and post mortem findings were therefore suggestive of poisoning due to ingestion of organophosphate treated toxic grains which were later supported by toxicological investigations.

Considering the organophosphate toxicity, peacocks were given injection of Atropine sulfate@0.2 mg/kg by intra muscular route and repeated at every four hours on three occasions. Response to therapy was observed in the form of gradual diminishing of clinical signs and uneventful recovery. The peacocks were then released in the field by forest department.

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Diagnosing Air gun pellet in brain of a monkey

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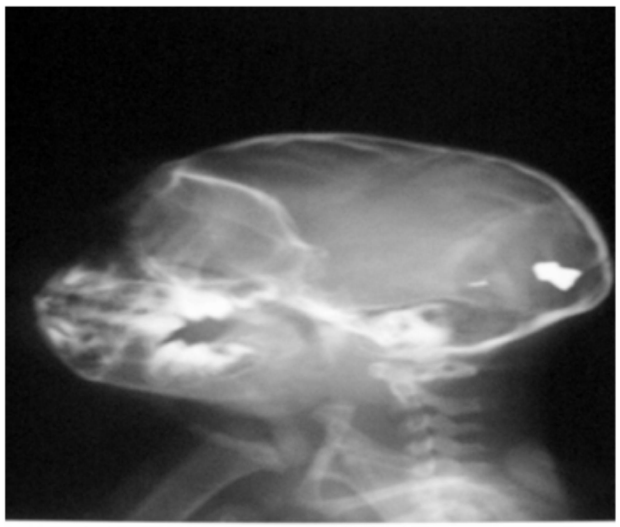
Abstract

A monkey with semicomatose condition with history of injury at left eye, unable to sit, falls down frequently with anorexia. Clinical examination revealed severely dullness and depression having hemiplagia of right side. The skull X ray revealed presence of air gun pellet in the posterior ventral part of brain while the whole skull was found intact. No apparent injury was observed except in left eye. Treatment started with Inj Prednisone 2 ml i/v, Inj. Melonex plus 2 ml i/m, Inj Mannitol @ 1 gm / kg Body Wt. i/v , Inj Nucetam (Piracetam) 200 mg i/v tid, Inj Potacef S 1 gm i/v with Inj DNS 5% 100 I/v and Inj Nervijen 1 ml i/v with anti septic dressing of wound for 10 days. At 12th day of treatment the monkey started to walk with support. At 45 days of treatment the monkey was able to run and climb over the trees.

Keywords: Air gun pellet Monkey, paraplegia,semicomatose, Skull X-ray.

The exploitation of land and forest resources by humans along with hunting and trapping for food and sport has led to the extinction of many species in India in recent times. Air-gun pellet injuries of the central nervous system (CNS) are rare but catastrophic events. They occur mostly in children and young adults and now days very common in wild animal. The entrance is usually either through the orbit or through the neck and the entry wound is so small that it may be disregarded on physical examination in the emergency room. Early recognition and correct management of the possible complications of Air-gun pellet injuries is important and may prevent a poor outcome (Jamshidi *et al*., 1997)

A monkey was presented to Teaching Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, Mhow with history that the monkey was found under semi comatose condition in a private Agriculture farm that is located about 120 Km away from college. On clinical examination a injury was observed at left eye, convulsions , unable to sit and falling down frequently. The body temperature was 96.2 ºF, severely dull and depressed having hemiplagia of right side. The skull X ray revealed presence of air gun pellet in the posterior ventral part of brain while the whole skull was found intact. No apparent injuries was observed except in left eye, indicate that the bullet was entered from left eye and traveled up to the posterior-ventral aspect of brain damaging left side of brain caused paralysis of right side of body.



Treatment started with Inj Prednisone 2 ml i/v, Inj. Melonex plus 2 ml i/m, Inj Mannitol @ 1 gm / kg body wt. i/v , Inj Nucetam 200 mg i/v tid, Inj Potacef S 1 gm i/v with Inj DNS 5% 100 I/v and Inj Nervijen[5] 1 ml i/v with anti septic dressing of wound for 10 days. The monkey started responding to treatment from 48 Hrs of the treatment. The improvement observed in clinical signs as monkey started to sit without support and taking food and water with assistance. At 12th day of treatment the monkey started to walk with support. At 45 days of treatment the monkey was able to run and climb over the trees, eye wound was completely healed up. Presently monkey is in the forest near to the Agriculture Farm and living a normal life with air gun pellet in the brain.

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The *Indian J. Vet. Med.* is published twice in a year, June and December. It contains review articles (guest), original/applied research articles, clinical observations, preliminary re-ports of scientific studies and short communications on Veterinary Medicine and Animal Health. In addition, the journal also publishes Letters to the Editor, Tips to Vets and other relevant information's.

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   a. For periodicals:

   Bartley, E.E., Wheatcroft, K.L., Claydon, T.J. Fountaine, F.C. and Fairish, D.V. 1951. Effect of feeding aureomycin to dairy calves. *J. Anim. Sci.* **10**: 1036-1038.

   ***b.* For books:**

   Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. VIII edn., Iowa State University Press, Iowa, USA, pp. 287-192.

   c. For chapter in a book:

   Thomas, J.R. and Charles, C.C. 1997. Calcium regulating hormones and diseases of abnormal mineral metabolism. In: *Clinical Biochemistry of Domestic Animals. Kaneko*, J.J., Harvey, J.W. and Bruss, M.L. (eds.). V edn., Academic Press, London, pp. 619-702.

   d. For thesis:

   Singh, S.K. 1998. Studies on clinico-biochemical changes in Downer cow syndrome. M.V.Sc. thesis.

Punjab Agricultural University, Ludhiana, India.

e. For proceedings of symposia/conference:

Shah, R.L., Kataria, J.M., Arya, S.C. and Verma, K.C. 1996. Study on inclusion body hepatitis in broiler chicks. *Proc. XX World Poult. Congress* held on Sept. 2-5,1996, New Delhi, Vol. IV,pp.313-314.

I. **Tables:** These should be as few as possible and typed on separate sheets and numbered in roman numeri-cal. Each table should have a brief and self-explanatory title. Table format should be in accordance with the format of *Indian J. Vet. Med.* that is containing grids and cell.

J. Figures: High-resolution (300-600 dpi or greater) and should be initially saved in a neutral data format such as JPEG. Illustrations should be numbered as cited in the sequential order in the text, with a legend on a separate sheet. The editors and publisher reserve the right to reject illustrations or figures based upon poor quality of submitted materials.

Abbreviations and Symbols: Metric system should be followed in the text. The quantities should be expressed in SI units. Contributor(s) are requested to use the following abbreviations.

Body weight	b wt	Litre	I
Calory	cal	Meter	m
Centimeter	cm	Microlitre	µl
Counts per minute	cpm	Millligram	mg
Cubic centimeter	cm^3	Millilitre	ml
Degree centigrade	°C	Minute(s)	min
Degree Fahrenheit	°F	Once a day	od
Decilitre	dl	Parts per million	ppm
Gram	g	Percent	%
Hour(s)	hr	Picogram	pg
Inch	in	Revolution per min	rpm
Intramuscular	im	Second(s)	sec
Intraperitoneal	ip	Square centimeter	cm^2
Intravenous	iv	Subcutaneous	sc
Kilo calories	kcal	Thrice a day	tid
Kilogram	kg	Year(s)	yr
Twice a day	bid	Volts	v

All other abbreviations should be spelled out when first used in the text.

Footnotes: These should be used only when absolutely essential. When used, they should be numbered in text, indicated by superscript numbers and kept as short as possible.

The British spellings must be followed throughout in the text and Oxford English Dictionary may be consulted in doubt.

Short communication

They should be in the same general format as full length papers, should contain between 2000-2500 words but not more than six type pages including tables and illustrations. The manuscript for this head should be

clearly marked 'Short Communication' at the right corner on the top of the first page of manuscript. The abstract (not more than 150-200 words), key words (not more than 3 words) and subheading, except for acknowledgement and references, should not be written in the manuscript. Each short communication should contain a definite conclusion of the findings.

The references should be given as per format for the research articles.

Clinical articles

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), Key words (not more than 3 words). The manuscript should contain history and impor-tant 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 treatment and conclusion. 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 and should bear subheadings. Each article should have significant clinical findings.

The references should be given as per format for the research articles.

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Indian Journal of Veterinary Medicine

Vol. 33 (June & December, 2013)

Author Index

Indian Journal of Veterinary Medicine

Vol. 33 (June & December, 2013)

Subject Index

ACKNOWLEDGEMENT TO THE REVIEWERS – 2013

Editorial board of Indian Journal of Veterinary Medicine would like to express their greatest appreciation and gratitude towards the reviewers of the Journal for the year 2013. We acknowledge that their valuable comments and insights have helped us to publish the best among the submitted article.

On behalf of Indian Society for Veterinary Medicine we sincerely thanks them for their time devoted and extremely thoughtful suggestions which immensely contributed to the improvement of the journal and overall for the greater cause that is for the development of Veterinary Sciences.

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Article statistics of IJVM Jan. to Dec. 2013

Total articles published in 2013	**:**	**46**
Research articles	:	13
Short communications	:	26
Clinical Articles	:	17
Total articles published in June issue (Vol. 33, No. 1, 2013)	**:**	**28**
Research articles	:	7
Short communications	:	13
Clinical Articles	:	8
Total articles published in December Issue (Vol. 33, No. 1, 2013)	**:**	**28**
Research articles	:	6
Short communications	:	13
Clinical Articles	:	9
Total article submitted for publication (Jan2013 to Dec 2013)	**:**	**103**
Articles accepted for publication	:	54
Articles rejected	:	49
Acceptance rate	:	52.43 %
Rejection rate	:	47.57 %

INDIAN JOURNAL OF VETERINARY MEDICINE

DECLARATION

FORM IV (See Rule 8)

1. Place of Publication	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.
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5. Editor's Name	:	Dr. S. Dey
Nationality	:	Indian
Address	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.
6. Proprietor's Name	:	Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India.

I, S. Dey, Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243122, Bareilly (UP) India hereby declare that the particulars given below are true to the best of my knowledge and belief.

Dated: 31st December, 2013 **(S. Dey)**

Contents