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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.

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## Evaluation of immune response to mesogenic strain of Ranikhet disease virus in poultry

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### Abstract

Seventy five day old Rhode Island Red chicks were equally divided into three groups consisting of 25 chicks in each group. All the birds were supplied with balanced feed and water ad-lib. Birds of Group-A were vaccinated with modified lentogenic F-strain of Ranikhet Disease vaccine through ocular-nasal route on 5<sup>th</sup> and 19<sup>th</sup> day of age and subcutaneous injection with modified R<sub>2</sub>B strain RD vaccine on 42<sup>nd</sup> day of age. The same vaccination Schedule was followed on the chickens of Group B. Group-C birds were kept as unvaccinated control. All the birds of Group A, B and C were challenged with velogenic strain of locally isolated RD virus at the age of 56<sup>th</sup> day and observed for next two weeks for any abnormality/mortality. Serum antibody titer of all birds of Group A, B and C was determined in all at the age of 5<sup>th</sup>, 19<sup>th</sup>, 42<sup>nd</sup> and 56<sup>th</sup> day and 60<sup>th</sup>, 64<sup>th</sup> and 68<sup>th</sup> day of age (post-challenge). The mean log<sub>2</sub> HI titre was higher (2.957 ± 0.043) in Group-A in comparison to group-B (2.460 ± 0.04) and group-C (0.00 ± 0.00) just before challenge. The Group A afforded 100% protection against challenge virus with highest antibody titre (mean log<sub>2</sub> 2.670 ± 0.03) on 12<sup>th</sup> day post-challenge in comparison to commercially available vaccine which provided 96 percent protection.

**Keywords:** Mesogenic strain vaccines, Rhode Island Red, Serum antibody titres

Newcastle disease, known in India as Ranikhet disease, is caused by Paramyxovirus type-1 serotype, has devastating effect on poultry flocks and causes huge economic losses to poultry industry. Vaccination is the most effective means of controlling RD and has been used throughout the world since 1940 (Bread and Hanson, 1984). In spite of regular vaccination with available vaccine, outbreaks continue to be reported even in vaccinated flocks in both organized and backyard flocks in West Bengal (Epidemiological Unit, IAH & VB, West Bengal, 2007-2008). To prevent such type of economic loss by sudden outbreaks of Ranikhet Disease (RD), a programme was undertaken to prepare attenuated lentogenic and mesogenic RD vaccine with better performance.

### Materials and Methods

#### Vaccines

Freeze dried R.D lentogenic F strain vaccine and mesogenic R<sub>2</sub>B strain vaccine procured from Institute of Animal Health & Veterinary Biologicals (IAH&VB), Kolkata-37, were serially passaged in 9 – 11 days old embryonated SPF fowl eggs, up to 5<sup>th</sup> passages for purification. HA titers were determined from allantoic fluids containing virus of every passage.

#### Preparation of vaccine

Fifth passage allantoic fluid 0.1 ml containing virus of lentogenic strain was inoculated in 5(five) 9 – 11 days old embryoning SPF fowl eggs. The allantoic

fluids were harvested from viable eggs at 120 hours post- inoculation, separately. The HA titre was determined from individual harvested fluid and pooled together. The HA titre was estimated again from pooled sample and termed as modified F strain virus. Similarly, the modified R<sub>2</sub>B strain virus was prepared from 5<sup>th</sup> passaged allantoic fluid of R<sub>2</sub>B (mesogenic) strain and was harvested at 90 hours post-inoculation.

#### Lyophilization of the prepared vaccine

Modified F-strain allantoic fluid (10 ml.) and 10 ml cryoprotectant (solution of equal volume of 10% sucrose and 5% lactalbumin hydrolysate) were mixed together. An aliquot of 1.0 ml was taken and lyophilized in 3.0 ml and 1.0 ml aliquot was lyophilized in 3.0 ml vials. Lyophilization of R<sub>2</sub>B strain allantoic fluid was done in the similar manner.

#### Calculation of vaccine dose

The vaccine dose was calculated by determining the EID<sub>50</sub> as per the method FAO (2002) and Reed and Muench (1938).

#### SPF Embryonated Chicken Eggs

SPF fowl eggs were procured from SPF Eggs Division, Venkey's (India) Ltd, Pune for purification / passaging and to determine the EID<sub>50</sub> of the virus.

#### Sterility Test

For confirmation of sterility following tests were

conducted:

The allanotic fluids containing virus were individually cultured in nutrient broth (Himedia laboratory, India) for 12 hours and then in nutrient agar (Himedia laboratory, India) for 24 hours at 37°C for bacterial growth. For fungal culture, the allanotic fluids were inoculated in Sabouraud's Dextrose agar (Himedia laboratory, India) at 37°C for 21 days and in PPLO agar (Himedia laboratory, India) at 37°C for 7 days for mycoplasma colony.

### Safety test

Twenty day-old-chicks, from SPF flock, were equally divided into two groups i.e. group-A, group-B, containing 10 chicks in each group and housed separately with balanced feed and water ad lib. Ten doses (i.e.  $10 \times 10^{6.5}$  EID<sub>50</sub>) of modified F strain vaccine was instilled supra-conjunctivally to each chick of group A at the age of 5 days and observed for next 21 days for any mortality or abnormality. Similarly birds of group-B was administered with ten doses (i.e.  $10 \times 10^5$  EID<sub>50</sub>) of modified R<sub>2</sub>B strain vaccine subcutaneously and observed for 3 weeks post-inoculation for mortality/any adverse clinical signs.

### Potency Test

Seventy five day old Rhode Island Red chicks were equally divided into three groups i.e. Group A, Group B and Group C having 25 chicks in each group and were housed separately with balanced feed and water ad lib. Chicks of group A were given oculonasal instillation of modified lentogenic F-strain vaccine with  $10^{6.5}$  EID<sub>50</sub> on 5<sup>th</sup> and 19<sup>th</sup> day of age and subcutaneous injection of modified R<sub>2</sub>B strain vaccine with  $10^5$  EID<sub>50</sub> on 42<sup>nd</sup> day. Chicks of group B were administered oculonasal instillation of commercial F-strain vaccine (I.A.H & V.B, West Bengal) on 5<sup>th</sup> and 19<sup>th</sup> day of age and subcutaneous injection of R<sub>2</sub>B strain vaccine (I.A.H & V.B, West Bengal) on 42<sup>nd</sup> day. Group C was kept as unvaccinated control. All the birds of three groups were challenged with  $10^6$  EID<sub>50</sub> velogenic strain (local isolate) of RD virus on 56<sup>th</sup> day of age and observed for next two weeks for any mortality/untoward reaction. HI titre was determined in the chicks of all three groups on 5<sup>th</sup> day, 19<sup>th</sup> day and 42<sup>nd</sup> day of age and 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> day of post-challenge according to the procedure suggested by Allan and Gough (1974).

### Statistical Analysis

The data collected through the study from various treatment groups were compared by one-way analysis of variance with SPSS 10.0 software. Differences among groups were considered significant at ( $P < 0.05$ .)

### Result and Discussion

The EID<sub>50</sub> and HA activity of the two commercial vaccines before passaging are presented in table 1.

**Table 1:** EID<sub>50</sub> and HA activities of two commercial vaccines before passaging

Vaccine source	EID <sub>50</sub>	HA Titre
Attenuated F strain vaccine	$10^{6.5}$	$2^6$
Attenuated R <sub>2</sub> B strain vaccine	$10^5$	$2^5$

**Table 2:** HA titre by serial passaging of F strain vaccine and R<sub>2</sub>B strain vaccine in SPF fowl eggs

Strain	HA titre				
	1 <sup>st</sup> passage	2 <sup>nd</sup> passage	3 <sup>rd</sup> passage	4 <sup>th</sup> passage	5 <sup>th</sup> passage
F Strain vaccine	$2^6$	$2^7$	$2^8$	$2^8$	$2^9$
R <sub>2</sub> B strain vaccine	$2^6$	$2^7$	$2^8$	$2^8$	$2^8$

By serial passaging it was evident that the HA titres of both the vaccines strain in 5<sup>th</sup> passage were  $2^9$  and  $2^8$  in F strain and R<sub>2</sub>B strain respectively. Similar observation was reported by Biswas (2011), who described that the HA titre of paramyxo virus increased by serial passages in SPF fowl eggs.

**Table 3:** HA titre of 5<sup>th</sup> passage virus in five SPF embryonated fowl eggs

Strain	HA titre					
	1 <sup>st</sup> egg	2 <sup>nd</sup> egg	3 <sup>rd</sup> egg	4 <sup>th</sup> egg	5 <sup>th</sup> egg	Pooled
F strain virus	$2^9$	$2^9$	$2^9$	$2^9$	$2^9$	$2^9$
R <sub>2</sub> B strain virus	$2^8$	$2^8$	$2^8$	$2^8$	$2^8$	$2^8$

HA titres in all this inoculated eggs were same i.e. for F strain vaccine  $2^9$  and the R<sub>2</sub>B strain vaccine  $2^8$ .

After lyophilisation, the HA titre decreased one log in both the modified vaccines. The EID<sub>50</sub> of both the modified F strain vaccine and R<sub>2</sub>B strain vaccine was  $10^{8.5}$  and  $10^8$  respectively. Similar type of findings were reported by Chettri (2010) who observed that after lyophilization of virus/vaccine, both the HA titre and



**Table 4: HA titre and EID<sub>50</sub> of modified F-strain vaccine and R<sub>2</sub>B strain Vaccine before and after lyophilisation**

Strain	Before lyophilisation		After lyophilisation.	
	HA	EID <sub>50</sub>	HA titre	EID <sub>50</sub>
Modified F strain vaccine	2 <sup>9</sup>		2 <sup>8</sup>	10 <sup>8.5</sup>
Modified R <sub>2</sub> B strain vaccine	2 <sup>8</sup>		2 <sup>7</sup>	10 <sup>8</sup>
FDRD F strain vaccine	2 <sup>6</sup>	10 <sup>6.5</sup>		
FDRD R <sub>2</sub> B strain vaccine	2 <sup>5</sup>	10 <sup>5</sup>		

**Table 5: Mean log<sub>2</sub> HI antibody titre of different groups at different days vaccinated against Ranikhet Disease**

Groups	Mean log <sub>2</sub> HI titres (Mean ± SE) (Pre-challenge)				Mean log <sub>2</sub> HI titres (Mean ± SE) (Post-challenge)		
	Before Primary Vaccination (5 <sup>th</sup> day of age)	Before boostering (19 <sup>th</sup> day of age)	Before mesogenic strain (42 <sup>nd</sup> day of age)	Just before challenge (56 <sup>th</sup> day of age)	4 <sup>th</sup> day (60 <sup>th</sup> day of age)	8 <sup>th</sup> day (64 <sup>th</sup> day of age)	12 <sup>th</sup> day (68 <sup>th</sup> day of age)
Group A	1.5000 ± 0.01	1.1700 <sup>az</sup> ± 0.03	2.1900 <sup>ay</sup> ± 0.045	2.9570 <sup>ax</sup> ± 0.043	2.0400 <sup>az</sup> ± 0.04	2.1600 <sup>ay</sup> ± 0.04	2.6700 <sup>ax</sup> ± 0.03
Group B	1.5000 ± 0.01	1.1100 <sup>az</sup> ± 0.045	1.8600 <sup>by</sup> ± 0.04	2.4600 <sup>bx</sup> ± 0.04	1.7100 <sup>bz</sup> ± 0.064	1.9500 <sup>by</sup> ± 0.067	2.2800 <sup>bx</sup> ± 0.089
Group C	1.5000 ± 0.01	0.3600 <sup>bx</sup> ± 0.04	0.0000 <sup>c</sup>	0.0000 <sup>c</sup>	0.0000 <sup>c</sup>	0.0000 <sup>c</sup>	0.0000 <sup>c</sup>

Note: 1. Superscript rows a, b, c., 2. Superscript column x, y, z., 3. Means value bearing no common superscript either in the rows or columns are significantly different (P<0.05) according to Duncan's multiple range test. Values are presented as mean ± SE.

EID<sub>50</sub> decreased. Therefore, dose per vial of aliquot was calculated on the basis of standard dose of lentogenic strain (i.e. 10<sup>6.5</sup>EID<sub>50</sub>, Hofacre, 1986) and mesogenic strain (i.e. 10<sup>5</sup>EID<sub>50</sub>, Alan *et al.*, 1973) vaccine.

No bacterial, fungal and mycoplasmal colony was detected in inoculated culture media. Therefore, it was concluded that the vaccine did not contain any exogenous contamination. In safety test, all the chicks of both the experimental and control group were found healthy after 21 days of observation with no untoward reaction.

**Table 6: Protection against challenge with virulent field strain of RDV**

Groups	Total Birds	Live Birds	Dead Birds	Mortality %	Protection Index
Group A	25	25	00	00	100
Group B	25	24	01	4	96
Group C	15	00	15	100	00

Before primary vaccination (5<sup>th</sup> day of life) all the RIR chicks of all groups showed protective level antibody HI titre i.e. 2<sup>5</sup> or mean 1.50 ± 0.01 which corroborates with the findings of Adene (2004). After 2 weeks of primary vaccination when the boosting was performed (19<sup>th</sup> day of age), the antibody titer was below protective level i.e. in group-A HI mean titre 1.17 ± 0.03, in group-B mean titre 1.11 ± 0.045, and in group C mean titre 0.36 ± 0.04. These mean antibody HI titre

raised significantly to the satisfactorily level (above protective level) except in control group before mesogenic strain vaccination (42<sup>nd</sup> day of age) and before challenged (56<sup>th</sup> day of age) i.e. in group-A mean HI titre 2.957 ± 0.043 and in group-B 2.460 ± 0.04 though in group-C titre was below detectable level. The findings of the present study corroborate with the results of Chandrasekar *et al.* (1989) who reported a gradual increase of HI antibody titres and a large response to second dose.

On post-challenge, the antibody titre decreased in vaccinated birds as compared to the non-vaccinated control group in which the HI antibody titre was below detectable level. On 4<sup>th</sup> day of post-challenge, the antibody titre suddenly showed decline but did not go below protective level in both the vaccinated group A (i.e. 2.04 ± 0.04) and group B (i.e. 1.71 ± 0.06) in comparison to pre-challenge titre i.e. 2.957 ± 0.04 and 2.460 ± 0.04 respectively. These findings concur with the observations of Tizard (1996) who suggested decrease in antibody titre due to neutralization of virus by circulating antibodies. The modified vaccine and the commercially available vaccine gave 100 percent and 96 percent protection respectively whereas, all (100%) the non-vaccinated birds died within 8<sup>th</sup> day of post-challenge. In survived birds, the antibody titre gradually increased in both of the group-A (i.e. 2.67 ± 0.03) and Group B (i.e. 2.28 ± 0.89) on 12<sup>th</sup> day post-challenge

and it was below pre-challenge titre. Although the commercial vaccine is approved as per OIE, 2008, yet the experimental vaccine proved superior.

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## Clinical effects of magnetic field and transcutaneous electrical nerve stimulation in dogs suffering from hind quarter neurological deficit

M.M. Ansari<sup>1\*</sup>, M. M. S. Zama<sup>2</sup>, S. Dey<sup>3</sup>, Puja Tiwari<sup>4</sup> and S. Khatri<sup>5</sup>

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### Abstract

Conventional drug therapy, CDT (n=8) alone and in combination with static magnetic field therapy, SMFT (n=8) and transcutaneous electrical nerve stimulation, TENS (n=8) was given in dogs suffering from hind quarter neurological deficit. For SMF therapy, 4 bipolar permanent magnet bars, each of 850 - 950 gauss were selected and used in 2 pairs, each lying on either side of the vertebral column with the opposite poles facing each other. The magnetic field was applied daily for 2 hours for 14 days. TENS at 100 Hz frequency, using 2 pole electrodes over the intervertebral foramen at lumbar region, was given daily for 10 minutes for 14 days. All the animals regained their normal postural reactions, except hopping reaction in hind limbs, by day 14 of the therapy. Hopping reaction was achieved in 9 dogs (2 in group I, 3 in group II and 4 dogs in group III) on day 14 and in rest of them on day 28. The dogs treated with TENS in combination with CDT showed early recovery followed by SMFT along with CDT and CDT alone.

**Keywords:** Dogs, Hind quarter weakness, Magnetic field, Neurological deficit, TENS.

Neurological disorders primarily involving the vertebral column and spinal cord are commonly encountered in small animal practice, especially in dogs. The most frequent manifestation of spinal cord affections is the weakness or neurological deficit of hind quarters. It is the loss of bilateral motor function of the rear limbs due to dysfunction of neural or muscular system. Such animals show difficulty to bear weight, paresis or paralysis of limbs associated with urinary and faecal incontinence (McGowan *et al.*, 2007).

The disease is also manifested by the clinical signs such as dragging the rear part of the body with pain sensation while trying to walk, rigid hyperextension of both hind limbs, unable to stand or can stand only for short periods of time generally without the normal arched back (Nelson and Couto, 2004). It may result as a sequel to the spinal cord disorder either by fall, jump from height, road traffic accident, dog bite over the vertebral column, malicious blow by stick, rod, stone, crush by heavy object, fracture and myoclonus form of canine distemper (Hoerlein, 1971, Berg and Boudrieau, 1992). The present study was undertaken to investigate the effect of magnetic field and TENS on clinical recovery of dogs suffering from hind quarter neurological deficit.

### Materials and Methods

A total of 24 dogs (2.5 months to 11 years of

age) of either sex suffering from hind quarter weakness presented to Referral Veterinary Polyclinic of Indian Veterinary Research Institute, Izatnagar were included for the study. All the animals were randomly divided in 3 equal groups (I, II and III) of 8 dogs each. Dogs in group I were treated with conventional drug therapy (CDT) alone. In addition to the conventional drug therapy, animals of groups II and III were also treated with static magnetic field therapy (SMFT) and transcutaneous electrical nerve stimulation (TENS), respectively. CDT was given for 14 days using methyl prednisolone acetate (Depomedrol\*), @ 30 mg/kg body weight intramuscular on first day and later on 15 mg/kg body weight I/M. on alternate days, Meloxicam (Melonex\*\*), @ 0.2 mg/kg body weight intramuscular daily, Gabapentine 300mg and Mecobalamine 500mcg combination (Neurokind- G\*\*\*), orally once daily and Injection of Vitamin B1, B6, B12 and Panthenol (Cyanocal-16\*\*\*\*), 2ml, I/M on alternate day. For static magnetic field therapy, 4 numbers of bipolar permanent magnet bars, each having average strength of 850 - 950 gauss, were selected. These magnets were divided into two pairs and embedded in a bandaged pad in such a way that when placed on the area of lumbar region, each magnet of a pair lies on either side of the vertebral column with the opposite poles facing each other. The distance between the magnets of the same side (right/left) was kept as 2.5 cm. The magnets embedded pad

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was applied on animals daily for 2 hours for 14 days. TENS at 100 Hz frequency, using 2 pole electrodes over the intervertebral foramen at lumbar region, was given daily for 10 minutes for 14 days.

On the day of presentation, the dogs were examined on the basis of history (treatment given by local veterinarian, etiology, duration of illness) and general appearance (posture, gait). Rectal temperature (RT), heart rate (HR), respiratory rate (RR) and different neurological parameters were recorded without giving any sedation or anesthesia on day 0, 3, 7, 14 and 28 of the treatment. All the examinations were done in quiet place. Wheel barrowing, hemi-standing, hemi-walking, hopping, placing and pain perception tests were conducted as per the procedure of Hoerlein, 1978, whereas, patellar, pelvic withdrawal, panniculus, anal and conscious proprioception reflexes as per Bali, 2000. General clinical improvement in individual cases was also assessed on day 28 as: Excellent (+++): when the dog made full recovery and there was no recurrence of signs, Good (++) : when a slight residual in-coordination of hind limb remained, Fair (+): when a moderate to severe hind limb in-coordination remained, and Poor (-): no improvement. Excellent and good results were taken as successful outcome. The data were subjected to one way ANOVA followed by Tukey test using SPSS 16.0 version for group, time and their interaction effects. The level of statistical significance for all comparisons was established at  $P < 0.05$ .

## Results and Discussion

Twenty one dogs (87.50%) were treated by referring veterinarians with varying combinations of drugs like corticosteroids, nervine tonic and non-steroidal anti-inflammatory drugs (NSAIDs) along with rest, before reporting to Polyclinics. However, the treatment yielded only limited response as also documented by Gopinathan (2006) in dogs with hind quarter weakness.

Out of 24, 16 dogs (66.66%) developed sudden episodes of hind quarter weakness following a jump or while climbing stairs, tussling with the dogs or during breeding. The dogs exhibited symptoms of pain as manifested by wide base stance, arching of back or kyphosis with or without protrusion of penis. The dogs were reluctant to move or pick up any object, refused to climb upstairs or board the car. All dogs had pain and

anxiety and awoke frequently during the night, and also cried while getting up. Eight percent dogs had inappetance and voluntary urination. Seven dogs (29.00%) had spasticity of limbs. Due to inability to flex the limbs they put them under the body. Bladder was difficult to palpate and only one dog had urine and faecal retention. Three dogs (12.50%) had flaccid hind limbs accompanied by urine and faecal incontinence. Bladder was easy to palpate. Similar clinical signs were also noticed by Sharma (2005) in hind quarter weakness.

Traumatic injury was the main known causative factor of the disease, as also reported by Gopinathan (2006) and Maiti *et al.* (2007). Among the known causes of trauma, fall from height was in 10 dogs (41.66%) followed by dog bite in 2 dogs (8.33%) and road traffic accident in 1 dog (4.16%). Eleven dogs (45.83%) had no history of physical trauma as the cause of the condition. Ettinger (2000) reported that many developmental disorders like generalized osteoporosis, hypovitaminosis “ $\leq$  and hypocalcaemia result in neuromuscular weakness in hind quarters.

Majority of the cases were brought to the Polyclinics within 1 week of commencement of the illness (20, 83.33%), whereas, 3 (12.50%) animals were reported after 1 week but within a month and 1 (4.16%) after 1 month of the illness. Brown *et al.* (1977) and Butterworth and Denny (1991) have also reported that the dogs with neurological deficit causing pain and also inability to bear weight and visible urinary/faecal incontinence are attended early by the owners as compared to those showing simple back pain alone or with other less severe deficits. Gopinathan (2006) also recorded that majority of the dogs suffering from hind quarter weakness were reported within a week of the illness, however, Sharma (2005) recorded it during 10-30 days.

The mean $\pm$ SE values of RT ( $^{\circ}$ C), HR (/min) and RR (/min) before and after therapy have been shown in table 1. The RT ( $^{\circ}$ C) showed a significant increase from day 14 in group III, significant decrease on day 14 in group I from the base value. In comparison to group I, higher values of RT ( $^{\circ}$ C), were recorded in groups II and III which were significantly ( $P < 0.05$ ) higher in group III on day 28. The tiny mineral ions in the blood become attracted to the magnetic field creating electrical currents in the blood stream. This, in turn, creates an increase in blood flow and heat in the area

(Steyn *et al.*, 2002). TENS also causes electric stimulation, muscle contraction, increase in blood supply and prompts the body to secrete endorphins and other natural pain killer (Bromiley, 1991). Slight increase in temperature in groups II and III following SMFT and TENS therapy may be due to stimulation of thermoregulatory centre of hypothalamus in the brain (Warren, 1976), increased concentration of epinephrine (Zhang *et al.*, 1979). Flushing out the pain causing factor due to increase blood supply, the temperature decreased on day 28, returning towards normal. The animals of group I showed decreasing values of rectal temperature, which may be due to anti-inflammatory effect of corticosteroids and NSAIDs. Glucocorticoids reduce fever and clinical evidence of inflammation (Bondy and

Cohn, 2002). Sharma (2005) also observed a non-significant decrease in RT after conventional therapy with glucocorticoids and NSAID.

The HR (/min.) showed a significant increase from day 3 in all groups from the base value. In comparison to group I, higher values of HR (/min.) were recorded in groups II and III which were significantly ( $P < 0.05$ ) higher in group III from day 14. The RR (/min.) showed an increasing trend in all the groups throughout the period of study. The values increased significantly ( $P < 0.05$ ) from day 7 in group II and from day 14 in group I as compared to baseline value. In comparison to group I, higher values of RR (/min.) were recorded in groups II and III which were non-

**Table 1:** Clinical parameters (mean $\pm$ SE) at different intervals in dogs with HQW subjected to different treatments.

Parameter	Gp	0 day	3 day	7 day	14 day	28 day
RT ( $^{\circ}$ C)	I	38.67 <sup>ab</sup> $\pm$ 0.00	38.65 <sup>ab</sup> $\pm$ 0.03	38.61 <sup>ab</sup> $\pm$ 0.01	38.61 <sup>aA</sup> $\pm$ 0.06	38.19 <sup>aA</sup> $\pm$ 0.07
	II	38.69 <sup>ab</sup> $\pm$ 0.04	38.73 <sup>ab</sup> $\pm$ 0.05	38.73 <sup>ab</sup> $\pm$ 0.04	38.76 <sup>bc</sup> $\pm$ 0.04	38.24 <sup>aA</sup> $\pm$ 0.03
	III	38.67 <sup>ab</sup> $\pm$ 0.00	38.69 <sup>ab</sup> $\pm$ 0.03	38.82 <sup>bc</sup> $\pm$ 0.01	38.87 <sup>cC</sup> $\pm$ 0.00	38.50 <sup>bA</sup> $\pm$ 0.05
HR(/min.)	I	98.50 <sup>aA</sup> $\pm$ 0.26	102.12 <sup>ab</sup> $\pm$ 0.44	103.00 <sup>abC</sup> $\pm$ 0.26	103.31 <sup>aC</sup> $\pm$ 0.16	103.37 <sup>aC</sup> $\pm$ 0.18
	II	98.00 <sup>aA</sup> $\pm$ 0.18	102.25 <sup>ab</sup> $\pm$ 0.16	103.50 <sup>cC</sup> $\pm$ 0.37	104.00 <sup>abC</sup> $\pm$ 0.18	104.37 <sup>abC</sup> $\pm$ 0.41
	III	98.37 <sup>aA</sup> $\pm$ 0.26	102.56 <sup>ab</sup> $\pm$ 0.60	104.75 <sup>aC</sup> $\pm$ 0.36	105.50 <sup>bc</sup> $\pm$ 0.32	105.25 <sup>bc</sup> $\pm$ 0.36
RR (/min.)	I	31.00 <sup>aA</sup> $\pm$ 0.75	32.87 <sup>aB</sup> $\pm$ 0.35	33.37 <sup>ab</sup> $\pm$ 0.26	34.00 <sup>ab</sup> $\pm$ 0.65	33.75 <sup>aB</sup> $\pm$ 0.70
	II	29.50 <sup>aA</sup> $\pm$ 0.73	33.00 <sup>ab</sup> $\pm$ 0.53	32.50 <sup>ab</sup> $\pm$ 0.32	32.75 <sup>ab</sup> $\pm$ 0.52	34.00 <sup>ab</sup> $\pm$ 0.75
	III	31.25 <sup>aA</sup> $\pm$ 0.75	33.50 <sup>aB</sup> $\pm$ 0.59	34.25 <sup>aB</sup> $\pm$ 0.88	34.37 <sup>aB</sup> $\pm$ 0.84	34.87 <sup>ab</sup> $\pm$ 0.78

Values in the same row without a common superscript letter A - C are significantly different ( $P < 0.05$ ). Values in the same column without a common superscript letter a - c are significantly different ( $P < 0.05$ )

**Table 2:** Per cent distribution of cases based on neurological examination in HQW dogs

A)	Postural reaction	Scoring			
		Normal		Abnormal	
	Wheel barrowing	100%		-	
	Hemistanding	100%		-	
	Hemiwalking	95.83%		4.16%	
	Hopping reaction	91.66%		8.33%	
	Placing reaction				
	Fore limb	100%		-	
	Hind limb	87.50%		12.50%	
B)	Proprioception deficit (Score)	0 (Absent) 50.00%	1 (Delayed response) 29.16%	2 (Normal) 20.83%	
C)	Reflexes (scoring)	-2 (Absent)	-1 (Diminished)	0 (Normal)	+1 (Increased)
	Patellar reflex	-	79.16%	16.16%	4.16%
	Pelvic withdrawal reflex	-	58.33%	37.50%	4.16%
	Anal reflex	-	75.00%	25.00%	-
	Panniculus reflex	-	66.66%	20.83%	12.50%
D)	Pain perception	Present 100%		Absent -	
	Superficial/deep				

significantly ( $P>0.05$ ) higher in group III followed by II on day 28. Increase in heart rate and respiration following therapy may be due to increased vasodilatation and angiogenesis in this area that led to increase pumping of heart (Young and Dyson, 1990; Hurley *et al.*, 2001). Further, the electric stimulation of medium frequency in groups II and III might be responsible for increased sympathetic stimulation that also resulted into an increase in body temperature. The increase in circulation and muscle contraction and stimulation to release of endogenous polypeptides have been reported by Bromiley (1991) and Johnson and Tabasam (2003). Increase in heart rate and respiration rate were in concurrence with the finding of Maiti *et al.* (2007) on ultrasound therapy in dogs.

The observations of detailed neurological examination performed on the day of presentation have been depicted in table 2. Wheel barrowing showed normal walking with head extended in normal position in all the animals. Hemi-standing was also normal in all dogs before the therapy. Twenty three (95.83%) dogs exhibited normal hemi-walking, whereas, 1 (4.16%) showed abnormal hemi-walking (group II) which became normal on day 14 after therapy. Hopping reaction was normal in forelimbs in all the dogs. However, 22 (91.66%) dogs showed abnormal hopping reaction in hind limbs. Out of them, 2 in group I, 3 in group II and 4 dogs in group III showed normal hopping reaction on day 14, whereas, rest of them on day 28. Placing (visual and tactile) response in forelimbs was normal in all the dogs. Twenty one (87.50%) cases showed abnormal placing of hind limbs; all of them became normal on day 7, except 1 in group I which was normal on day 14.

The proprioception deficit (absent, 0) was observed in 12 dogs (3 in each groups I & III and 6 in group II). The response after 3 seconds (delayed, 1) was recorded in 7 (3 in each groups I & III and 1 in group III), whereas the response within 3 seconds (normal, 2) was observed in 5 dogs (2 in each groups I and III and 1 in group II) out of 24 animals. Deficit showed normal response on day 7 in 14 (3 in group I, 7 in group II and 4 in group III) dogs and 5 dogs (3 in group I, 2 in group III) showed normal response on day 14. Four dogs (2 in group I and 1 each in group II & III) out of 24 dogs affected with hind quarter weakness had normal patellar reflex, whereas, 19 (6 each in groups

I & III and 7 in group II) showed diminished and 1 (group III) showed increased reflex. Patellar reflex returned to normal in 16 dogs (4 in group III and 6 each in groups II & III) on day 7, and 4 dogs (2 in group I and 1 each in groups II & III) on day 14. Pelvic withdrawal reflex was found normal in 9 dogs (3 in group I, 1 in group II and 5 in group III), diminished in 14 (4 in group I, 7 in group II and 3 in group III) and increased in 1 (1 in group I). In 13 dogs, the reflex returned to normal on day 7 (4 in group I, 6 in group II and 3 in group III) and in 2 dogs (1 each in groups I & II) on day 14. Anal reflex was normal in 6 dogs (3 in group I, 1 in group II and 2 in group III). Eighteen dogs (5 in group I, 7 in group II and 6 in group III) exhibited diminished reflex which returned to normal in 17 dogs (4 in group I, 7 in group II and 6 in group III) on 7 day and one dog (group I) on day 14. Panniculus reflex was normal in 5 dogs (3 in group I, 1 each in groups II and III), diminished in 16 dogs (3 in group I, 7 in group II and 6 in group III) and increased in 3 dogs (2 in group I and 1 in group III). Panniculus reflex returned to normal in 18 dogs (4 in group I and 7 each in groups II and III) on 7 day, and in 1 dog (group I) on day 28.

Neurological examination provided very useful information regarding severity and exact location of injury. However, there was individual variation in the sensitivity among the animals showing similar clinical symptoms. All the animals regained their normal postural reactions, except hopping reaction in hind limbs, by day 14 of the therapy. Hopping reaction was achieved in 9 dogs (2 in group I, 3 in group II and 4 dogs in group III) by day 14 and in rest of them by day 28. It indicated that SMFT and TENS enhanced the recovery process when given along with conventional treatment.

Superficial and deep pain sensation along vertebral column was monitored and it was present in all the dogs. Intact pain sensation, on day of presentation, in all the dogs may be one of the major factors for the excellent to good improvement in all the dogs, barring one. Loss of deep pain perception is a bad prognostic sign, and surgery is seldom recommended when deep pain perception is absent for more than 48 hours (Wheeler, 1989). The other reason for the improvement recorded was that majority of the dogs were exhibiting diminished (LMN) hind limb spinal

reflexes, and only few exaggerated (UMN) reflexes. Parent (2000) reported that with the worsening of disease, the UMN became more affected and the paresis may be developed into paralysis.

Overall results indicated that there was a moderate to severe hind limb in-coordination from day 2 to 6. From day 7 onwards, dogs could stand with slight hind limb in-coordination and by day 14 they walked with near normal gait and normal clinical response. The dogs could stand and walk with complete clinical recovery on day 28. However, 1 dog in group I exhibited only mild improvement till day 28. Over all clinical improvement was excellent in 15 dogs (62.50 %), good in 8 dogs (33.33 %) and fair in 1 dog (4.16%). Overall results indicated the success rate as 95.83%. Only mild improvement in one dog till day 28 may be due to the advance symptoms of the disease and its delayed presentation to the Polyclinics (after 2 weeks of illness).

Further, critical review of clinical improvement in individual animals suggested that excellent and early neurological recovery was recorded in dogs subjected to transcutaneous electrical stimulation along with CDT followed by SMFT along with CDT and CDT alone.

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## Amelioration of *Lantana camara var aculeata* poisoning in crossbred Jersey cows

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### Abstract

Eight crossbred Jersey cows were presented to the Teaching Veterinary Clinical Complex of College of Veterinary Science and Animal Husbandry, O.U.A.T, Bhubaneswar, with clinical signs of complete off-fed, jaundice, photosensitization and fissure in skin, ear tip, muzzle and oral commissures, keratoconjunctivitis, corneal opacity with partial to complete loss of vision. The cows had history of eating *Lantana camara var aculeata*. The detailed clinical examination of these cows revealed atony of rumen, severe photosensitization. The visible mucous membranes were severely icteric and in five out of eight cows there was loss of vision. Laboratory analysis of blood revealed severe leucocytosis with increased level of total bilirubin and analysis of rumen liquor revealed reduction in protozoal counts. Basing on history, clinical examination and laboratory findings the cases were clinically diagnosed as *Lantana camara* poisoning. For amelioration of the toxicity it was found that oral use of Charcoal, Meboliv (Indian Herbs Ltd) syrup, *Saccharomyces* with metoclopramide hydrochloride, DNS (5%), and pheniramine maleate, eye drop containing ofloxacin and dexamethasone could cure these abnormalities in 100% cases. However loss of vision was not completely recovered with this therapeutic regimen but substantial improvement in vision was recorded in two out of five cases.

**Keywords:** Amelioration, Crossbred Jersey cows, *Lantana camara* poisoning

*Lantana camara var aculeata* is a wild weed and native to many parts of Orissa. The cows normally do not prefer to eat this plant; however, occasionally they fed on tender green leaves of *L. camara var aculeata* during July and August. It has three varieties and all are reported to cause different degrees of toxicity. Out of these *L. camara var aculeata*, red flowered one, is the most toxic (McSweeney, 1960). Among the cows the exotic and crossbred cattle are more susceptible to toxicity than indigenous cattle (Dwivedi *et al.*, 1972). Hepatotoxicity, jaundice, photosensitization and ruminal stasis have been reported in bovine (Radostits *et al.*, 1995). Loss of vision in lantana toxicity in bovine has not been reported earlier from this subcontinent. Many a times in rural parts of coastal Orissa, the condition has been a major cause of health hazards and induce morbidity and mortality, losses to poor farmers due to complexity in the toxic constituents i.e. Lantadene A, B, C and D – a pentacyclic triterpenoid, which does not have any specific antidote. Therefore control measures of the problem are largely depends upon in time diagnosis, stoppage of feeding and amelioration of the toxic effects. We therefore desired to undertake this study

to develop a package for amelioration of various toxic effects of *Lantana* in cross bred Jersey cows.

### Materials and Methods

The present study was conducted in eight crossbred Jersey cows clinically affected with *L. camara* toxicity. Detailed history of the cases revealed that they are being reared around Bhubaneswar, have eaten plenty of *L. camara var aculeata* plants in their grazing field. Blood samples were collected before medication and after clinical recovery or at time of death for the hematological examination. Parameters like hemoglobin, total leukocyte count were estimated using standard methods (Schalm *et al.*, 1975) and biochemical indices indicative of hepato-biliary function i.e. total bilirubin was estimated following the method of Varley 1980. The rumen fluid was collected through the stomach tube and its pH was estimated using broad range BDH paper. Rumen protozoan activity was measured as per the method suggested by Mishra & Singh, 1974. All the animals received the same ameliorative measures formulated on the basis of clinical and laboratory changes. The affected animals were treated with. (a) Charcoal @ 3gm/ kg b.wt. as drench once orally (b) Metoclopramide hydrochloride @ 0.3 mg/ kg b.wt intramuscularly twice daily for two days (c) Dextrose Normal Saline Solution @ 10 ml/ kg b.wt. twice daily intravenously for three consecutive days (d) Syrup Liver Stimulant (Meboliv) @ 25 ml once orally for nine days

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(e) Pheneramine maleate @ 1mg/ kg b.wt intramuscularly once daily for three days (f) *Saccaromyces* preparations (Yeastacc boli, Alltech) @ one bolus/ 100kg b.wt. twice daily for three days. The cows exhibiting vision abnormality were provided eye drop containing Ofloxacin & Dexamethasone @ four drops twice daily for five days in each eye. The owners were advised to keep their cows away from sun light and lantana plants till recovery, to provide adequate fresh drinking water and to dress the skin lesions regularly with Himax Ointment (Natural Remedies). All the cows were observed closely till recovery or death.

The assessment of the effect of ameliorative measures was made on the basis of score of clinical signs and the effect on haemato-biochemical changes.

The data were analyzed statistically as per methods of Snedecor and Cochran (1975).

### Results and Discussion

The results of the haemato-biochemical and rumen protozoan changes before and after treatment are given in Table No. 1. The appetite was started improving on third day post treatment. However, animals regain complete appetite between 4<sup>th</sup> and 7<sup>th</sup> day post treatment. This may be due to replacement of glucose, electrolyte and fluid, as well as restoration of the normal functions of damaged hepatocytes through liver stimulants, replacing rumen protozoan activity by *Saccaromyces* and enhanced excretion of toxins through faeces and urine as well as reduction in absorption of toxins from the gastrointestinal tract (Bhide and Akhter, 1991). All the animals were recovered from photosensitization (Fig-1), and skin eruptions (Fig – 2 & 3) between 6 and 8

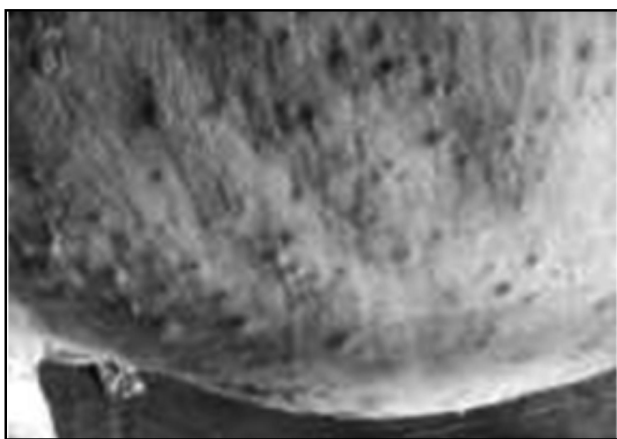


Fig.1 showing photosensitization



Fig.2 Showing eruptions of skin in oral commissures



Fig.3 Showing eruptions in muzzle and hyper salivation



Fig.4 Showing corneal opacity.

days of post-treatment. It may be due to enhanced rate of elimination of phylloerythrin in bile, inhibiting further absorption of toxins from the alimentary tract as well

**Table 1: Hemato-biochemical and rumen protozoan changes before and after treatment**

Parameters	Before Treatment	After Treatment (10 <sup>th</sup> day)
Hb (gm %)	10.4± 0.12	10.91± 0.06
TLC (10 <sup>3</sup> /Cmm.)	13.8 ± 0.21	7.92± 0.18**
Total Billirubin (mg/dl.)	2.48± 0.16	0.78± 0.05 **
Rumen liquor pH	10.50 ± 0.19	7.0± 0.2 *
Rumen protozoa	0-2/field	4-8 /field
Ruminal mortality/ 5min	1.2 ± 0.12	5.5 ± 0.12 **

\*denotes values differ significantly ( $P \leq 0.05$ ); \*\* denotes values differ significantly ( $P \leq 0.01$ )

as enhanced rate of excretion of plant residues from rumen and small intestine through fluid therapy, prokinetics and adsorbents. Similar findings were reported by Mc lennon and Amos (1989). The ruminal mortality along with peristalsis in these cows were significantly reduced, however these values were increased 3<sup>rd</sup> day onwards in majority of the treated animals. Dhillon and Paul, 1970 and Hussain and Roychoudhury 1992 have also reported effects of lantana toxicity on rumen function. However, the improvement of the abnormal rumen function through the present therapy appears to be interesting on clinical point of view. The definite cause of reduced rumen motility is not known however it might be due to the high alkaline pH of rumen liquor (Hussain and Roychoudhury, 1992) and reduced rumen microbial population by toxic effects of Lantana. During this study the ruminal motility came to normal within 4 to 6 days of treatment which may be due to increased microbial population along with decreased salivary secretion resulting in normalization of rumen pH as well as ruminal motility. This also supports the earlier findings



**Fig.5** Showing keratoconjunctivitis

of Kronfeld (1980).

The increased serum total billirubin and decreased in blood hemoglobin concentration are due to hemolytic effects of Icterogenin, a toxic principle from Lantana (Hari *et al.*, 1973), hepatobiliary disorder (Dhillon and Paul, 1970; Hussain and Roychoudhury 1992), paralysis of gallbladder, closure of bile canaliculi (Radostits *et al.*, 2000). The normalization of total serum billirubin and haemoglobin after treatment in the present study have indicated elimination of icterogenins from circulation along with normalization of hepatobilliary function with normal release of bile from bile duct into gastrointestinal tract. The total leukocyte count decreased to normal after treatment might be due to reduction in inflammatory process in the body system by different toxins of Lantana plant. This is in agreement with the findings of Ali *et al.* (1996)

The exact mechanism of corneal opacity (Fig 4) with loss of vision as detected in the present study is not clear. However, it might be due to effect of toxins of Lantana on the cranial nerve or higher centers in brain. The keratoconjunctivitis (Fig 5) might be due to localized contact reaction of Lantana in the eye during grazing and mechanical injury due to heavy scratching by the hoof. The parenteral antihistaminic along with localized application of antibiotics and corticosteroid seems to have ameliorative potential in this present study. Although three animals showing partial blindness were recovered after 2-3 weeks where as two animals showing complete loss of vision could not respond to this therapy warrants further detailed study on effect of lantana toxin on nervous system especially on cranial nerves in bovine.

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## Effect of vitamin E and Selenium on haemogram and oxidative stress parameters in poultry

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### Abstract

Aim of the present experiment was to study the effect of continuous oral supplementation of vitamin E and Se for a period of 30 days in broiler chicks on oxidative stress and haematological parameters. One day old healthy vaccinated broiler chicks (Strain-Vancobb) were randomly divided into two equal groups (n=6) as T<sub>1</sub> and T<sub>2</sub>. Chicks were maintained up to the age of 45 day and sacrificed on 46<sup>th</sup> day. From 15<sup>th</sup> to 45<sup>th</sup> day of age, chicks of group T<sub>2</sub> were fed Vitamin E + Selenium (Tocoseal®). Vitamin E and Selenium powder was orally given @ 600 mg/kg body weight after dissolving in olive oil at the rate of 600mg Vitamin E and Selenium/0.5ml of olive oil directly into proventriculus. Chicks of vehicle control group (T<sub>1</sub>) were fed olive oil (at the dose of 0.5 ml/kg body weight) daily for the same period. Blood was collected on day 15 and 45 from wing vein for estimation of haematological parameter. Liver and kidney tissues were collected from sacrificed birds of both the groups for biochemical studies. Vitamin E and Selenium treated group (T<sub>2</sub>) showed no significant change in TEC, PCV and clotting time in chicks. However, values of TLC and Hb were significantly lower compared to control chicks (T<sub>1</sub>). Similarly, vitamin E and Se did not affect SOD, catalase, AST and ALT enzymes activity in liver and kidney tissue, but it increased the level of GSH and concomitantly decreased lipid peroxidation level in liver and kidney tissue of broiler chick of group T<sub>2</sub>.

**Keywords:** Broiler chicks, Haematology, Oxidative stress, Selenium, Vitamin E

Vitamin E is one of the antioxidants widely used in poultry diets and has been reported as a major antioxidant in plasma membranes of all cells and sub-cellular organs, functioning as a chain-breaker and free radical scavenger. Poultry cannot synthesize vitamin E and its concentration is reduced under stress conditions. Similarly, Selenium (Se) also plays an important role in the antioxidant defence system due to its requirement by the Se-dependent glutathione peroxidises (GSHPx), which is involved in cellular antioxidant protection. It has been suggested that there is a synergistic relationship between Se and vitamin E, because GSHPx continues the work of vitamin E by detoxifying hydroperoxides (Surai, 2002b). The production of reactive free radicals (ROS) as by-products of metabolism that have the potential to damage or destroy cellular structures is in a dynamic equilibrium under normal conditions in living organisms. This dynamic equilibrium is provided by a balance between antioxidants and pro-oxidants (Koinarski *et al.*, 2005). However, stress factors such as nutritional (low digestible feed, polyunsaturated fatty acids, mycotoxins and oxidized oil, vitamin E and Se deficiency, vitamin A excess, heavy metals and other toxicants), environmental (high or low ambient temperatures, transportation, etc.) and pathogenesis of numerous diseases including parasitic infections, have a negative impact on this antioxidant/pro-oxidant balance (Surai, 2002b; Koinarski *et al.*, 2005). The

imbalance between an antioxidant and pro-oxidant system is named oxidative stress. In commercial poultry production, oxidative stress has been associated with the deterioration of many physiological functions including health, growth, reproduction and immunity. Intracellular defence mechanisms against ROS-induced damage may be classified as non-enzymatic (ascorbate,  $\alpha$ -tocopherol, carotenoids and glutathione) or enzymatic antioxidants, such as superoxide dismutase, catalase and glutathione peroxidises (Surai, 2002a; b). Keeping in view the above facts, the present work aimed to study the effect of vitamin E and Se supplementation on oxidative stress and haemogram in broiler chicks.

### Materials and Methods

Vitamin E and Se was used as Tocoseal® supplied by M/S Nandan remedies 311/8, Nagendra road, Kolkata. Each 10 g of Tocoseal® contained 5 g of Tocopherol acetate and 30 mg of sodium selenite.

### Design of experiment

Experiment was conducted using 12 healthy vaccinated broiler chicks (one day old, Strain- Vancobb). They were randomly divided into two equal groups (n=6) as T<sub>1</sub> and T<sub>2</sub>. Chicks in group T<sub>1</sub> was kept as vehicle control while T<sub>2</sub> was experimental group. Chicks of both groups were maintained up to the age of 45 day and sacrificed on 46<sup>th</sup> day. Chicks were maintained on

balanced broiler starter feed (Metabolic energy 2890 Kcal /Kg and crude protein 22.1%) for first 4 weeks followed by broiler finisher feed (Metabolic energy 2945 Kcal /Kg and crude protein 19.10%) for the remaining period of experimentation as per recommendation of BIS, (1992). Fresh water and feed was supplied *ad lib*. From 15<sup>th</sup> to 45<sup>th</sup> days of age chicks of group T<sub>2</sub> were fed orally Vitamin E + Selenium @ 600 mg/kg body weight after dissolving in olive oil @ 600mg Tocosel® /0.5ml of olive oil directly into proventriculus. Chicks of vehicle control group (T<sub>1</sub>) were fed olive oil @ 0.5 ml/kg body weight daily for the same duration. On 15<sup>th</sup> and 45<sup>th</sup> day of age, blood (3ml) was collected with EDTA (5%) from wing vein for haematological parameter estimation. Liver and kidney tissues were collected from sacrificed birds of each group for biochemical study.

#### **Hematological study**

Total erythrocyte count (TEC), Total leucocyte count (TLC) (Natt and Herick, 1952), Hemoglobin (Hb) level (Coffin, 1953), haematocrit value (PCV) by Wintrobe hematocrit tube (Schalm *et al.*, 1975), Clotting time (Schalm *et al.*, 1975) were estimated and expressed as SI unit.

#### **Estimation of biochemical parameter**

Immediately after sacrifice, liver and kidney tissue were collected in ice-cold normal saline for estimation of various biochemical parameters. Tissues were minced, washed in chilled distilled water and potassium hydroxide solution (1.15%w/v) to remove all blood clots and then blotted. 10% homogenate was prepared by using tissue homogeniser (Remi RQ127A) maintaining cold condition. Homogenate was centrifuged in refrigerated centrifuge machine (4°C) at 6000 rpm for 20 minute (Remi-C-24) and supernatant was collected. This supernatant was utilised for estimation of reduced glutathione (Griffith, 1980), Superoxide dismutase (Misera and Fridovitch, 1972), Aspartate transaminase (AST), Alanine transaminase (ALT) (Yatizidis, 1960), Catalase (Abei, 1974) and protein (Wooton, 1974). Homogenate (10%) was prepared from liver and kidney tissue of each chick by adding 0.1 ml of butylated hydroxyl toluene (BHT) in ethanol and 9.9 ml of 1mM EDTA to 1 g of tissue for estimation of lipid peroxidation. Trichloroacetic acid was added to it, then centrifuged at 6000 rpm for 20 min. Supernatant was

used for lipid peroxidation estimation (Nair and Turner 1984).

#### **Statistical analysis**

Mean and standard error were calculated and data were analysed using standard methods of Snedecor and Cochran (1968). Differences at P<0.05 (at least) were considered to be significant.

#### **Results**

From the perusal of Table 1 depicting haemogram of chicks in group T<sub>1</sub> and T<sub>2</sub>, it is evident that vitamin E and Selenium caused no significant change in TEC, PCV and clotting time following daily consecutive oral administration for 30 days. However, values of TLC and Hb were significantly lower compared to control chicks (T<sub>1</sub>).

From the results of oxidative stress and biochemical parameters in liver and kidney tissues of broiler chick of the experiment (Table 2), it is evident that vitamin E and Se supplementation had non-significant effect on activities of superoxide dismutase (SOD), catalase, AST and ALT enzymes in both liver and kidney tissue of broiler chicks following oral administration for consecutive 30 days. However, it significantly increased the level of reduced glutathione (GSH) and concomitantly decreased lipid peroxidation (LPO) level in liver and kidney tissue of broiler chick in group T<sub>2</sub>.

#### **Discussion**

In present study oral feeding of vitamin E and Se @ 600mg/kg body weight increased haemoglobin % and TLC significantly but no significant changes were observed in PCV, TEC and clotting time as compared to control group (T<sub>1</sub>) chicks. There are variable findings in available literatures regarding the effect of vitamin E and Se on haematological parameters. Cay and Nazirogin (1999) observed a significant increase in TLC value but no significant changes in RBC, haemoglobin percentage and packed cell volume in male Wistar rats treated with vitamin E and selenium by intraperitoneal route for 5 weeks. Trans *et al.* (2000) in their experiment observed no significant effect of vitamin E and Selenium supplementation for 45 days from 1<sup>st</sup> day in male broiler chicks on haematological parameter (red blood cell, haemoglobin, haematocrite and mean corpuscular volume).

**Table 1: Effect of vitamin E and Se on haemogram before and after 30 days oral administration in broiler chick. (Mean  $\pm$  SE, n=6)**

Parameters	15 <sup>th</sup> day		45 <sup>th</sup> day	
	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>
Haemoglobin (gm/L)	84.00 $\pm$ 2.6	83.00 $\pm$ 2.4	92.00 $\pm$ 3.5	103.00 $\pm$ 2.4
PCV %	0.26 $\pm$ 0.009 <sup>a</sup>	0.26 $\pm$ 0.012 <sup>a</sup>	0.29 $\pm$ 0.009 <sup>a</sup>	0.32 $\pm$ 0.008 <sup>a</sup>
Clotting time (in sec.)	170.00 $\pm$ 11.76	150.00 $\pm$ 11.88	160.00 $\pm$ 7.80	150.00 $\pm$ 10.5
TEC (10 <sup>12</sup> /L)	2.30 $\pm$ 0.130	2.33 $\pm$ 0.18	3.01 $\pm$ 0.24	3.90 $\pm$ 0.25
TLC (10 <sup>9</sup> /L)	19.05 $\pm$ 0.69	18.50 $\pm$ 0.75	21.80 $\pm$ 1.05	26.80 $\pm$ 0.76

The values with dissimilar superscript vary significantly ( $P \leq 0.05$ ).

**Table 2: Effect of vitamin E and Se on oxidative stress and biochemical parameters before and after 30 days oral administration in liver and kidney tissue of broiler chick. (Mean  $\pm$  SE, n=6)**

Parameters	Liver		Kidney	
	T <sub>1</sub>	T <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>
SOD (Units/mg protein)	0.58 $\pm$ 0.03	0.67 $\pm$ 0.03	0.56 $\pm$ 0.03	0.65 $\pm$ 0.03
Catalase (nmole of H <sub>2</sub> O <sub>2</sub> decomposed/minute/mg protein)	0.18 $\pm$ 0.007	0.20 $\pm$ 0.02	0.09 $\pm$ 0.02	0.11 $\pm$ 0.02
GSH (n mole of reduced glutathione/mg of wet tissue)	3801.00 $\pm$ 128.44	4777.60 $\pm$ 194.10	2201.80 $\pm$ 153.63	2962.26 $\pm$ 149.68
LPO (n mole of malonaldehyde/gm of wet tissue)	4.98 $\pm$ 0.35	2.10 $\pm$ 0.17	5.12 $\pm$ 0.49	2.20 $\pm$ 0.150
AST ( $\mu$ g of pyruvic acid formed/hr/mg protein)	250.40 $\pm$ 12.62	262.08 $\pm$ 11.22	317 $\pm$ 9.34	320.45 $\pm$ 11.57
ALT ( $\mu$ g pyruvic acid formed/hr/mg protein)	18.45 $\pm$ 1.08	20.25 $\pm$ 1.21	10.22 $\pm$ 1.05	12.0 $\pm$ 1.21

The values with dissimilar superscript vary significantly ( $P \leq 0.05$ ).

In oxidative stress parameters, no significant change was observed in SOD, catalase, but there was significant increase in GSH level and consequently decrease in LPO in both liver and kidney tissue of group T<sub>2</sub> as compared with vehicle control group (T<sub>1</sub>) chicks. AST and ALT levels were also non-significantly different in both the groups. Ozturk-Urek *et al.* (2001) also found that feeding of selenium and vitamin E @ 0.07 mg Se and 70 mg  $\alpha$ -tocopherol/kg of diet to chicks significantly decreased LPO and increased glutathione peroxidase in tissue but they did not affect the SOD and catalase activity in different tissue of chicks. Aydemir *et al.* (2000) also reported that combined supplementation of Vitamin E and Se decreased the LPO (46%) but it did not affect the catalase and SOD enzyme activity in chicken erythrocyte. Scott *et al.* (1977) also observed that rats on a high vitamin E intake 500 mg/kg had a significantly higher tissue GSH level while selenium intake had no effect on GSH level. Cadenas *et al.* (1995) reported that Vitamin E supplementation in diet (15, 150, 1500mg vitamin E/kg diet) of male guinea pig caused decrease in lipid peroxidation level in different

tissue but did not affect antioxidant enzyme and non-enzymatic antioxidants such as SOD, catalase, GSH-Peroxidase, GSH-reductase and GSH.

Vitamin E is involved in removal of free radical and prevent their peroxidative effect on unsaturated lipid of membrane and thus help in maintaining integrity of membrane. Chromanol ring of tocopherols donates its phenolic hydrogen to reduce the free radical and is itself oxidised to the quinone form. Vitamin E appears to be the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipid. Phospholipid of mitochondria, endoplasmic reticulum and plasma membrane possess affinity for  $\alpha$ -tocopherol and vitamin E appear to concentrate on these sites. Tocopherol acts as anti-oxidants, breaking free radical chain reaction as a result of their ability to transfer phenolic hydrogen to a peroxy free radical of a peroxidised poly-unsaturated fatty acid (Mays, 1996). Both vitamin E and selenium act to protect membrane lipid but in different fashion. Selenium works through glutathione and glutathione

peroxidase system to increase destruction of peroxides while vitamin E is believed to prevent oxidation of unsaturated fatty acid (Hockstra, 1970). Thus in present study vitamin E and Se decreased normal lipid peroxidation level and thus increased GSH level in treated group as compared to control.

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## Effect of curcumin and alpha-tocopherol in histologically confirmed cases of chronic hepatitis in dogs

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### Abstract

Chronic hepatitis is a frustrating disease with inevitable progression to cirrhosis, and thus has a poor prognosis. Definitive diagnosis of this condition invariably requires liver biopsy. The aim of the present study was to find out the efficacy of curcumin and alpha-tocopherol in histologically confirmed cases of chronic hepatitis in dogs. The clinical cases were confirmed through serum biochemistry, liver biopsy and ultrasonographic findings. Out of 39 confirmed cases, 21 were treated by curcumin and 18 with alphotocopherol. On the basis of the results obtained in this study, it is concluded that therapy with curcumin or alphotocopherol produced equally good clinical response (75 per cent each) and significantly ( $P \leq 0.01$ ) reduced enzyme levels subsequent to therapy. Curcumin or alphotocopherol can be used as therapeutic agents to reduce the progression of chronic hepatitis. As seen from the enzyme and biochemical assays, curcumin had more effect than alphotocopherol.

**Keywords:** Alpha-tocopherol, Chronic hepatitis, Curcumin, dogs.

The combined measurement of alkaline phosphatase (ALP/SAP) and serum bile acid (SBA) is very sensitive and specific for detecting liver diseases and bile acid estimation could be used for screening chronic hepatitis (Twedt, 1998; Sterczer *et al.*, 2001). The liver biopsy is always required for definitive diagnosis (Rutgers and Haywood, 1988; Twedt, 1998; Sterczer *et al.*, 2001). In canine chronic hepatitis, treatment remains symptomatic and largely aimed at slowing progression to irreversible fibrosis rather than specific treatment of the underlying cause. Where the initiating factor is unknown or where it is known but chronic changes are already present, the aim is to intervene at the next stage to arrest fibrogenesis and prevent irreversible hepatic fibrosis (Watson, 2004).

Alphotocopherol protect the liver from many types of hepatic oxidant damage and it can be given @ 5 to 10 IU/kg per day to dogs with chronic hepatitis (Twedt, 1998). Vitamin E is an antioxidant that is recommended at a dose of 50 to 600 IU per day. Oxidant injury to hepatic mitochondria has been reported in Bedlington terriers with copper storage hepatopathy (Sokol *et al.*, 1994). The use of antioxidants as adjunctive therapies to reduce fibrosis in dogs with CH has long been recommended, with vitamin E most commonly being used (Center, 2000). Curcuminoids are effective in suppressing the hepatic microvascular inflammatory response to lipopolysaccharide and may be a natural alternative anti-inflammatory substance

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(Lukita – Atmadja *et al.*, 2000). Curcumin induces the contraction of human gall bladder (Rasyid and Lelo, 1999). The effect of contraction is on dose dependent manner (Rasyid *et al.*, 2002). Curcumin alleviates the severity of hepatic inflammation in experimental steatohepatitis induced by the methionine, choline deficient diet, an effect likely to be mediated via inhibition of NF- $\kappa$ B activation and dependent proinflammatory genes (Leclercq *et al.*, 2004). The copper (II)-Curcumin complex, possesses superoxide dismutase activity, free radical neutralizing ability and antioxidant potential (Barik *et al.*, 2005).

The purpose of this study was to find out the therapeutic effect of curcumin and alpha tocopherol in hoistologically confirmed cases of CH in dogs. To the best of our knowledge it may be the first report on therapeutic studies on curcumin in chronic hepatitis dogs.

### Materials and Methods

The sick dogs brought to small animal outpatient unit at Madras Veterinary College, Chennai with clinical signs suggestive of liver disease were screened by serum biochemistry, liver biopsy and ultrasonographic findings. Six animals brought from the Chennai city police for routine health check-up acted as healthy control. The health status was analyzed by their records and physical and haematological examination.

Confirmed cases were randomly divided in to



two groups and subjected to clinical trial with  $\alpha$  – tocopherol (Vitamin E) or curcumin. Alpha tocopherol was given at a dose of 5 to 10 IU / kg body weight per day (Twedt, 1998). Curcumin was given at a dose rate of 20 mg / kg body weight twice a day (Rasyid and Lelo, 1999; Rasyid *et al.*, 2002; Mekala *et al.*, 2006). The curcumin capsules were purchased from Mount Sahiya, Kottayam, Kerala as 300 mg capsules (99 per cent pure). The owners were encouraged to report any unwanted symptoms at the earliest. Clinical cases of chronic hepatitis were randomly allotted to the treatment trials with curcumin or alphotocopherol for a period of two months or more (ie. until clinical improvement was noticed). Monitoring of these cases was done by biochemical evaluation at two weeks interval. The data on the last visit after the reported clinical recovery was used for statistical analysis. The parameters were studied before and after the trial in both the groups.

Initially, Amoxycillin-cloxacillin was administered for 5-7 days along with supportive intravenous fluid therapy and oral vitamins B therapy. Other symptomatic therapy included control of coagulopathy, ascites and GI ulceration. Further, the owner's were advised to feed their dogs with a highly digestible diet containing high biological value proteins, high carbohydrate and moderate fat. Also complete blood count and serum biochemistry were done before and after treatment.

The serum biochemical parameters were analysed by semi auto analyzer through commercial kits.

Blind percutaneous transabdominal technique as described by Center (1996a) was followed and 14G true-cut disposable biopsy needle was used. In few cases, necropsy samples were collected because of owner's inconvenience or failure of attempts at treatment or owing to the end stage of disease. The samples were collected and stored in 10% formalin and processed routinely. The changes were read under light microscope. The data from 15 animals in each group, which had both before and after treatment values were subjected to paired 't' test for evaluating the treatment efficacy. One way ANOVA was used to compare the means of serum biochemical changes between the groups by SPSS software version 15.

## Results and Discussion

Total 39 cases were confirmed as chronic hepatitis through histopathological and serum biochemical changes. In rest of the cases, tissue samples could not be collected mostly due to the owner's non compliance for undertaking biopsy or necropsy. No bleeding tendencies or untoward effects were noticed during or after the biopsy procedure. The histopathological changes in this study were achieved by acquisition of biopsy/necropsy specimen based on the histopathological observations as outlined by Sevelius (1995). The ultrasonographic changes of control and diseased animals were followed by the guidelines of Nyland *et al.* (2002). Most cases of chronic hepatitis don't have etiology that can be readily diagnosed. They seemed to be idiopathic. Therefore,

**Table 1:** Mean  $\pm$  S.E. values of serum biochemical parameters in treatment trial groups of chronic hepatitis (before treatment)

Parameters	Control(n=10)	Curcumin(n=15)	Alphotocopherol(n=15)
ALT (U/L)	30.77 $\pm$ 3.30 <sup>a</sup>	154.12 $\pm$ 25.78 <sup>b</sup>	128.90 $\pm$ 25.06 <sup>b</sup>
SAP (U/L)	92.21 $\pm$ 7.53 <sup>a</sup>	379.98 <sup>b</sup> $\pm$ 69.08	386.11 $\pm$ 64.45 <sup>b</sup>
GGT (U/L)	3.81 $\pm$ 0.42 <sup>a</sup>	19.44 $\pm$ 3.09 <sup>b</sup>	16.61 $\pm$ 2.42 <sup>b</sup>
Total Bilirubin (mg/dl)	0.45 $\pm$ 0.03 <sup>a</sup>	1.82 $\pm$ 0.20 <sup>b</sup>	1.45 $\pm$ 0.21 <sup>b</sup>
Direct Bilirubin (mg/dl)	0.28 $\pm$ 0.02 <sup>a</sup>	1.16 $\pm$ 0.14 <sup>b</sup>	0.96 $\pm$ 0.16 <sup>b</sup>
Total Protein (gm/dl)	7.21 $\pm$ 0.14 <sup>b</sup>	6.28 0.32 <sup>a</sup>	6.22 $\pm$ 0.29 <sup>a</sup>
Albumin (gm/dl)	3.27 $\pm$ 0.10 <sup>b</sup>	2.12 $\pm$ 0.12 <sup>a</sup>	2.14 $\pm$ 0.13 <sup>a</sup>
Globulin (gm/dl)	4.03 $\pm$ 0.14	4.23 $\pm$ 0.28	4.00 $\pm$ 0.24
BUN (mg/dl)	21.13 $\pm$ 1.14	28.69 $\pm$ 4.79	24.99 $\pm$ 3.13
Creatinine (mg/dl)	0.95 $\pm$ 0.07	1.03 $\pm$ 0.11	1.04 $\pm$ 0.16
Glucose (mg/dl)	105.60 $\pm$ 60	100.01 $\pm$ 4.19	98.18 $\pm$ 3.78
Cholesterol (mg/dl)	221.42 $\pm$ 12.09	218.68 $\pm$ 16.74	238.38 $\pm$ 19.57

Mean bearing the different superscript in the same row differ significantly ( $P \leq 0.01$ ).

**Table 2:** Serum biochemical parameters (Mean  $\pm$  S.E) in treatment trial groups of chronic hepatitis (after treatment)

Parameters	Control(n=10)	Curcumin(n=15)	Alphatocopherol(n=15)
ALT (U/L)	30.77 $\pm$ 3.30 <sup>a</sup>	68.99 $\pm$ 6.76 <sup>b</sup>	70.35 $\pm$ 10.10 <sup>b</sup>
SAP (U/L)	92.21 $\pm$ 7.53 <sup>a</sup>	196.56 $\pm$ 21.45 <sup>ab</sup>	295.32 $\pm$ 61.52 <sup>b</sup>
GGT (U/L)	3.81 $\pm$ 0.42 <sup>a</sup>	12.38 1.91 <sup>b</sup>	14.29 $\pm$ 2.10 <sup>b</sup>
Total Bilirubin (mg/dl)	0.45 $\pm$ 0.03	1.07 $\pm$ 0.10	1.64 $\pm$ 0.62
Direct Bilirubin (mg/dl)	0.28 $\pm$ 0.02	0.68 $\pm$ 0.06	1.03 $\pm$ 0.37
Total Protein (mg/dl)	7.2 $\pm$ 0.148	6.54 $\pm$ 0.29	6.43 $\pm$ 0.16
Albumin (gm/dl)	3.27 $\pm$ 0.10 <sup>b</sup>	2.75 $\pm$ 0.08 <sup>a</sup>	2.72 $\pm$ 0.20 <sup>a</sup>
Globulin (gm/dl)	4.035 $\pm$ 0.14	3.84 $\pm$ 0.25	3.82 $\pm$ 0.14
BUN (mg/dl)	21.13 $\pm$ 1.14	29.85 $\pm$ 7.02	28.84 $\pm$ 5.36
Creatinine (mg/dl)	0.95 $\pm$ 0.07	1.51 $\pm$ 0.48	1.16 $\pm$ 0.15
Glucose (mg/dl)	105.60 $\pm$ 3.48	99.86 $\pm$ 2.32	104.72 $\pm$ 4.26
Cholesterol (mg/dl)	221.42 $\pm$ 12.09	221.02 $\pm$ 7.60	228.43 $\pm$ 10.98

Mean bearing the different superscript in the same row differ significantly ( $P \leq 0.01$ ).

**Table 3:** Effect of treatment on serum biochemical parameters

Parameters	Curcumin Therapy (n = 15)		Alphatocopherol Therapy (n = 15)	
	Pre-therapy	Post therapy	Pre-therapy	Post therapy
ALT (U/L)	154.13 $\pm$ 25.7	69.00 $\pm$ 6.76**	128.90 $\pm$ 25.06	70.35 $\pm$ 10.10**
SAP (U/L)	379.98 $\pm$ 69.08	196.57 $\pm$ 21.45**	386.12 $\pm$ 64.45	295.33 $\pm$ 61.52
GGT (U/L)	19.45 $\pm$ 3.09	12.39 $\pm$ 1.91**	16.61 $\pm$ 2.42	14.30 $\pm$ 2.10
Total Bilirubin (mg/dl)	1.83 $\pm$ 0.20	1.08 $\pm$ 0.10**	1.45 $\pm$ 0.21	1.65 $\pm$ 0.62
Direct Bilirubin (mg/dl)	1.17 $\pm$ 0.14	0.68 $\pm$ 0.06**	0.97 $\pm$ 0.16	1.04 $\pm$ 0.37
Total Protein (mg/dl)	6.29 $\pm$ 0.32	6.55 $\pm$ 0.29	6.22 $\pm$ 0.29	6.43 $\pm$ 0.29
Albumin (gm/dl)	2.12 $\pm$ 0.12	2.76 $\pm$ 0.08**	2.15 $\pm$ 0.13	2.72 $\pm$ 0.20**
Globulin (gm/dl)	4.24 $\pm$ 0.28	3.84 $\pm$ 0.25	4.00 $\pm$ 0.24	3.83 $\pm$ 0.14
BUN (mg/dl)	28.70 $\pm$ 4.79	29.86 $\pm$ 7.02	24.99 $\pm$ 3.13	28.85 $\pm$ 5.36
Creatinine (mg/dl)	1.04 $\pm$ 0.11	1.52 $\pm$ 0.48	1.04 $\pm$ 0.16	1.16 $\pm$ 0.15
Glucose (mg/dl)	100.02 $\pm$ 4.19	99.86 $\pm$ 2.32	98.18 $\pm$ 3.78	104.72 $\pm$ 4.26
Cholesterol (mg/dl)	218.69 $\pm$ 16.74	221.03 $\pm$ 7.60	244.51 $\pm$ 19.40	228.43 $\pm$ 10.98

\*Significant ( $P \leq 0.01$ ), \*\*Highly Significant ( $P \leq 0.01$ )

treatment depends on addressing pathology findings on the liver biopsy such as inflammation and necrosis. Hence, the treatment remains symptomatic and largely aimed at slowing progression to irreversible fibrosis rather than specific treatment to the underlying cause (Watson, 2004). There are a number of reviews of recommended treatment for canine chronic hepatitis aiming to halt progression (Dill-makey, 1995; Center, 1996b and 2000).

In this study, 21 were treated with Curcumin and 18 with Alphatocopherol. There was poor owner compliance in 5 of Curcumin trial and 2 of Alphatocopherol trial. Consequently 16 cases each received treatment with Curcumin / Alphatocopherol.

Out of them, 75 per cent (12 cases each) of cases in each trial improved clinically and rest of them either died or did not make any appreciable progress during the study period.

The serum enzymes like ALT, SAP and GGT were significantly ( $P \leq 0.01$ ) increased almost 4-5 times in chronic hepatitis in both Curcumin and Alphatocopherol group (Table 1). Their levels decreased (Table 2) to 2-3 times the control group after treatment with curcumin or alphatocopherol ( $P \leq 0.01$ ). The post treatment means of all the three enzymes in both treatment groups were still elevated when compared to the control group. The post treatment elevated levels were statistically significant ( $P \leq 0.01$ ) with respect to

all three enzymes in alphotocopherol group and only with ALT and GGT in curcumin group (Table 2). Within group the decrease in ALT, SAP and GGT after treatment were significant ( $P \leq 0.01$ ) for all the three enzymes in curcumin group. Whereas, the decrease in ALT alone was significant in alphotocopherol group (Table 3). Significant ( $P \leq 0.01$ ) reduction in all the serum enzyme values (ALT, SAP and GGT) in curcumin group and significant reduction in ALT on alphotocopherol group were indicative of satisfactory therapeutic value of both curcumin and alphotocopherol in the treatment of chronic hepatitis. However, it should be noted that curcumin had comparatively more therapeutic efficacy than alphotocopherol as seen from the enzyme assays of this study. It is thought that changes in liver initially affect the hepatocytes which lead to secretion of ALT to the serum; only later when the inflammation spread to the bile duct can elevate levels of SAP be detected in the serum (Speeti *et al.*, 1996). Center (2007) stated that ALP and GGT are membrane bound enzymes and located in biliary membrane and hence they are elevated in cholestatic disorders. It may be interpreted from these observations that curcumin has a better effect on cholestatic disorders than alphotocopherol. Occurrence of chronic cholangiohepatitis in four cases of Curcumin group and three cases of Alphotocopherol group also supported this interpretation.

Total bilirubin and direct bilirubin were found elevated significantly ( $P \leq 0.01$ ) before treatment both in curcumin and alphotocopherol group (Table 1). After treatment their levels decreased and post treatment means and control means were on par (Table 2). However, their decrease was significant only in curcumin group (Table 3). These observations further supported the interpretation of better efficacy of curcumin on cholestatic disorders.

Serum total protein and albumin levels were found decreased significantly before treatment in both curcumin ( $6.28 \pm 0.32$  and  $2.12 \pm 0.12$  g/dl) and alphotocopherol group ( $6.22 \pm 0.29$  and  $2.14 \pm 0.13$ ) when compared to control. The treatment increased their levels to  $6.54 \pm 0.29$  gm/dl and  $2.75 \pm 0.08$  gm/dl in curcumin group and to  $6.43 \pm 0.16$  gm/dl and  $2.72 \pm 0.20$  gm/dl in alphotocopherol group and the post treatment means were on par with control group (Table 2). The increases in serum albumin levels were significant ( $P \leq 0.01$ ) and the same with total protein was

insignificant in both the treatment groups (Table 3). Treatment with curcumin or alphotocopherol lead to improvement in the protein status and thus restored their levels nearer to the control group (Table 2). Pre and post treatment means of serum albumin in both the groups indicated significant ( $P \leq 0.01$ ) improvement (Table 3). Thus the improvement in serum protein status can be attributed to improvement in the albumin synthesis function of liver as a result of restoration in hepatic function. The improvement in serum bilirubin and serum albumin status can be considered very significant in monitoring the treatment response in chronic hepatitis. The levels of serum globulin during disease and after treatment varied insignificantly from that of the control. BUN, creatinine, glucose and cholesterol levels of pre and post treatment means varied insignificantly ( $P \leq 0.01$ ) from control group (Table 1 and 2) and between them (Table 3) during disease and after treatment with curcumin or alphotocopherol. Hence they are considered unimportant parameters in the monitoring of therapy on chronic hepatitis in dogs.

On the basis of the results obtained in this study, it is concluded that therapy with Curcumin or Alphotocopherol produced equally good clinical response (75 per cent each) and significantly reduced enzyme levels subsequent to therapy. Curcumin or Alphotocopherol can be used as therapeutic agents to reduce the progression of chronic hepatitis. As seen from the enzyme and biochemical assays, Curcumin can be reported to be more effective than Alphotocopherol.

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## Efficacy of oral vitamin E supplementation in bovine clinical mastitis

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### Abstract

The present investigation was carried out to evaluate ameliorative potential of oral supplementation of vitamin E in bovine clinical mastitis. Six cases of clinical mastitis in cross-bred cows selected on the basis of physical examination of udder and milk and confirmation by cultural examination of milk were randomly divided into two groups. Animals of group I were administered enrofloxacin @ 5 mg/kg body weight daily by intramuscular route for 5 days. Animals of group II were supplemented with vitamin E @ 1000 I.U. orally along with enrofloxacin injection daily for 5 days. Three lactating cows showing negative CMT reaction and SCC  $<2 \times 10^5$  cells/ml were considered as healthy control. Therapeutic efficacy was assessed on basis of CMT, SCC in quarter's milk on day 0, 4, 7 and 14 of the therapy and measurement of oxidative stress indices in blood on day 0, 7 and 14 of the therapy. On therapy, there was significant ( $P < 0.05$ ) reduction in CMT point score and SCC in quarter's milk within a group but the reduction in values in group II was significantly ( $P < 0.05$ ) higher than that of group I indicating earlier clinical response. The oxidative stress indices revealed a significant ( $P < 0.05$ ) upregulation in antioxidant levels and insignificantly higher reduction in lipid peroxidation in mastitic cows supplemented with vitamin E, suggesting the ameliorative potential of vitamin E in bovine clinical mastitis and thus, may be incorporated in its therapeutic regimen along with antibiotic for earlier clinical response.

**Keywords:** Amelioration, Bovine Clinical mastitis, Oral supplementation, Vitamin E

Mastitis is one of the most economic disease of dairy animals accounting for a loss of Rs. 60532 millions/annum to dairy industry in India (Dua, 2001). It is characterized by an influx of somatic cells, primarily polymorphonuclear neutrophils, into the mammary gland which destroy the invading microbe via oxygen-dependent and oxygen-independent systems. Simultaneously they damage the secretory epithelium of mammary gland via reactive oxygen species and proteolytic enzymes, resulting in decreased milk production (Zhao and Lacasse, 2008). Even though antibiotics are very useful to treat the infection, they cannot prevent oxidative damage caused by release of toxic radicals of tissue breakdown and reactive molecules (Blum *et al.*, 2000). These toxic radicals can be neutralized by antioxidants like vitamin E, vitamin C, selenium, copper, zinc etc. (Sharma, 2007).

Vitamin E is an important lipid soluble antioxidant that protects the cells against free radical-initiated lipid peroxidation (Halliwell and Gutteridge, 2007). Low levels of the plasma and milk vitamin E has been observed in bovine mastitis (Atroshi *et al.*, 1987) and its supplementation has been shown to reduce incidence and duration of clinical mastitis in dairy cows

(Smith *et al.*, 1984). Therefore, the present investigation was carried out to evaluate ameliorative potential of oral supplementation of vitamin E in clinical cases of bovine mastitis.

### Materials and methods

**Selection of animals:** Six cases of acute clinical mastitis in cross-bred cows (crosses of Jersey/Brown Swiss/Haryana/Holstein Friesian) maintained under identical feeding and management practices at the Cattle and Buffalo farm, Indian Veterinary Research Institute, Izatnagar, India were selected based on physical examination of udder and milk. Intramammary infection was confirmed by cultural examination of milk and requisite biochemical tests as per Quinn *et al.* (2004) and antibiotic sensitivity of each isolate was determined by disc diffusion method as per Bauer *et al.* (1966).

**Therapeutic regimen:** Animals were randomly divided into two groups of 3 animals each. Animals of group I were administered enrofloxacin (selected on the basis of antibiotic sensitivity) @ 5 mg/kg body weight daily by intramuscular route for 5 days. Animals of group II were supplemented with vitamin E @ 1000 I.U. orally along with enrofloxacin injection daily for 5 days. Three lactating cows showing negative CMT reaction and SCC  $<2 \times 10^5$  cells/ml were kept in group III as healthy control.

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**Table 1:** Mean CMT point score and SCC in quarter's milk in different groups in response to therapy (Mean±S.E.)

Parameter	Group	d0	d4	d7	d14
CMT point score	I	3.67±0.33 <sup>ab</sup>	2.67±0.33 <sup>bb</sup>	1.83±0.17 <sup>cc</sup>	0.92±0.08 <sup>db</sup>
	II	3.83±0.17 <sup>ab</sup>	2.17±0.17 <sup>bb</sup>	1.17±0.17 <sup>cb</sup>	0.80±0.10 <sup>cb</sup>
	III	0.00±0.00 <sup>A</sup>	0.00±0.00 <sup>A</sup>	0.00±0.00 <sup>A</sup>	0.00±0.00 <sup>A</sup>
SCC (10 <sup>5</sup> /ml)	I	86.13±0.99 <sup>ab</sup>	42.83±2.11 <sup>bc</sup>	24.07±2.60 <sup>cc</sup>	8.40±0.62 <sup>dc</sup>
	II	85.13±2.01 <sup>ab</sup>	28.90±1.61 <sup>bb</sup>	11.30±1.33 <sup>cb</sup>	3.67±0.46 <sup>db</sup>
	III	1.87±0.06 <sup>A</sup>	1.85±0.05 <sup>A</sup>	1.85±0.07 <sup>A</sup>	1.86±0.06 <sup>A</sup>

(Mean±S.E.) for a parameter with superscripts a, b, c, d differ significantly ( $P \leq 0.05$ ) in a row

(Mean±S.E.) for a parameter with superscripts A, B, C differ significantly ( $P \leq 0.05$ ) in a column

**Table 2:** Oxidative stress indices in blood in different groups in response to therapy (Mean±S.E.)

Oxidative stress indices	Group	d0	d7	d14
LPO (nmol MDA/mg Hb)	I	3.280±0.101 <sup>ab</sup>	3.170±0.044 <sup>bb</sup>	2.823±0.068 <sup>bb</sup>
	II	3.177±0.103 <sup>ab</sup>	2.733±0.056 <sup>bb</sup>	2.670±0.069 <sup>bb</sup>
	III	2.453±0.060 <sup>A</sup>	2.447±0.035 <sup>A</sup>	2.477±0.068 <sup>A</sup>
GSH (µmol conjugate/ml packed RBC)	I	0.066±0.007 <sup>B</sup>	0.068±0.006 <sup>C</sup>	0.069±0.004 <sup>B</sup>
	II	0.072±0.006 <sup>ab</sup>	0.092±0.004 <sup>bb</sup>	0.103±0.006 <sup>ba</sup>
	III	0.108±0.009 <sup>A</sup>	0.118±0.008 <sup>A</sup>	0.105±0.003 <sup>A</sup>
CAT (µmol H <sub>2</sub> O <sub>2</sub> decomposed/min/mg Hb)	I	69.57±2.20 <sup>ab</sup>	118.75±5.05 <sup>bc</sup>	124.00±4.87 <sup>bb</sup>
	II	69.45±3.14 <sup>ab</sup>	156.38±4.64 <sup>bb</sup>	165.01±3.55 <sup>ba</sup>
	III	180.30±2.52 <sup>A</sup>	178.25±3.14 <sup>A</sup>	178.07±4.56 <sup>A</sup>
SOD (µmol MTT formazan/mg Hb)	I	0.455±0.005 <sup>ab</sup>	0.475±0.007 <sup>ab</sup>	0.517±0.007 <sup>bc</sup>
	II	0.446±0.007 <sup>ab</sup>	0.503±0.011 <sup>bb</sup>	0.554±0.007 <sup>cb</sup>
	III	0.583±0.007 <sup>A</sup>	0.577±0.006 <sup>A</sup>	0.583±0.005 <sup>A</sup>

(Mean±S.E.) for each index with superscripts a, b, c differ significantly ( $P \leq 0.05$ ) in a row

(Mean±S.E.) for each index with superscripts A, B, C differ significantly ( $P \leq 0.05$ ) in a column

**Evaluation of therapeutic efficacy:** The clinical efficacy was assessed on the basis of California Mastitis Test (CMT) and Somatic cell count (SCC) in quarter's milk on day 0, 4, 7 and 14 of the therapy as per Schlam and Noorlander (1957) and Schlam *et al.* (1971) respectively. Antioxidant potential was assessed on the basis of blood oxidative stress indices on day 0, 7 and 14 of the therapy. Lipid peroxidation in terms of malondialdehyde (MDA) production was measured in 10% hemolysate as per Placer *et al.* (1966). Reduced glutathione (GSH) was measured in 50% RBC suspension by dithio-bis-2 nitro benzoic acid (DTNB) method as per Prins and Loos (1969). Superoxide dismutase (SOD) and catalase (CAT) activities were measured in 10% hemolysate as per Madesh and Balasubramanian (1998) and Bergmeyer (1983) respectively.

**Statistical analysis:** Data were analyzed by

two-way analysis of variance (ANOVA) using statistical software package SPSS 16.0.

## Results

Microbes isolated from milk of cows affected with clinical mastitis were *Streptococcus agalactiae* followed by Micrococci and Coliform and all isolates were sensitive to enrofloxacin, the antibiotic used in the study. Mean CMT point score and SCC in quarter's milk in different groups in response to therapy are depicted in table 1. On commencement of therapy, there was significant ( $P < 0.05$ ) reduction in CMT point score and SCC in quarter's milk within a group. But the reduction in values in group II i.e. vitamin E supplemented group on day 7 was significantly ( $P < 0.05$ ) higher than that of group I.

Oxidative stress indices in blood in different groups in response to therapy are depicted in table 2.

There was significant ( $P < 0.05$ ) increase in erythrocytic malondialdehyde production in mastitic cows as compared to healthy control. On therapy, there was significant reduction in malondialdehyde production within each group but the reduction was insignificantly higher in vitamin E supplemented group. There was significant ( $P < 0.05$ ) decrease in GSH concentration, SOD and catalase activities in mastitic cows. On therapy, there was observed significant ( $P < 0.05$ ) increase in GSH concentration in vitamin E supplemented group. Also a significant ( $P < 0.05$ ) increase in SOD and catalase activities was observed within each group on therapy but the increase was significantly ( $P < 0.05$ ) higher in vitamin E supplemented group.

### Discussion

Mean CMT point score and SCC recorded in milk of mastitic cows correspond with clinical mastitis as per Schlam *et al.* (1971). Milk SCC is a reflection of the inflammatory response to intramammary infection. Following intramammary infection, there is a massive influx of immune cells, primarily polymorphonuclear neutrophils into the milk which kill bacteria and when the infection subsides, they usually return to normal within a few weeks (Schukken *et al.*, 2003). The reduction in CMT point score and SCC in quarter's milk within a group on therapy reflects the positive clinical response. But the significantly higher reduction in values in vitamin E supplemented group on day 7 indicates an earlier clinical response to therapy. This might be due to the enhanced phagocytosis and decreased nitric oxide production by milk polymorphonuclear cells with supplementation of vitamin E and selenium (Mukherjee, 2008), thus eliminating the infection and reducing the inflammatory response.

The increase in erythrocytic malondialdehyde production in mastitic cows was similar to finding by Ranjan *et al.* (2005), reflecting inflammation related oxidative damage. There was reduction in malondialdehyde production within each group but the insignificantly higher reduction in vitamin E supplemented group might be due to inactivation of both lipid and lipid peroxy radicals by donating its phenolic hydrogen (Blokhina *et al.*, 2003), thus arresting the autocatalytic membrane lipid peroxidation. The decrease in blood GSH concentration, SOD and catalase activities in mastitic cows reflects a compromise in antioxidant

defense of the body, related to increased consumption to counteract reactive oxygen species produced by neutrophils. On therapy, the significantly higher increase in GSH concentration, SOD and catalase activities in vitamin E supplemented group might be explained on the basis of the upregulatory effect of vitamin E on antioxidant defense system of the animal (Mukherjee, 2008). Upregulation in SOD activity results in conversion of superoxide anion ( $O_2^-$ ) produced by activated neutrophils to hydrogen peroxide ( $H_2O_2$ ) which are acted upon by catalase to form water (Sordillo and Aitken, 2009), thereby correlates the increase in catalase activity. Sharma *et al.* (2010) also observed the enhanced catalase activity on supplementation of vitamin E and selenium in bovine staphylococcal mastitis. Increased GSH production may be associated with enhanced glutathione peroxidase activity on vitamin E and selenium supplementation (Mukherjee, 2008), demanding more GSH for reduction of  $H_2O_2$  and lipid hydroperoxides (Sordillo and Aitken, 2009). Thus, supplementation of vitamin E along with antibiotic therapy revealed the upregulation in antioxidant activity and reduction in oxidative damage in bovine clinical mastitis.

### Conclusion

The present study revealed significantly ( $P < 0.05$ ) higher reduction in CMT point score and SCC in quarter's milk along with significant upregulation in antioxidant levels and decrease in lipid peroxidation in mastitic cows supplemented with vitamin E, suggesting the ameliorative potential of oral supplementation of vitamin E in clinical mastitis. Hence it may be incorporated in therapeutic regimen of bovine clinical mastitis along with antibiotic for earlier clinical response.

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## Effect of hypogalactia on clinico haematological parameters in cross bred cows

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### Abstract

A 90 day trial was conducted to evaluate the comparative *in vivo* response to three different indigenous herbal galactagogues: Chandrasur (*Lepidium sativum*), Shatavari (*Asparagus racemosus*), and Methi (*Trigonella foenum-graceum*) combined with the area-specific mineral mixture and probiotics (live yeast culture). All cows remained free from infection as evidenced by the clinico-haematological profile. The improved blood picture also pointed to boosting of health status under the holistic nutraceutical regimens.

**Keywords:** Clinico-haematological profile, Herbal galactagogues, Hypogalactia.

Hypogalactia is prolonged nutritional deficiency state where the lactating cow or buffalo gives sub-optimal milk production primarily because of hypocalcaemia (Waghmare *et al.*, 2000; Patel and Jadhav, 2003), hypophosphataemia (Das *et al.*, 2003), and hypomagnesaemia (Radostits *et al.*, 2007). Macro-mineral deficiencies often exist concurrent with negative energy balance, NEB (Underwood and Suttle, 2001). In India, nearly 75 million marginal farmer households (70% < 1 hectare in size) produce only about 4 litres milk unit<sup>-1</sup> day<sup>-1</sup> (FAO, 2010 Report). In the current socio-economic milieu in India and many other developing countries across the world, the only viable solution is to boost the rural agro-based economy through improved livestock productivity.

Galactagogue-cum-therapeutic potency of coarsely powdered indigenous herb Chandrasur or garden cress, GC (*Lepidium sativum*) dry seeds (Shori *et al.*, 2004), Shatavari or shatmul ( *Asparagus racemosus*) dry rhizomes (Kumar *et al.*, 2008), Methi (*Trigonella foenum-graceum*) dry seeds (Tomar *et al.*, 1996) is well -established. Efficacy of area-specific mineral mixtures in different agro-climatic regions is also well-documented (Sharma *et al.*, 2002; Joshi *et al.*, 2007). Potential of direct-fed microbials (probiotics) such as live yeast culture on improved lactation performance is on record (Dutta *et al.*, 2009). However, synergistic effect of herbal galactagogues with area-specific mineral mixture (ASMM) and probiotics remained to be elucidated. The present study aims to fill this lacuna.

### Materials and Methods

Jabalpur (23.1° N lat. and 79.5° E long., 410.87

meters above MSL) is located near the geographical centre of India. The climate, typically subtropical, witnesses average annual precipitation of ~1240 mm, mainly during the monsoons (June-September). The soils vary markedly in colour and texture; black clay predominates. The 90 day trial, including the preparatory period, was conducted from December 1 to March 31, 2012 extending from winter to early spring season. Max. and min. ambient temperature in winter averaged 26.3° C and 9.6° C, respectively. The corresponding figures in the spring season were 36.2° C and 18.8° C. The max. and min. relative humidity (%) ranged from 82.9 - 33.1 in winter, and from 52.1 - 17.2 in the spring season.

Total 150 crossbred cows, irrespective of genotype, parity status, stage of lactation, and free from any detectable clinical signs of disease were short-listed following epidemiological studies. These comprised animals in advanced gestation, and cows at freshening, and at different stages of lactation in the selected dairy units. Hypogalactic cows were carefully identified. Spot Modified California mastitis test (MCMT) was conducted on aseptically collected coded milk samples to rule out mastitis cases. Further, Rothera's test (Oser, 1979) for detection of ketone bodies in the urine samples was applied to eliminate metabolic ketoacidosis.

Therapeutic trial on hypogalactic cows was conducted in periurban dairy units (University Livestock Farm, Adhartal) and one privately owned Dairy Farm, in Gwarighat, Jabalpur. Total 42 hypogalactic cows were randomized into seven equal Treatment groups, each comprising 6 animals. Control group T<sub>1</sub> - without herbal galactagogue (s) - received adequate amount of the ICAR (1998) standard basal ration: DCP 15, TDN 70, area-specific mineral mixture, ASMM (evolved by the

Institute's subject-matter specialists in the Department of Animal Nutrition) @ 25 g, and probiotics (live yeast culture) 15 g animal<sup>-1</sup> day<sup>-1</sup>. Additionally, the six Treatment groups received in the morning hours PO coarsely ground dry herbal galactagogues, singly (T<sub>2</sub> Chandrasur 100g; T<sub>3</sub> Shatavari 100g; T<sub>4</sub> Methi 100g) or in combination (T<sub>5</sub> Chandrasur 70g + Methi 30g; T<sub>6</sub> Shatavari 70 g + Methi 30g; T<sub>7</sub> Chandrasur 70g + Shatavari 30g) animal<sup>-1</sup> day<sup>-1</sup>, uniformly mixed in the concentrate mixture.

Physical examination of the individual cow and body parts, especially the mammary gland/ different quarters (for detection of asymmetry/ inflammation/ abrasions/ lesions/ induration), alterations in the posture/ aberrations in the behavioural profile; clinical parameters (rectal temperature RT, pulse rate PR, respiratory rate RR), abdominal palpation and auscultation of the thoracic cavity (Radostits *et al.*, 2007) was carried out before and after the specified remedial therapies. Five ml jugular vein blood sample was aseptically collected from each cow in the morning hours at the specified intervals: day 0, 30, and 60. Blood was quantitatively transferred into chemically clean, dry heparinized labelled glass vials containing  $\beta$ -heparin (20 U ml<sup>-1</sup>), and haematological attributes: TEC (x 10<sup>6</sup>  $\mu$ l<sup>-1</sup>), Hb concentration (g dl<sup>-1</sup>) and PCV (%) were determined with Auto blood cell analyzer (Abascus Daitron, Austria) and the DLC (%), manually on modified Wright-stained blood films (Jain, 1986).

The data were subjected to ANOVA test (Snedecor and Cochran, 1994) to partition the sources

of variation. Then, Duncan's Multiple Range Test was applied to identify the significant differences between the Mean values of a given parameter under the different Treatments, uniformly at 5% level of probability.

## Results

Rectal temperature, pulse rate, and respiratory rate remained within the respective physiological limits.

ANOVA of the data on total erythrocyte count revealed highly significant differences attributable to different remedial therapies. Treatments T<sub>2</sub> (Shatavari 100 g), T<sub>5</sub> (Chandrasur 70g + Methi 30g), and T<sub>7</sub> (Chandrasur 70g + Shatavari 30g), each in combination with ASMM 25g and probiotics 15g PO animal<sup>-1</sup> day<sup>-1</sup> produced a noteworthy increase in TEC value on day 30. This favourable response persisted on day 60 (Table 1).

In regard to Hb concentration, the effects of treatments/ intervals were highly significant. Observed increases in Hb values in Treatments T<sub>2</sub>, T<sub>5</sub>, and T<sub>7</sub> closely paralleled changes in the TEC. For packed cell volume% effects of different treatments (P < 0.05) as well as intervals (P < 0.01) were significant. Observed increases in the PCV% in all Treatment groups on day 30 were further augmented on day 60.

In ANOVA of the data on differential leucocyte count, neutrophil % (DLC-N) revealed significant differences because of treatments (P < 0.05) and intervals (P < 0.01). An increasing trend was clearly discernible (Table 2). Eosinophil % (DLC-E) (showed significant

**Table 1.** Haematological profile\*of hypogalactic crossbred cows under different remedial therapies at varying intervals

Intervals/ Parameters/	TEC ( x 10 <sup>6</sup> $\mu$ l <sup>-1</sup> )			Hb concentration (g dl <sup>-1</sup> )			PCV (%)		
	Intervals (d)			Intervals (d)			Intervals (d)		
	0	30	60	0	30	60	0	30	60
T <sub>1</sub> = Basal ration, BR	11.2±0.5	10.4±0.9	11.1±0.9	10.4±0	8.4±0.4	8.6±0.4	24.1±0.5	23.5±0.5	23.8±0.4
T <sub>2</sub> = BR+Chandrasur 100g	12.4±0.4	12.9±0.6	13.0±0.6	9.7±0.3	10.2±0.4	10.4±0.2	27.7±0.8	29.5±0.6	30.7±0.5
T <sub>3</sub> = BR+Shatavari 100g	9.7±0.4	10.4±0.3	10.8±0.4	6.7±0.4	7.2±0.4	7.2±0.4	27.8±1.0	30.2±0.8	31.7±0.8
T <sub>4</sub> = BR+Methi 100g	11.2±0.4	11.4±0.8	11.7±0.8	9.0±0.3	9.0±0.4	9.0±0.4	24.3±0.4	24.5±0.6	25.5±0.6
T <sub>5</sub> = BR+Chandrasur 70g+Methi 30g	12.4±0.6	13.0±0.7	13.2±0.7	10.0±0.3	10.4±0.2	10.6±0.2	25.0±1.1	28.8±0.8	30.2±0.7
T <sub>6</sub> = BR+Shatavari 70g+Methi 30g	8.7±0.4	9.5±0.4	9.7±0.5	7.0±0.5	7.6±0.4	7.7±0.4	26.5±0.8	27.8±0.6	29.3±0.7
T <sub>7</sub> = BR+Chandrasur 70g+Shatavari 30g	12.5±0.4	13.2±0.4	13.3±0.4	9.0±0.3	9.7±0.2	9.9±0.2	28.5±1.3	30.7±1.0	31.3±0.8

ASMMM @ 25g + Probiotics (YC) @15 g animal<sup>-1</sup>d<sup>-1</sup> offered uniformly in all seven groups, \*Mean values  $\pm$  SE

**Table 2 .** Leucocyte profile\* of hypogalactic crossbred cows under different remedial therapies at varying intervals

Parameters/Treatments	Neutrophils %			Eosinophils %			Lymphocytes %			Monocytes %		
	Intervals (d)			Intervals (d)			Intervals (d)			Intervals (d)		
	0	30	60	0	30	60	0	30	60	0	30	60
T <sub>1</sub> = Basal ration, BR	20.5	22.2	24.3	4.0	3.2	4.0	39.8	42.7	45.0	4.7	4.5	5.0
T <sub>2</sub> = BR+Chandrasur 100g	17.3	17.8	18.8	3.7	3.3	4.5	47.2	48.7	49.5	3.2	3.3	4.0
T <sub>3</sub> = BR+Shatavari 100g	21.3	24.3	25.3	4.0	4.3	4.3	46.8	49.0	49.8	4.5	4.3	4.5
T <sub>4</sub> = BR+Methi 100g	20.0	22.7	25.0	4.0	4.2	4.7	41.2	43.2	44.7	4.5	4.7	5.3
T <sub>5</sub> = BR+Chandrasur 70g+Methi 30g	16.5	17.5	19.8	3.2	3.3	3.7	45.8	46.5	46.7	2.8	2.8	3.3
T <sub>6</sub> = BR+Shatavari 70g+Methi 30g	22.2	23.0	24.5	3.0	2.3	2.5	46.3	48.3	49.2	3.3	2.7	3.0
T <sub>7</sub> = BR+Chandrasur 70g+Shatavari 30g	17.7	19.3	20.3	3.8	4.3	4.2	47.5	49.3	50.5	3.2	3.8	4.2

ASMM @ 25g + Probiotics (YC) @15 g animal<sup>-1</sup>day<sup>-1</sup> offered uniformly in all seven groups, \*Mean values (%) of differential leucocyte count

differences because of treatments/ intervals. However, changes in values remained within the physiological range. Lymphocyte % (DLC-L) revealed significant differences because of treatments/ intervals, the changes in values remaining within the normal range. Monocyte % (DLC-M) showed significant differences ( $P < 0.05$ ) because of treatments but the values remained within the physiological range.

## Discussion

In functional hypogalactia, the lactating cow or buffalo gives less than the expected milk production. In view of the significant cumulative economic losses, holistic nutraceutical regimens are gaining pertinence. The present study was designed to delineate clinico-haematological profile of hypogalactic cows in relation to the prevailing managerial conditions, and to evaluate the relative efficacy of different therapeutic regimens incorporating various indigenous herbal galactagogues, combined with ASMM and probiotics. Environmental factors like heat stress in the tropics cause accelerated accumulation of deleterious reactive oxygen species, ROS in animal tissues (Kumar *et al.*, 2008). High ambient temperature, often coupled with elevated relative humidity, induces panting with accelerated expulsion of CO<sub>2</sub> in the exhaled air. This leads to depletion of the buffering capacity of salivary inflow. Reduced DMI suppresses rumination. The importance of adequate measures to minimize heat stress is, therefore, evident. Presence of certain phytochemicals in Shatavari that act as potent adaptogens (Kumar *et al.*, 2008) assumes pertinence in this context.

Evaluation of the clinical profile is important

in the routine health check-up of lactating dairy animals. In a variety of infectious diseases, pyrexia is a characteristic symptom (Kahn and Line, 2010). Thus, significant changes in the clinical profile of dairy animals reflect challenges to homeostatic mechanisms. In the present Therapeutic trial on hypogalactic crossbred cows, no noteworthy change in the normal values of clinical parameters: RT, PR and RR in all specified groups indicate freedom from exposure to any infectious agent, or induction of metabolic disorder. This observation is corroborated by similar findings in some periurban dairy units in Mhow, west Madhya Pradesh (Shori, 2004).

Haemogram was employed to monitor nuances in the *in vivo* response elicited by different nutraceutical regimens (T<sub>2</sub>-T<sub>7</sub>) comprising three different herbal galactagogues, singly or in combination. It is noteworthy that Treatments T<sub>2</sub> (Chandrasur 100g), T<sub>5</sub> (Chandrasur 70g + Methi 30g), and T<sub>7</sub> (Chandrasur 70g + Shatavari 30g) combined with ASMM 25g + probiotics (YC) 15g PO animal<sup>-1</sup>day<sup>-1</sup> consistently augmented the TEC and Hb values along with increased PCV% in the blood circulation. This observation indicates improved nutritional/ health status of cows under the specified Treatment groups, propitious to enhanced lactation performance in contrast to the declining trend in Hb concentration observed in buffaloes at freshening (Hagawane *et al.*, 2009). Though mineral supplementation *per se* elicited a favourable biological response in anaemic grazing cattle (Samant *et al.*, 1995), no comparable report on synergistic effect of herbal galactagogues is forthcoming.

Differential leucocyte count (DLC) is useful in

monitoring patho-physiological states in farm animals like infections of bacterial or viral aetiology, reproductive disorders, and stressful conditions. Total absence of any significant change in the relative proportion (%) of different leucocytes in circulation corroborates continuing normal health status of the cows under study.

Improved blood picture pointed to augmented nutritional and health status of the animals under different holistic nutraceutical regimens.

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## **Efficacy of eprinomectin pour on against gastrointestinal parasites of goats**

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### **Abstract**

The efficacy of eprinomectin pour on was evaluated against gastrointestinal parasites (*Haemonchus spp.*, *Trichostrongylus spp.*, *Cooperia spp.*) in goats. Efficacy of the drug was evaluated on the basis of EPG count of the faecal sample, clinical recovery, and restoration of haematological parameters. Eprinomectin pour on @ 1ml/10kg body weight was 96.98 percent effective against gastrointestinal parasites in goats.

**Keywords:** Eprinomectin pour on, Gastrointestinal parasites, Goat .

Gastrointestinal nematode is the most common problem in goats affecting productivity. Like other animals, goats also commonly suffer from helminthic infection which affects the health of the animals and afterwards causes death due to heavy infection. To control this infection in goats, a variety of anthelmintics have been tried with varying results (Roy *et al.* 1990, Roy and Galdhar, 2004). Frequent use of suppressive deworming has resulted in widespread anthelmintic resistance among goat (Wanyangu *et al.*, 1996). Resistance of benzimidazoles has been documented in certain caprine parasites. In general, a strain of parasite resistant to one benzimidazoles drug quickly develops resistance to other benzimidazoles or pro benzimidazoles (Smith, 2002).

Eprinomectin a semi-synthetic compound of the avermectin family intended for the treatment of internal and external parasites in cattle including lactating cows. So far in India no reports are available regarding the efficacy of eprinomectin pour on against gastrointestinal nematodes in goats. Keeping this point in mind, the present study was undertaken to evaluate the efficacy of eprinomectin pour on against gastrointestinal nematodiasis in goats and to observe its side effects, if any.

### **Materials and Methods**

For this study, nondescript 16 goats of either sex, of different ages suffering from naturally infected gastrointestinal nematodes (*Haemonchus spp.* 70%, *Trichostrongylus spp.* 21% and *Cooperia spp.* 9%) were selected for this study. Clinical signs manifested were that of partial loss of appetite, pale conjunctival mucous membranes, and poor body conditions. The goats were

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randomly divided into two groups Group A comprising 10 goats were treated with 0.5% w/v eprinomectin pour on solution @1ml/10kg body weight. The drug was applied topically by pouring along the midline of back from the mid shoulder to the tail salting. Group B comprising 6 goats served as untreated control.

Eggs per gram (EPG) of faeces were determined for each goat of both the groups before treatment (Soulsby, 1989). The second EPG of faeces were done on 21st day of post treatment to assess the parasitic load. The eggs of *Haemonchus spp.*, *Trichostrongylus spp.* and *Cooperia spp.* were identified on the basis of their morphology, shape and size. For haematological studies 1ml of blood was collected in clean and sterile glass vial containing EDTA and haematological parameters like Haemoglobin (Hb), Packed Cell Volume (PCV), Total erythrocyte count (TEC) and Total leukocyte count (TLC) were estimated in automated haematology blood cell counter (MS93-Vet). Statistical analysis of the experimental data was done as per the methods of Snedecor and Cochran (1967). Therapeutic efficacies of the drugs were evaluated on the basis of reduction/absence of egg per gram of faeces, disappearance of clinical signs and restoration of haematological profiles.

### **Results and Discussion**

The results of the trial are given in table 1. The mean EPG of *Haemonchus sp.*, *Trichostrongylus spp.* and *Cooperia spp.* in group A became low on 21st day of post treatment indicating percent efficacy of 96.98%. On the other hand, untreated goats showed gradual rise in EPG. Following treatment, overall improvement of feeding habit and body condition with regard to the alertness and growth rate was noticed. Hoste *et. al*

**Table 1.** Eggs per gram of faeces in treated animals.

Group	No. of goats	Treatment	EPG before treatment	EPG after treatment	Efficacy %
A	10		1418±117.40 <sup>a</sup>	42.80 ±8.07 <sup>b</sup>	96.98
B	06		1386.66 ±186.59 <sup>a</sup>	1435.00 ±200.01 <sup>a</sup>	

Means having similar superscript do not differ significantly (P<0.05)

**Table 2:** Showing changes in haematological parameters following therapy in clinical cases of gastroenteritis parasites in goats.

Parameters	Groups	Days of observation		
		0	7	21
PCV(%)	A	18.92±1.52 <sup>ab</sup>	22.35±1.52 <sup>bc</sup>	25.77±1.52 <sup>c</sup>
	B	17.12±1.96 <sup>ab</sup>	16.68±1.96 <sup>a</sup>	15.28±1.96 <sup>a</sup>
Hb (gm%)	A	8.12±0.15 <sup>a</sup>	8.78±0.15 <sup>b</sup>	10.08±0.15 <sup>c</sup>
	B	8.13±0.19 <sup>ab</sup>	8.15±0.19 <sup>a</sup>	7.96±0.19 <sup>a</sup>
TEC (x10 <sup>6</sup> /cu.mm)	A	5.00±0.07 <sup>a</sup>	5.09±0.07 <sup>a</sup>	5.53±0.07 <sup>b</sup>
	B	4.95±0.10 <sup>a</sup>	4.92±0.10 <sup>a</sup>	4.83±0.10 <sup>a</sup>
TLC (x10 <sup>3</sup> /cu mm)	A	13.00±0.48 <sup>a</sup>	11.86±0.48 <sup>a</sup>	9.69±0.48 <sup>b</sup>
	B	11.85±0.63 <sup>a</sup>	12.28±0.63 <sup>a</sup>	12.89±0.63 <sup>a</sup>

Means having similar superscript do not differ significantly (P<0.05)

(2004) also reported the efficacy of eprinomectin pour on against *Hamonchus spp* and *Trichostrongylus sp.* in sheep.

The results of haematological parameters reveal the mean Hb, PCV and TEC values significantly lower and increased values of TLC was observed in helminth infected goats (Table-2.). The mean value of Hb, PCV and TEC increased, and TLC values decreased slowly after therapy. Eprinomectin has no direct effect on Hb, PCV, TEC and TLC but has effective anthelmintic activity without any toxicity in goats. This could be due to absence of worm burden which helped to improve body conditions. There were no side effects of this drug on the host. Hence eprinomectin pour on is recommend as a drug of choice against gastrointestinal parasitic infection in goats because of its efficacy and easy administration. It is the only endectocide approved for use during lactation with a zero milk withdrawal period.

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## Clinico haematological and biochemical profile of Chegu pashmina goats

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### Abstract

The mean value of body temperature of Chegu pashmina goats was  $101.53 \pm 0.17$  °F with heart rate of  $69.41 \pm 2.54$ /min and respiration rate of  $37.50 \pm 3.00$ /min. Haemogram revealed haemoglobin value as  $10.85 \pm 0.32$  g/dl and packed cell volume as  $30.85 \pm 1.28$  %. Total erythrocyte and leucocyte counts were  $16.57 \pm 0.47 \times 10^6$  / cu mm and  $13.68 \pm 0.92 \times 10^3$  / cu mm respectively. Differential leucocyte count revealed neutrophils as  $24.33 \pm 1.63$ %, lymphocytes as  $67.17 \pm 0.48$ %, monocytes as  $3.33 \pm 0.21$  %, eosinophils as  $4.66 \pm 0.33$  % and basophils as  $0.5 \pm 0.22$  %. The plasma enzymatic activity of Alanine amino-transferase and Aspartate amino-transferase was  $33.38 \pm 2.65$  IU/L and  $21.00 \pm 3.40$  IU/L. The blood glucose and total plasma protein levels were  $54.94 \pm 2.60$  mg/dl and  $8.44 \pm 0.33$  g/dl whereas the total plasma bilirubin, blood urea nitrogen, and plasma creatinine values were  $0.60 \pm 0.095$  mg/dl,  $10.19 \pm 1.29$  mg/dl and  $1.15 \pm 0.038$  mg/dl respectively.

**Keywords:** Biochemistry, Chegu, Clinical signs, Goats, Haematology, Pashmina

The total livestock population of Himachal Pradesh is 52.11 lac with a goat component of 23.8% viz. 12.4 lac comprising of two breeds viz. Gaddi and Chegu goats (2007 census). Chegu goats produce a finest and warmest natural fibre called "Pashmina". The true breeding tract of these goats is confined to the cold desert region of Lahaul-Spiti and Kinnaur area in Himachal Pradesh having a height > 3500 meters from mean sea level. The present paper puts on record some clinical and haemato-biochemical observations on Chegu goats of Himachal Pradesh.

### Materials and methods

The present study was undertaken on Chegu pashmina goats brought from higher hills (MSLH > 4500 meters) around Dubling / Namgia villages of Kinnaur district to Agricultural University campus, Palampur (MSLH 1240 meters) of Kangra district of Himachal Pradesh. Routine clinical observations were recorded. Heparinised venous blood samples were collected within 5 days after arrival of the goats at Palampur (H.P.). The blood samples were analyzed for Hb, PCV, TEC, TLC, DLC and erythrocytic indices by using standard methods (Benjamin, 1985). The blood biochemical analysis for plasma enzymatic activity of Alanine amino-transferase (ALT), Aspartate amino-transferase (AST), plasma glucose, total plasma protein, total bilirubin, blood urea nitrogen (BUN) and plasma creatinine were done by using reagent kits\* on RA-50.

### Results and Discussion

The area (MSLH > 3500 meters), where the Chegu pashmina goats are reared in Himachal Pradesh,

is difficult to access. Hence, no comprehensive scientific intervention has been done, so far, to record the reference values of various body parameters. A few authors (Kumar *et al.*, 2000 ; Sharma *et al.*, 2005 ; Chauhan *et al.*, 2010), however, placed on record mean values of some haemato-biochemical parameters of these animals, but, with a wide variations, that too, in goats maintained at a totally different climatic conditions and at a very low lying area (MSLH 1200 meters). Hence, true picture of various parameters, in this rare species of pashmina goats, is still lacking.

During the present investigation, the mean value of body temperature in Chegu pashmina goats was  $101.53 \pm 0.17$  °F which was slightly lower than that of Gaddi goats of Palampur area of Himachal Pradesh. However, the heart rate of  $69.41 \pm 2.54$ /min and respiration rate of  $37.50 \pm 3.00$ /min were comparatively higher probably because the goats were accustomed to live at higher altitude where ambient oxygen was comparatively much lower (Karim *et al.*, 2010). Haemoglobin was  $10.85 \pm 0.32$  g/dl with PCV value of  $30.85 \pm 1.28$  % which were more or less similar to the observations of Rastogi *et al.*, (1997) who recorded Hb as  $8.43 \pm 0.38$  g/dl and PCV as  $27.1 \pm 1.2$  % in nomadic Gaddi goats of Himachal Pradesh. The TEC value was slightly higher ( $16.57 \pm 0.47 \times 10^6$  / cu mm) during the present study as against the mean value of  $12.62 \pm 0.97 \times 10^6$  / cu mm recorded by above authors. Sharma *et al.* (2005) also reported Hb value, in Chegu goats, stationed at Palampur, as  $9.79 \pm 0.23$  g/dl with PCV value as  $30.85 \pm 1.28$  % which were almost similar to the present findings. However, the TEC was markedly higher  $16.57 \pm 0.47 \times 10^6$  / cu mm as compared to the  $9.90 \pm 0.43$

$\times 10^6$ /cu mm mean value recorded by the above authors. This difference might be because of persistent effect of earlier habitat of higher hills where the atmospheric oxygen concentration was comparatively lower and in response the goats had higher TEC values along with higher heart and respiration rates which are supposed to occur naturally at higher altitude (Benjamin, 1985; Karim, *et al.*, 2010). Moreover, the goats appeared to be anaemic as was evident from slightly pale color of the conjunctival mucous membrane and low haemoglobin ( $10.85 \pm 0.32$  g/dl). The TLC value ( $13.68 \pm 0.92 \times 10^3$ /cu mm) was also towards the higher limit but within normal range. The DLC values were within normal range (Benjamin, 1985). The MCV and MCH mean values were comparatively lower whereas MCHC value was markedly higher (Table 1) when compared with the reports of Sharma *et al.* (2005) who observed MCV as  $39.32 \pm 2.11$  fl, MCH as  $10.26 \pm 0.51$  pg and MCHC as  $26.19 \pm 0.71$  %.

**Table 1:** Erythrocytic indices in Chegu pashmina goats

S. No.	Parameters	Value (Mean $\pm$ S.E.)
1.	MCH	$6.41 \pm 0.096$ pg
2.	MCHC	$40.84 \pm 0.39$ %
3.	MCV	$15.83 \pm 0.32$ fl

The plasma enzymatic activity of Alanine amino-transferase *viz.*  $33.38 \pm 2.65$  IU/L was markedly higher whereas the activity of Aspartate amino-transferase was lower *viz.*  $21.00 \pm 3.40$  IU/L than the values reported by Kumar *et al.*, (2000) who recorded corresponding values as  $24.88 \pm 2.76$  IU/L and  $101.28 \pm 4.39$  IU/L in Chegu goats maintained at

**Table 2:** Plasma biochemical status in Chegu goats

S. No.	Parameters	Values (Mean $\pm$ S.E.)
1	ALT	$33.38 \pm 2.65$ IU/L.
2	AST	$21.00 \pm 3.40$ IU/L
3	Glucose	$54.94 \pm 2.60$ mg/dl
4	Total protein	$8.44 \pm 0.33$ g/dl
5	Total Bilirubin	$0.60 \pm 0.095$ mg/dl
6	BUN	$10.19 \pm 1.29$ mg/dl
7	Creatinine	$1.15 \pm 0.038$ mg/dl

Palampur (HP). However, the total plasma protein value and glucose values (Table 2) were matchable with that of above authors. The total plasma bilirubin, blood urea nitrogen, and plasma creatinine values ( $0.60 \pm 0.095$  mg/dl,  $10.19 \pm 1.29$  mg/dl and  $1.15 \pm 0.038$  mg/dl respectively) were in conformity with those of other animal species (Radostits *et al.*, 2007).

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## Ectoparasite infestations in anaemic goats

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### Abstract

Ectoparasitism is an important problem resulting in anaemia and transmission of vector born diseases in livestock. The present study reveals the prevalence of ectoparasitism in anaemic goats and identification of various parasites responsible for anaemic changes. Among 250 anaemic goats, 54 animals (21.6 per cent) were infested with ectoparasites. Ticks were the most common ectoparasite found in goats (38.89 per cent), followed by lice (27.78 per cent), mites (25.93 per cent) and fleas (7.40 per cent).

**Keywords:** Anaemia, Ectoparasitism, Goat, Prevalence.

Ectoparasitism limits production in the goat industry in different ways. External parasites feed on body tissues and skin irritation produced by these parasites result in discomfort and irritation to the animal. Ectoparasites also transmit many diseases. They reduce weight gains and milk production. In general, infested livestock cannot be efficiently managed to realize optimum production levels. According to Yakhchali, (2006) ectoparasitism was the major cause for anaemia in goats. Present study was undertaken to assess the prevalence of the various ectoparasitic infestations in goats contributing to anaemic conditions.

### Materials and methods

Two thousand five hundred and fifteen goats presented to University Veterinary Teaching Hospitals Mannuthy and Kokkalai and 400 animals belonging to different organized farms of Thrissur district were screened for anaemia by examination of conjunctival mucosa and estimation of levels of haemoglobin and volume of packed red cells (VPRC). Two hundred and fifty anaemic goats were selected and subjected to detailed examination for presence of ectoparasitic infestations. Ticks, fleas and lice were collected and identified. The ticks were removed from host skin for identification by using a steel forceps. Five percent formalin (5 parts concentrated formaldehyde solution plus 95 parts water) was used for the preservation. The ticks were treated with boiling 10% KOH solution to dissolve soft viscera. The specimen was then mounted in 10% KOH for immediate examination and identified. Lice and fleas were identified based on the morphology. For identifying mites, deep skin scrapings were collected from goats with skin lesions and examined under microscope after boiling with 10% Potassium hydroxide.

### Results

Among 250 anaemic goats, 54 animals (21.6 per cent) were infested with various ectoparasites. Ticks were the most common ectoparasite found in goats (38.89 per cent). Ticks collected from the animals were identified as *Haemaphysalis bispinosa* in 62 per cent of cases and *Haemaphysalis spinigera* in 38 per cent of cases. Lice were present in 27.78 per cent animals which were identified as *Linognathus spp.*. Fleas were present in 7.40 per cent animals and the fleas were mainly *Ctenocephalids spp.* Mites were observed in 25.93 per cent goats. Out of 14 cases of mange, *Demodex* was identified in three cases, *Sarcoptes* in six cases and *Psoroptes* in five cases.

Major clinical signs noticed in ectoparasitic infested goats were pale mucous membranes, restlessness and poor growth rate. Ectoparasitic infestation was predominantly on face, neck, belly, ear, and thigh regions in descending order.

### Discussion

From the present study, it is clearly evident that ectoparasitism is an important cause of anaemia in goats and the blood loss caused by them is severe. This high prevalence of ectoparasites may be due to geographical and seasonal peculiarities of the area. Favorable climates, improper management, lack of awareness of farmers and poor animal health extension services are believed to have contributed for the widespread occurrence of ectoparasites as suggested by Sertse and Wossene (2006).

Pale mucous membranes, restlessness, rough hair coat and lower growth rate were observed in

majority of ectoparasite infested goats. Anish *et al.* (2006) observed restlessness, weakness, poor growth rate and dull rough coat in kids infested with cat fleas. Pale mucous membranes, tachycardia, tachypnoea and hypothermia were also observed.

The goats with poor body condition were 4.3 times at risk for sarcoptic mange, 2.1 times for *Linognathus* spp. and 1.6 times for tick infestation than goats of good body condition (Sertse and Wossene, 2006). Dimri *et al.* (2006) identified the ectoparasites of goats as ticks (*Boophilus*, *Hyalomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus* and *Amblyomma*), lice (*Damalinia*, *Haematopinus*, *Solenopotes* and *Linognathus*) and mites (*Sarcoptes*, *Psoroptes* and *Demodex*).

It is evident that the role of ectoparasitism is high in developing anaemia in goats and appropriate treatment and control measures are to be adopted for elimination of these parasites and to alleviate the symptoms of anaemia .

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## Seroconversion of sentinel animals to bluetongue virus and its association with *Culicoides* abundance in Bidar, Karnataka

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### Abstract

The monthly monitoring of seronegative goats and cattle to detect the possible seroconversion to bluetongue virus revealed that the highest percentages of seroconversions had occurred during the month of February and March 2003. In addition, seroconversions were also noticed in the month of August 2002 and during the months of October to December 2002. The higher abundance of *Culicoides* midges was also noticed during the period of seroconversion. Thus, the study indicated that the bluetongue virus is actively circulating between the host, agent and vectors.

**Keywords:** : AGID, BTV, *Culicoides* midges, Sentinel animals.

Bluetongue (BT) is an infectious, non contagious vector borne viral disease of livestock. Vector species are confined to the genus *Culicoides*. The word sentinel originated from the French word "Sentinelle" and Italian "Sentinella" meaning watch over – to keep a guard. The concept of sentinel heard for the study of Orbivirus was first used in Australia in 1970. Later it was used by many scientists and described the sentinel herd as the "backbone" of the epidemiological studies for the routine surveillance of the virus activity and associated vector transmission of Bluetongue virus (BTV). In the present study an attempt was made to monitor the sentinel animals for about stipulated period of six months for natural infection by BTV and their association with abundance of *Culicoides* midges.

### Materials and Methods

Two batches of seronegative apparently healthy animals which were maintained at Veterinary College, Bidar were used for sentinel animal study to monitor the possible seroconversion to BTV.

Batches of 20 apparently healthy bluetongue seronegative goats aged between 4 to 5 months were monitored during July 2002 to December 2002. A second batch of seronegative animals consisting of 6 apparently healthy adult goats aged between 4 to 6 years and 7 apparently healthy young cattle aged between 1 to 4 years were monitored for seroconversion during January 2003 to June 2003.

The blood samples of these animals were collected in monthly intervals and serum samples were separated. All the serum samples of these sentinel animals were subjected to agar gel immunodiffusion

(AGID) test to detect the precipitating antibodies to bluetongue virus.

The *Culicoides* midges were collected near the sheds of these sentinel animals at Veterinary College, Bidar at fortnightly intervals using down drought light trap (220 volts) equipped with 8 W black light tube. *Culicoides* abundance and dominant species were recorded as per the procedure described by Udupa (2001).

### Results

Two batches of bluetongue seronegative apparently healthy goats and cattle maintained at Veterinary College, Bidar were monitored at monthly intervals for a period of six months each, covering twelve months period from July 2002 to June 2003. In a total of 20 young goats monitored for seroconversion to bluetongue virus in the first batch, none of them seroconverted during July 2002. Three (15%) goats were found to be seroconverted during August, none during September and only one (5.88%) during October 2002. During November 2002, three out of sixteen (18.75%) showed positive results to AGID followed by one out of thirteen (7.69%) during December 2003.

In the second batch, adult goats and young cattle were monitored for seroconversion during January 2003 to June 2003. Among six adult goats kept for monitoring, none of them seroconverted during January 2003. During February 2003, four out of six (66.67%) goats were seroconverted and in March 2003 the remaining two goats showed seroconversion reaching to 100 per cent. Among seven young cattle one each cattle seroconvert during February and March 2003. The remaining cattle

continued to be seronegative throughout the study period.

The abundance of *Culicoides* midges were recorded during October second fortnight of 2002 to August first fortnight of 2003 and the dominant species recorded during the period was *C. imicola* and *C. oxystoma*. The seroconversion of sentinel animals to BTV and its association with *Culicoides* abundance is depicted graphically (Fig.)

### Discussion

Among first batch of young goats' seroconversion to bluetongue were found to occur during the months of August, October, November and December 2002, with higher percentage of seroconversion during August and November 2002. In the second batch of adult goats and young cattle seroconversion to bluetongue were found to occur during the months of February and March 2003. The occurrence of seroconversion in these animals during these two periods (August to December 2002 and February to March 2003) indicated that BTV was actively circulating in this farm during these periods. Thus activity of BTV was observed during south west monsoon, post south west monsoon and winter seasons. Similar findings were reported by Mohammed and Taylor (1987) and Kakker *et al.* (1996). This finding correlates with the reports on the outbreaks of bluetongue in Karnataka, where diseases were recorded in the months of September to March (Srinivas and Ramachandran, 1991). Even Nadagouda (1997) reported that, November to March were the months of outbreak of the bluetongue disease in Northeastern zone of Karnataka.

In the present study, the maximum numbers of seroconversion were congregated during the months of February and March 2003 in adult goats and young

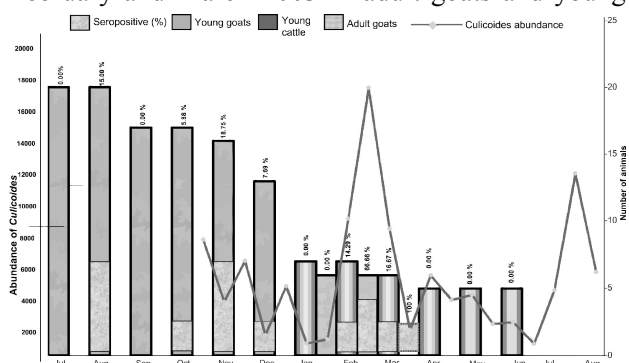


Figure 1: Relationship between seroconversion pattern and abundance of *Culicoides*

cattle, during which period there was peak upsurge in abundance of *Culicoides* midges when collected in the vicinity of farm. In addition, there were substantial numbers of *Culicoides* midges noticed during October 2002 through January 2003. Even though, the pattern of abundance of *Culicoides* during July 2002 to September 2002 was not available, substantial increase in abundance was noticed during July and August in the following year. In addition, the dominant species of *Culicoides* such as *C. imicola* and *C. oxystoma*, which are potential and suspected vectors of bluetongue respectively, were found in higher abundance during the period from October 2002 to March 2003 near the sheds of sentinel animals. Similar findings were reported by Udupa (2001). Thus, present finding indicated the positive association between abundance of *Culicoides* midges and risk of seroconversion to BTV and thus BTV might actively circulate between the host and vectors. Hence, sentinel animal study for bluetongue may be used for monitoring the activity of virus and forecasting the disease.

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## Effect of feed additives on haemato biochemical changes in *Salmonella typhimurium* infected poultry

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### Abstract

*Salmonella typhimurium* produced deleterious effect on haematobiochemical parameters. *Withania somnifera* improved haematological parameters only after there was self restriction of the infection. *W. somnifera* and *A. sativum* significantly improved biochemical parameters after 25<sup>th</sup> DPI.

**Keywords:** Haemato-biochemical changes, *Salmonella typhimurium*.

Among the different diseases occurring in poultry, Salmonellosis cause serious losses to the poultry industry in terms of mortality, reduced growth and loss of egg production. Antibiotic growth promoters are used at sub-therapeutic doses in feeds in order to improve quality of product and control zoonotic pathogens. Antibiotics are no longer desirable because of concerns about the development of drug resistant bacteria and antibiotic residues in food (Jensen, 1998). Thus natural herbs could be expected to serve as a safer alternative as growth promoters due to their lower cost of production, reduced risk of toxicity and environment friendliness. Among various herbal options, *Ashwagandha* (*Withania somnifera*) and *Garlic* (*Allium sativum*) are also added as feed additives because of wide spectrum of activities like anti-inflammatory, anti-microbial, anti-stress, antioxidant, immuno-modulatory, hepatoprotective and haemopoietic properties

### Materials and Methods

The present study was conducted on **150, day-old** broiler chicks. After an incubation period of seven days, all the chicks were randomly divided into four groups viz group I, II, III and IV. Group I chicks were challenged orally with  $2 \times 10^8$  *S. typhimurium*/0.5ml of normal saline. Group II and group III chicks were challenged orally with  $2 \times 10^8$ /0.5ml of normal saline and were fed *Withania somnifera* and *Allium sativum*, respectively mixed in feed @1% while group-IV chicks served as control.

Blood samples (3-4ml) were collected from randomly selected six birds of each group at 3, 7, 10, 14, 18, 21, 25, 28 and 35 days post infection in vials containing EDTA @ 2mg/ml of blood as an anticoagulant for haematological and without

anticoagulant for biochemical study. The haematological parameters Haemoglobin (g/dl), Packed Cell Volume (%), Total Erythrocytic Count (million/mm<sup>3</sup>), Total Leukocyte Count (thousand/mm<sup>3</sup>), Differential Leukocyte Count, Mean Corpuscular Volume (fl), Mean Corpuscular Hemoglobin (pg) and Mean Corpuscular Haemoglobin Concentration (g%) were investigated as per standard methods described by Schalm *et al.* (1975). Biochemical investigation (Total serum protein (g/dl), albumin (g/dl), globulin (g/dl), A:G ratio (%), Apartate aminotransferase (IU/ml), Alanine aminotransferase (IU/ml) and serum creatinine (mg/dl) were done using standard kits from Span Diagnostic Ltd. The data generated was subjected to one way ANOVA statistical analysis as per method described by Snedecor and Cochran (1967).

### Results and Discussion

The haematological values (Hb, PCV and TEC) decreased significantly ( $P < 0.05$ ) from 7<sup>th</sup> DPI (Hb. at 7<sup>th</sup> DPI; gr. I: 5.06, gr. II: 5.03, gr. III: 5.07, gr. IV: 6.70) in all the treatment groups than control group birds up to the last observation but in group II birds these values increased significantly than group I from 25<sup>th</sup> DPI (Hb. at 25<sup>th</sup> DPI; gr. I: 4.13, gr. II: 4.53, gr. III: 4.17, gr. IV: 7.83) while non-significant change was observed between group I and group III birds. The decreased haematological values in infected birds corresponded with the findings of Assoku and Penhale (1978) in fowl typhoid and Metwally (2009) in different *Salmonella* serotypes. Assoku and Penhale (1978) opined that decreased haematological values were due to effect of endotoxin of *Salmonella* which immunologically modify the erythrocytes causing them to be eliminated from the circulation. The increased

haematological values in group II birds corresponded with the findings of Kumar *et al.* (2006) in cockerels and Daisy *et al.* (2008) in broilers. *W. somnifera* stimulates stem cell proliferation and increases bone marrow cellularity (Mishra *et al.*, 2000). In present study, *Allium sativum* did not improve haematological values, which is in agreement with Ahmad *et al.* (2010) in broilers affected with aflatoxicosis.

MCV values revealed significant increase in all infected groups than control group from 7<sup>th</sup> DPI till the end of the study. Group II birds performed better and the MCV values were significantly lower ( $P < 0.05$ ) than group I and III from 25<sup>th</sup> DPI. MCH and MCHC values did not differ significantly between treatment group and control group birds throughout the experimental study.

This indicated that the anemia encountered in present study was of macrocytic normochromic type. The results of present study corresponded with Assoku and Penhale (1978) in *Salmonella gallinarum* infection in chicken.

Enumeration of total leukocyte counts revealed a significant increase in all the infected groups from 3<sup>rd</sup> day PI (TLC at 3<sup>rd</sup> DPI: gr. I: 24.33, gr. II: 23.66, gr. III: 24.33, gr. IV: 20.66) up to the last observation. Maximum count was recorded at 18<sup>th</sup> DPI when the values were about 2 times the normal values (TLC at 18<sup>th</sup> DPI: gr. I: 50.00, gr. II: 48.87, gr. III: 49.67, gr. IV: 25.30). These findings are in accordance with the observations of Metwally (2009) in different *Salmonella* infections, Assoku and Penhale (1978) in chicken and turkey poults infected with *Salmonella gallinarum*. Leukocytosis has been attributed to bone marrow hyperplasia (Assoku and Penhale, 1978) and massive tissue necrosis. Non-significantly lower values of TLC were observed in birds treated with *W. somnifera* and *A. sativum* than group I birds. Heterophils and monocytes increased significantly ( $P < 0.05$ ) in all the infected groups from 3<sup>rd</sup> DPI up to the last observation, the highest values were observed on 18<sup>th</sup> DPI. Similar haematological changes had been reported in birds infected with *S. typhimurium* by Metwally (2009). Heterophilia may be attributed to acute and chronic inflammatory diseases (Coles, 1986) and degenerative changes in the internal organs. Among treatment groups, group II and group III birds revealed non-significant change than group I birds.

Serum proteins, albumin and A:G ratio decreased significantly in all the infected groups from 3<sup>rd</sup> DPI up to the last observation while globulins increased significantly ( $P < 0.05$ ) in all the treatment groups from 7<sup>th</sup> DPI than control group birds up to the 28<sup>th</sup> DPI. (Albumin values at 18<sup>th</sup> DPI: gr. I: 0.28, gr. II: 0.32, gr. III: 0.29, gr. IV: 3.16 and at 35<sup>th</sup> DPI: gr. I: 1.72, gr. II: 2.20, gr. III: 2.11, gr. IV: 3.12). These findings are in agreement with Jensen (1998) in *S. typhimurium* and Kokoshorarov (2006) in *S. gallinarum* infection in chicken. Blood *et al.* (1994) opined that hypoproteinemia might be due to renal diseases, liver damage and congestive heart failure. In the present study, there was decreased appetite, diarrhoea and damage to liver and kidney tissue as was evident in histopathological examination of these tissues. Hyperglobulinemia is associated with chronic diseases and bacterial septicemia (Coles, 1986). Group II and III birds revealed significantly increased ( $P < 0.05$ ) values of serum protein, albumin and A:G ratio from 25<sup>th</sup> DPI than group I while non-significant effect was observed on globulin values. *W. somnifera* repairs liver damage and induces liver to synthesize proteins Kumar *et al.* (2006). The increase in serum proteins were observed by Metwally (2009) in albino rats and Fadlalla *et al.* (2010) in broilers.

Serum enzymes (AST, ALT and creatinine) values increased significantly in all the treatment groups than control group from 3<sup>rd</sup> DPI up to the last observation (AST values at 18<sup>th</sup> DPI: gr. I: 121.00, gr. II: 120.00, gr. III: 120.0, gr. IV: 45.66 and AST values at 35<sup>th</sup> DPI: gr. I: 61.66, gr. II: 50.66, gr. III: 53.00, gr. IV: 44.33). These results are in agreement with Kokosharov and Goranov (1997) and Santos *et al.* (2002) who found increase in AST, ALT and creatinine respectively in broilers infected with *S. gallinarum* and *S. dublin* infection in calves. Serum AST, ALT and creatinine increases in hepatic, muscular dystrophy and renal damage. Group II and group III birds revealed significantly decreased values of AST, ALT and creatinine from 25<sup>th</sup> DPI than control group birds. Hepatoprotective and renoprotective effects of *W. somnifera* and *A. sativum* had been demonstrated by Ahmad *et al.* (2005) and Ajayi *et al.* (2009). *W. somnifera* and *Allium sativum* may stabilize cell membrane, protect the cells against deleterious agents and free radical-mediated toxic damages and accelerate the regenerative capacity of cells. This is reflected in

the reduction of levels of serum enzymes.

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## Haematological changes in immunomodulated calves after vaccination with *Brucella abortus* with s-19 strain

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### Abstract

The present study was carried out at Livestock Research Station; Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar with an objective to study the haematological changes in immunomodulated cattle and buffalo calves by vaccination against brucellosis. Total thirty six calves were selected at random and divided into three groups comprising of twelve calves in each group. Gr. I served as control, given only Bruvac injection @ 2 ml sub cutaneously, Gr. II calves were treated with injection Levamisole hydrochloride @ 2.5 mg/Kg body weight intra muscularly thrice at 48 hr interval from the day of vaccination. Gr. III calves were given chelated zinc @ 300 mg/day each for 40 days before and 60 days after vaccination. Increasing trend in TLC, TEC and PCV values was observed up to 30 days post vaccination in all the three groups of calves however, values of neutrophils decreased significantly ( $P < 0.01$ ) from the day of vaccination to 15 days and 30 days post vaccination in calves of Gr. II

**Keywords:** Brucellosis, Chelated zinc, Haematology, Levamisole.

Brucellosis is caused by infection with *Brucella abortus* and is clinically characterized by abortion in late pregnancy and subsequent infertility. Vaccination for brucellosis is rarely practiced in India; information on haemato biochemical changes of such calves is also meager. Hence it was presumed that immunomodulation along with vaccination against brucellosis may afford better protection.

### Materials and Methods

Thirty six calves of Kankrej cattle and Mehsana buffaloes aged 4-8 months, weighing 100-120 Kg. maintained at LRS, S.D.Agricultural University, Sardarkrushinagar were selected randomly and divided into three groups. Each group comprised of six cattle calves and six buffalo calves. Group-I calves were given inj. Bruvac\* @ 2 ml. (s/c) and kept as control. Group-II calves were injected with 7.5 % Levamisole hydrochloride @ 2.5 mg./Kg. body weights (i/m) thrice at 48 hour interval from the day of vaccination. Group-III calves were given chelated zinc @ 300 mg./ day/head for 40 days before and 60 days after vaccination. Blood was collected (in heparinized vacutainer and in vacutainer with clot accelerator for biochemical parameters) on 0, 15, 30 and 60 days after vaccination. Hematological examinations include Hb (G/dl), Total erythrocyte count (TEC:  $\times 10^6$ /cmm), Total leukocyte count (TLC:  $\times 10^3$ /cmm), Differential leukocyte count (DLC) and Packed cell volume (PCV). PCV was measured by microhematocrit method as per Jain (1979) and data were analyzed using t test.

### Results and Discussion

Hematological changes in Kankrej cattle calves and Mehsana buffalo calves are presented in Table-I and Table- II, respectively.

Total leukocyte count in control cattle calves show values of 6.75 on the day of vaccination increasing to 7.37 on day 30 post vaccination and then declining to 6.91 on 60 day post vaccination. In Levamisole treated group, TLC values increased progressively from the day of vaccination to 60 day post vaccination. In zinc supplemented group also, TLC values increased from the day of vaccination.

Among buffalo calves, TLC in control group show value of 7.06 on the day of vaccination, increasing to 7.46 on 15 days post vaccination and then declining to 7.23 on 60 days post vaccination. In Levamisole treated calves, values show progressive increase in TLC values from 7.04 on the day of vaccination to 8.59, 8.48 and 7.29 on 15, 30 and 60 days post vaccination, respectively were observed.

In general, TLC values elevated from 15 to 3 days post vaccination in control group and up to 60 days post vaccination in Levamisole treated calves. Rise in TLC among control calves till 30 days post vaccination has been reported by Villalba *et. al.* (1990). TLC values in levamisole treated calves showed progressive rise from the day of vaccination to 60 days post vaccination. Levamisole possesses immunomodulatory effects, stimulating haemopoietic



**Table 1:** Hematological changes observed in immunomodulated Kankrej calves following immunization with *Brucella abortus* s- 19 vaccine.

Parameter	Interval (days)	Group- I (n=6)	Group- II (n=6)	Group- III (n=6)
TLCX10 <sup>3</sup> /cmm	0	6.75 ± 0.23	6.80 ± 0.94	6.68 ± 0.43
	15	7.18 ± 0.69	7.85 ± 0.89	7.91 ± 0.44
	30	7.37 ± 0.63	8.02 ± 0.81	7.73 ± 0.62
	60	6.91 ± 0.55	8.48 ± 1.12	7.82 ± 0.71
TEC X10 <sup>6</sup> /cmm	0	7.05 ± 0.36	7.37 ± 0.97	7.58 ± 1.24
	15	7.09 ± 0.21	7.88 ± 0.92	7.63 ± 0.87
	30	7.17 ± 0.37	7.91 ± 0.81	8.02 ± 0.80
	60	6.99 ± 0.42	7.53 ± 0.62	7.65 ± 0.77
Hb %	0	10.65 ± 0.68	10.67 ± 0.80	10.82 ± 0.79
	15	11.13 ± 0.45	10.93 ± 1.08	11.17 ± 0.73
	30	11.15 ± 0.69	11.32 ± 1.13	11.55 ± 0.51
	60	11.00 ± 0.87	11.12 ± 1.13	10.57 ± 0.73
PCV %	0	35.22 ± 1.28	35.10 ± 2.22	35.65 ± 2.39
	15	35.68 ± 1.50	36.93 ± 1.94	36.91 ± 2.52
	30	36.30 ± 1.28	38.05 ± 2.95	37.53 ± 2.29
	60	35.02 ± 1.32	37.60 ± 2.66	37.40 ± 2.02
Neutrophils	0	26.33 ± 0.88	26.67 ± 0.67	25.50 ± 0.43
	15	24.00 ± 0.86	20.67 ± 1.05**	23.50 ± 0.76
	30	22.83 ± 1.30	21.17 ± 0.70**	23.00 ± 1.15
	60	24.67 ± 1.05	23.00 ± 1.81	24.17 ± 1.40
Lymphocytes	0	69.17 ± 0.60	69.00 ± 0.63	69.17 ± 0.48
	15	71.67 ± 0.84	75.33 ± 0.95**	71.50 ± 1.18
	30	72.83 ± 1.45	74.83 ± 0.70**	72.17 ± 1.08
	60	70.50 ± 1.09	73.67 ± 1.73	71.00 ± 1.12
Monocytes	0	2.33 ± 0.56	2.33 ± 0.33	2.50 ± 0.22
	15	2.17 ± 0.17	2.17 ± 0.31	2.33 ± 0.42
	30	2.67 ± 0.49	2.00 ± 0.21	2.17 ± 0.31
	60	2.33 ± 0.33	1.76 ± 0.37	2.33 ± 0.33
Eosinophils	0	1.83 ± 0.31	1.67 ± 0.21	2.50 ± 0.43
	15	1.67 ± 0.33	1.50 ± 0.22	2.00 ± 0.26
	30	1.50 ± 0.22	1.17 ± 0.17	2.17 ± 0.17
	60	1.33 ± 0.31	1.33 ± 0.21	1.67 ± 0.21
Basophils	0	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21
	15	0.50 ± 0.22	0.33 ± 0.21	0.67 ± 0.21
	30	0.17 ± 0.17	0.50 ± 0.22	0.50 ± 0.22
	60	0.67 ± 0.21	0.00 ± 0.00	0.83 ± 0.31

\*\* Significant at P<0.01

system (Hennesy et. al., 1987). Increasing trend of TLC following levamisole administration was also reported by Qureshi *et. al.* (2001) in buffalo calves.

Values of Hb, PCV and TEC showed non

significant increasing trend from the day of vaccination to 30 days post vaccination in all the groups of cattle calves. Mottelib *et. al.*(1975) also reported increase in PCV and TEC values after vaccination in HF calves. However these values fluctuated in buffalo calves.

**Table 2:** Haematological changes observed in immunomodulated buffalo calves following immunization with *Brucella abortus* S- 19 vaccine.

Parameters	Interval (days)	Group-I (n=6)	Group-II(n=6)	Group-III(n=6)
TLCX10 <sup>3</sup> /cmm	0	7.06 ± 0.71	7.04 ± 0.70	7.22 ± 0.64
	15	7.46 ± 0.63	7.28 ± 0.70	8.59 ± 0.66
	30	7.36 ± 0.66	9.64 ± 0.79*	8.48 ± 0.48
	60	7.23 ± 0.85	7.11 ± 0.89	7.29 ± 0.99
TEC X10 <sup>6</sup> /cmm	0	6.93 ± 0.99	7.23 ± 1.04	7.45 ± 0.81
	15	7.04 ± 1.08	7.45 ± 0.96	7.94 ± 0.97
	30	6.90 ± 1.26	7.39 ± 0.74	7.87 ± 0.73
	60	7.04 ± 0.77	7.43 ± 0.54	7.36 ± 0.82
Hb %	0	11.39 ± 0.91	11.50 ± 1.10	11.65 ± 1.25
	15	11.29 ± 1.16	11.63 ± 1.26	12.26 ± 1.00
	30	11.07 ± 1.09	11.20 ± 1.14	12.05 ± 1.25
	60	11.09 ± 0.96	11.37 ± 1.16	11.70 ± 1.47
PCV %	0	36.90 ± 1.76	37.32 ± 2.56	37.40 ± 2.20
	15	36.69 ± 1.71	37.33 ± 2.58	38.40 ± 2.64
	30	36.43 ± 1.65	37.08 ± 2.34	38.18 ± 2.73
	60	36.83 ± 2.19	37.13 ± 2.61	37.70 ± 2.09
Neutrophils	0	31.67 ± 1.78	27.50 ± 0.43	27.83 ± 0.75
	15	28.50 ± 0.86	24.50 ± 0.99*	24.83 ± 0.91
	30	27.50 ± 0.97	25.00 ± 1.29	25.33 ± 0.88
	60	27.83 ± 1.46	24.50 ± 1.65	25.67 ± 1.56
Lymphocytes	0	63.82 ± 1.52	67.50 ± 0.43	67.67 ± 1.15
	15	67.50 ± 1.14	71.00 ± 1.21*	70.67 ± 0.56
	30	67.17 ± 1.30	70.83 ± 1.22*	70.17 ± 0.83
	60	67.83 ± 1.91	70.50 ± 1.65	70.00 ± 1.39
Monocytes	0	2.17 ± 0.20	2.33 ± 0.21	2.33 ± 0.42
	15	1.67 ± 0.40	2.00 ± 0.26	2.17 ± 0.31
	30	2.00 ± 0.32	2.17 ± 0.31	1.83 ± 0.31
	60	1.83 ± 0.37	2.33 ± 0.33	1.83 ± 0.31
Eosinophils	0	1.83 ± 0.37	2.33 ± 0.21	1.83 ± 0.31
	15	1.67 ± 0.37	2.00 ± 0.26	2.00 ± 0.37
	30	2.50 ± 0.24	1.83 ± 0.31	2.17 ± 0.31
	60	1.83 ± 0.37	2.17 ± 0.31	1.83 ± 0.31
Basophils	0	0.50 ± 0.24	0.33 ± 0.21	0.33 ± 0.21
	15	0.67 ± 0.37	0.50 ± 0.22	0.33 ± 0.21
	30	0.83 ± 0.37	0.17 ± 0.17	0.50 ± 0.22
	60	0.67 ± 0.20	0.50 ± 0.22	0.67 ± 0.21

\*\* Significant at P&lt;0.05

Increase in the values of PCV and TEC in levamisole treated group of buffalo calves has been reported by Qureshi *et al.* (2001).

Among cattle calves, DLC in control calves

revealed non significant reduction in neutrophils per cent from 31.67 on the day of vaccination to 27.83 on 60 days post vaccination. Similarly, in zinc supplemented calves from 25.50 on the day of vaccination to 23.00

on 30 days post vaccination. Reduction in values of neutrophils was significant ( $P < 0.01$ ) in levamisole treated calves, from 26.67 on the day of vaccination to 20.67 on 15 days post vaccination and 21.17 on 30 days post vaccination. Similar trend of reduction in values of neutrophils was observed in buffalo calves.

In cattle calves, lymphocyte in control calves showed non significant increase from 69.17 per cent on the day of vaccination to 72.83 on 30 days post vaccination. Similarly, in zinc supplemented calves from 69.17 on the day of vaccination to 72.17 on 30 days post vaccination. However in levamisole treated calves, highly significant ( $P < 0.01$ ) increase from 69.00 on the day of vaccination to 75.33 on 15 days post vaccination and 74.83 on 30 days post vaccination was observed. Similar trend of rise in values of lymphocyte was observed in buffalo calves. Lymphocytosis was reported among levamisole treated cattle by Bermann *et. al.* (2001) and among buffaloes by Qureshi *et. al.* (2001). Relative Lymphocytosis on 15 to 30 days post vaccination in calves of all the three groups of cattle and buffaloes observed in the present study support findings of Villalba *et. al.* (1990) in Brucella infected dogs.

Values for mean monocyte, eosinophil and basophil did not differ significantly in any of the groups of cattle as well as buffalo calves.

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## Clinico-epidemiological study on bubaline ketosis in Chhattishgarh

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### Abstract

Urine and milk nitroprusside test revealed an overall incidence of 15.28% (120/785) in buffalo population of Durg region of Chhattishgarh. The highest incidence was 57.50% in 7-9 years age group, 4<sup>th</sup> to 6<sup>th</sup> lactation and during 0-1 month postpartum. Sudden and unexpected drop in milk yield, selective feeding, wasting body condition, depression, anorexia, acetone smelling breath, constipation and nervous signs were the major clinical signs.

**Keywords:** Buffaloes, Ketosis, Epidemiology, Symptoms.

Ketosis is a metabolic disease of substantial economic significance and is responsible for declined milk production even before the clinical form and impaired reproductive efficiency (Anderson *et al.*, 1991). Excessive negative energy makes animals more susceptible to ketosis (Rukkwamsuk *et al.*, 1999). Evidence suggests that the Ketosis has been mainly investigated in bovines as compared to bubaline. Keeping in view the paucity of literature on bubaline ketosis, the present study was designed to investigate the epidemiology and symptomatology of bubaline ketosis in Durg region of Chhattishgarh.

In the present study 785 lactating buffaloes from organized and unorganized farms from villages in vicinity of Durg *viz.* Anjora, Anda, Chandrekhuri, Khapri, Nehru Nagar, Thanod and Tigra were screened for ketosis. Screening was performed on the basis of history and symptomatology. Confirmation was done by urine and milk nitroprusside tests *viz.* modified Rothera's test and Ross test. The occurrence of ketosis was correlated with age, stage of lactation and lactation number.

Out of 785 buffaloes, 120 were diagnosed as ketotic, thus indicating an overall incidence of 15.28% in the area which is much higher than the incidence of 2.92% reported in bubaline population of Tamilnadu by Thirunavukkarasu *et al.* (2010). Agewise the highest incidence (57.50%) was in 7-9 years age group followed by 23.33% in 9 years and above and lowest (19.16%) in 5-7 years age group. Lactation wise, the maximum susceptibility (45.5) was in 4<sup>th</sup> lactation followed by 3<sup>rd</sup> (25.80%), 2<sup>nd</sup> (15.0%), 5<sup>th</sup> (10.0%); and 6<sup>th</sup> and above (4.16%). Anantwar and Singh (1993) recorded 72.15% incidence of bubaline ketosis in the age group

of 6-9 years and 3<sup>rd</sup>- 4<sup>th</sup> lactation. Similarly, the incidence was highest (40.0%) during 0-1 month postpartum followed by 25.83% during 1-2 month, 17.50% during 2-3 , 8.33% during 3-4, 5.83% during 4-5 and 2.5% during 5-6 month postpartum.

The most prominent clinical signs was sudden and unexpected drop in milk yield (100%) followed by selective feeding (76.66%), wasting body condition (48.33%), depression (31.66%), anorexia (23.33%), acetone smelling breath (18.33%), constipation (12.50%) and nervous signs (2.5%). The present findings are in conformation of that of Mir and Malik (2003).

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## Modified serum agglutination test for diagnosis of brucellosis in bovine

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### Abstract

In the present study an attempt was made to evaluate the efficacy of modified serum tube agglutination test for diagnosis of brucellosis. A total of 81 specimen of serum collected from organized and un-organized dairy farm in and around Jammu region were subjected to standard serum tube agglutination test (STAT) and modified STAT. The results of the modified STAT revealed 9 (11.12%), 58 (71.60%) and 14 (17.28%) samples as doubtful, negative and positive, respectively, while standard STAT revealed 22(27.16%), 47 (58.02%) and 12 (14.81%) as doubtful, negative and positive, respectively. The positive and negative predictive value of modified STAT was 0.857 and 1 respectively with sensitive of 100% and specificity of 97.10% when compared with standard STAT. The overall agreement between the two tests was 97.53 % with kappa value of 0.908 showing almost perfect agreement between the two tests. The study revealed that the results of modified STAT were more clear and easy to read as compared to standard STAT.

**Keywords:** Brucellosis, Diagnosis, Modified STAT.

Brucellosis is considered as one of the most widespread zoonoses in the world by the FAO, WHO and OIE (Schelling *et al.*, 2003). For its diagnosis, serum tube agglutination test (STAT) is widely used but *Brucella* organisms reveal cross-reaction with other bacteria like *E.coli* O:116 and O:157, *Francisella tularensis*, salmonella, *Pseudomonas maltrophilia*, *Vibrio cholera* and *Yersinia enterocolitica* serotype O:9 (Radostits *et al.*, 2000). In the present study we have used modified STAT for diagnosis of brucellosis in bovines and its relative efficacy (sensitivity, specificity and overall agreement) was compared to Standard STAT, which is widely used for routine screening of bovine brucellosis.

Total 81 blood samples from 50 of buffaloes and 31 of cattle having history of frequent abortions, retention of placenta and repeat breeding were subjected to Standard STAT as per the method suggested by Alton *et al.*, (1975). Whereas modified STAT was done as per OIE (1996). In modified STAT, EDTA solution (pH 7-2 i.e., 3.72g/L) was used. The antigen along with positive reference serum for STAT was procured from the Division of Biological Products, IVRI, Izatnagar. A titer of 80 IU per ml and above was considered positive, below 80 to 40 IU as doubtful and below 40 IU as negative for brucellosis for both standard and modified STAT. The sensitivity, specificity and overall agreement of modified STAT with standard STAT were analyzed (Samad *et al.*, 1994) along with Kappa statistic (Thrusfield, 1995).

Through modified STAT, 9 (11.12%), 58 (71.60%) and 14 (17.28%) samples were found doubtful, negative and positive, respectively, while standard STAT revealed 22(27.16%), 47 (58.02%) and 12 (14.81%) as doubtful, negative and positive, respectively. Our findings are in agreement with that of Nazir *et al.* (2005), who reported that EDTA added modified STAT reduces doubtful samples by 17.7% as compared to standard STAT. The findings are also in accordance with that of Carin *et al.* (1984), who reported that EDTA added STAT did not result in a decrease in the antibody of infected cattle but it decreased the titer of non specific reactions and was preferable to the standard STAT.

Modified STAT showed a sensitivity 100% and specificity of 97.10% when compared with standard STAT. The positive predictive value of 0.857 and negative predictive value of 1 were revealed by modified STAT as compared to standard STAT, with overall agreement of 97.53% between the two tests. A similar statistic was also revealed by Kappa statistics ( $\kappa$ ) as kappa value 0.908 showed almost perfect agreement between the two tests. Romakhov *et al.* (1990) studied the value of EDTA test for classifying doubtful reactions in comparison to the standard STAT and confirmed it by testing on serum samples from healthy, infected and vaccinated cattle. Macmillan *et al.* (1985) and Radostits *et al.* (2000) stated that agglutination reaction was sufficiently affected by the action of EDTA and that non-specific reaction with *Brucella* could be reduced by addition of EDTA to the diluents. However Corbel

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**Table 1:-** Comparative evaluation of modified and standard STAT

Test	No. of Samples	Antibody titers		
		Negative	Doubtful	Positive
Modified –STAT	81	58 (71.60 %)	9 (11.12 %)	14 (17.28%)
STAT	81	47 (58.02 %)	22 (27.16 %)	12 (14.81 %)
Difference		+ 11 (13.58%)	-13 (16.04%)	+2 (2.46 %)

Less than 40 IU as negative , Between 40 – 80 IU as doubtful and 80 IU or above positive

(1985) reported that the serum in which agglutinating activity was entirely attributable to EDTA-labile agglutinins, a complete or almost complete loss of titer occurred in the presence of a chelating agent like EDTA.

It can be concluded that the modified STAT has excellent degree of sensitivity and specificity when compared with standard STAT and its results are more clear and easy to read than standard STAT and can be used as an alternate to standard STAT in diagnosis of brucellosis in field.

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## Clinico biochemical, haematological and therapeutic studies on gastro intestinal helminthiasis in cattle

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### Abstract

Total 45 parasitized animals exhibited symptoms of anaemia with reduced ruminal motility and rough hair coat. The haematology revealed significantly ( $P < 0.05$ ) decreased Hb concentration ( $6.39 \pm 0.22$  g%, PCV ( $24.75 \pm 0.88\%$ ) and TEC ( $4.65 \pm 0.11 \times 10^6 / \mu\text{l}$ ) with normocytic normochromic anaemia. Plasma mineral analysis revealed marginal hypomagnesaemia ( $1.69 \pm 0.04$  mg/dl), hypophosphatemia ( $4.52 \pm 0.16$  mg/dl), hyponatraemia ( $133.06 \pm 2.32$  mEq/l), hypokalaemia ( $3.96 \pm 0.13$  mEq/l) with hypocuprosis ( $< 0.80$  ppm) in 25 animals and iron deficiency ( $< 130 \mu\text{g/dl}$ ) in 5 animals. Blood gas and acid base status showed no alteration. Treatment with oral and parenteral haematinics showed significant ( $P < 0.05$ ) effective against trematodal (*Fasciola sp.* and Amphistomes) and nematodal (Strongyle and *Trichuris sp.*) infection in anaemic animals.

**Keywords:** Anaemia, Cattle, Gastro-intestinal Helminths.

Parasitic infections of livestock have worldwide distribution. In some countries it is considered as major cause of economic losses. In India, the problem of livestock parasitism has affected the viability and expansion of livestock industry in many ways like retarded growth, low productivity (milk, wool, and meat), depressed reproduction and suppressed resistance of the animals. Production losses due to helminthiasis may run into millions of rupees (Shah and Chaudhary, 1995). The present paper reports the therapeutic efficacy and alteration in clinical, haematological and biochemical parameters associated with gastrointestinal helminthiasis in cattle of Palam Valley of Himachal Pradesh.

### Materials and Methods

Total 152 crossbred cattle aged 3 – 8 years with haemoglobin concentration 8g% or less were screened. Out of these, 45 adult crossbred cattle of either sex positive for gastro – intestinal Gastrointestinal helminthiasis in cattle- Katoch, Mandial and Katoch

helminthic ova (Table 1) were investigated. Faecal sample (10 - 15 grams) from each animal was collected for faecal egg count (eggs per gram of faeces) by Mc Master technique and by technique described by Soulsby (1982) at various intervals (Table 1). Another group of 25 cattle, negative for helminthic ova / cyst and clinically healthy was taken as control. Heparinized venous blood samples were collected from parasitized and healthy animals and analysed for Haemoglobin (Hb), Packed Cell Volume (PCV), Total erythrocytic count

(TEC), Total leucocytic count (TLC), Differential leucocytic count (DLC) and Erythrocytic indices (Jain, 1986). Plasma calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) were estimated by Atomic Absorption Spectrophotometer (Model 3100, Perkin Elmer, U.S.A.). Plasma Sodium and Potassium were measured using Flame photometer (Systronics Mediflame, 127) whereas, plasma inorganic phosphorus by photometric method of Taussky and Shorr (1953). Blood gases and acid base status viz. partial pressure of oxygen ( $p\text{O}_2$ ), carbon dioxide ( $p\text{CO}_2$ ), pH, plasma bicarbonate ( $\text{HCO}_3\text{A}$ ), standard bicarbonate ( $\text{HCO}_3\text{S}$ ) and total carbon dioxide ( $\text{TCO}_2$ ) were determined by using blood gas analyser (Eschweiler System 2000 ECO – BGA). The animals positive for trematodes were treated with Oxyclozanide <sup>1</sup> @ 15 mg/kg b wt orally and nematodes positive animals with

Fenbendazole <sup>2</sup> @ 5 mg/kg b wt orally as a single doses with Oral haematinics <sup>3</sup> @ 1 dose orally, bid for 8 days + Inj. Iron Dextran <sup>4</sup> @ 10 ml deep im ad x 2 injections in highly anaemic animals (Hb  $< 6$  g%) and only oral haematinics in moderately anaemic animals (Hb 6 - 8g%). Supportive therapy consisted of multiminerals and multivitamins <sup>5</sup> @ 50 grams daily.

The data obtained were subjected to ‘One way analysis of variance and Tukey – Kramer multiple comparison tests’ at 5 per cent level of significance by using computer software Instat (Russel, 1990).

### Results and Discussion:

The quantitative microscopic faecal

examination of parasitized animals revealed the presence of ova of *Fasciola sp.* in 16, Amphistomes in 19, Strongyles in 8 and *Trichuris sp.* in 2 animals with respective mean values of epg as  $675.22 \pm 74.87$ ,  $673.68 \pm 48.87$ ,  $625 \pm 59.01$  and  $500 \pm 100$ .

**Table 1.** Faecal egg count (Mean  $\pm$  S.E) of gastrointestinal helminths in parasitized animals

S.No.	Type of Helminth	Number of animals infected	Faecal egg count (epg)
1	<i>Fasciola sp.</i>	16	$675.22 \pm 74.87$
2	Amphistome	19	$673.68 \pm 48.87$
3	Strongyle	8	$625 \pm 59.01$
4	<i>Trichuris sp.</i>	2	500 100

*Fasciola sp.*, Amphistomes, Strongyle, and *Trichuris sp.* have been reported as the common parasites of livestock of Himachal Pradesh (Krishna *et al.*, 1989). Eggs per gram of faeces ( $500 \pm 100$  to  $675.22 \pm 74.87$ ) noticed during the study was markedly lower than the faecal egg load ( $1175 \pm 964.36$  to  $2581.25 \pm 291.72$ ) reported by Bhongade *et al.* (1993) in Nagpur area, however it is higher than the range of 50 to 300 for *Fasciola sp.* and Amphistomes already reported in the cattle of Palam Valley of Himachal Pradesh by Jithendran and Bhat (1999). These variations might be due to differences in preventive and curative health care strategies and varied managerial practices adopted in the area.

The animals were anaemic, debilitated with reduced rumen motility and swelling in the intermandibular space in few cases. The clinical symptoms observed in the present study were similar to the observations of Katoch (1999) and Singh *et al.* (2006). The heart rate was slightly higher ( $79.64 \pm 1.67$ ) than anaemic animals ( $71.28 \pm 0.53$ ). The slightly higher heart rate might be due to compensation made by the animal body with the attempt to supply oxygen in required amount to the tissues (Radostits *et al.*, 1994). However after treatment, the heart rate was restored towards normalcy.

In parasitized animals the Hb, PCV and TEC values were significantly lower than healthy animals. The significantly ( $P < 0.05$ ) lower mean values of Hb ( $6.39 \pm 0.22$  g%), PCV ( $24.75 \pm 0.88\%$ ) and TEC ( $4.65 \pm 0.11 \times 10^6/\mu\text{l}$ ) indicated moderate anaemia (Benjamin, 1997) which simulate the findings of Srinivasan and Samuel (1999). However Sharma *et al.* (2005) reported comparatively higher haemoglobin values in parasitized anaemic animals. A significant ( $P < 0.05$ ) decrease in TLC ( $6.37 \pm 0.11 \times 10^3/\mu\text{l}$ ) noticed during the present study which corroborated the

findings of Srinivasan and Samuel (1999). The differential leucocytic count did not show any alteration which agrees with observations of Wadhwa *et al.* (2001). The erythrocytic indices reflected normal values of MCV

**Table 2 :** Haemogram in healthy and parasitized animals (Mean  $\pm$  S.E.)

S.No.	Parameters	Healthy animals		Parasitized animals	
		Before treatment		After treatment	
				Day 4	Day 8
1	Hb ( g% )	$11.23^d \pm 0.21$	$6.39^a \pm 0.22$	$7.45^b \pm 0.09$	$9.13^c \pm 0.14$
2	PCV ( % )	$37.84^d \pm 0.90$	$24.75^a \pm 0.88$	$28.18^b \pm 0.38$	$31.2^c \pm 0.42$
3	TEC (x $10^6/\mu\text{l}$ )	$6.99^d \pm 0.14$	$4.65^a \pm 0.11$	$5.21^b \pm 0.04$	$5.78^c \pm 0.08$
4	TLC (x $10^3/\mu\text{l}$ )	$8.10^d \pm 0.14$	$6.37^a \pm 0.11$	$6.92^b \pm 0.08$	$7.54^c \pm 0.11$
5	MCV (fl)	$53.88^a \pm 0.53$	$52.69^a \pm 1.06$	$54.20^a \pm 0.72$	$54.27^a \pm 0.61$
6	MCH (pg)	$16.10^b \pm 0.14$	$13.59^a \pm 0.20$	$16.68^b \pm 0.81$	$15.80^b \pm 0.09$
7	MCHC (%)	$29.80^{cd} \pm 0.30$	$26.19^a \pm 0.53$	$27.80^b \pm 0.31$	$29.35^c \pm 0.36$
8	DLC ( % )				
	N	$23.48^a \pm 0.69$	$24.51^a \pm 0.32$	$24.6^a \pm 0.29$	$25.02^a \pm 0.34$
	L	$72^a \pm 0.88$	$70.33^a \pm 0.87$	$70.58^a \pm 0.78$	$70.4^a \pm 0.79$
	E	$2.17^a \pm 0.28$	$2.6^a \pm 0.29$	$2.36^a \pm 0.24$	$2.4^a \pm 0.25$
	M	$2.33^a \pm 0.28$	$2.52^a \pm 0.17$	$2.4^a \pm 0.16$	$2.16^a \pm 0.21$
	B	Nil	Nil	Nil	Nil

Values in a row having same superscript do not differ significantly (  $P < 0.05$  ).



**Table 3.** Plasma minerals concentration( Mean  $\pm$  S.E.) in healthy and parasitized animals

S.No.	Parameters	Healthy animals		Parasitized animals	
		Before treatment		After treatment	
				Day 4	Day 8
1	Calcium (mg/dl)	10.83 <sup>ab</sup> $\pm$ 0.15	9.94 <sup>ac</sup> $\pm$ 0.20	10.44 <sup>a</sup> $\pm$ 0.16	10.90 <sup>b</sup> $\pm$ 0.12
2	Magnesium(mg/dl)	2.52 <sup>d</sup> $\pm$ 0.09	1.69 <sup>a</sup> $\pm$ 0.04	1.92 <sup>b</sup> $\pm$ 0.03	2.15 <sup>c</sup> $\pm$ 0.04
3	Phosphorus(mg/dl)	5.08 <sup>b</sup> $\pm$ 0.06	4.52 <sup>a</sup> $\pm$ 0.16	5.0 <sup>b</sup> $\pm$ 0.06	5.24 <sup>b</sup> $\pm$ 0.04
4	Sodium (mEq/l)	140.08 <sup>a</sup> $\pm$ 1.52	133.06 <sup>a</sup> $\pm$ 2.32	135.15 <sup>a</sup> $\pm$ 1.59	138.4 <sup>a</sup> $\pm$ 1.33
5	Potassium (mEq/l)	4.74 <sup>b</sup> $\pm$ 0.17	3.96 <sup>a</sup> $\pm$ 0.13	4.45 <sup>b</sup> $\pm$ 0.07	4.75 <sup>b</sup> $\pm$ 0.06
6	Iron ( $\mu$ g/dl)	231.08 <sup>cd</sup> $\pm$ 2.65	167.46 <sup>a</sup> $\pm$ 7.87	213.37 <sup>b</sup> $\pm$ 3.28	217.57 <sup>b</sup> $\pm$ 2.54
7	Copper (ppm)	1.23 <sup>c</sup> $\pm$ 0.03	0.79 <sup>a</sup> $\pm$ 0.04	0.90 <sup>a</sup> $\pm$ 0.02	1.04 <sup>b</sup> $\pm$ 0.02
8	Zinc (ppm)	1.15 <sup>c</sup> $\pm$ 0.03	0.94 <sup>a</sup> $\pm$ 0.03	1.1 <sup>bc</sup> $\pm$ 0.04	1.10 <sup>bc</sup> $\pm$ 0.03

Values within a row having superscript with at least one common letter do not differ significantly at 5% level ( $P < 0.05$ ).

(52.69  $\pm$  1.06 fl) and MCHC (26.19  $\pm$  0.53 g/ dl) indicating normocytic normochromic anaemia which might be because of chronic depression of erythropoiesis due to helminthiasis (Jain, 1986). This simulates the observations of Egbe – Nwiyi and Chaudrai (1996) who reported non-responsive normocytic normochromic anaemia in cattle suffering from fascioliasis in Borno state of Nigeria.

The lower levels of plasma Mg (1.69  $\pm$  0.04 mg/dl), P (4.52  $\pm$  0.16 mg / dl), Na (133.06  $\pm$  2.32 mEq/l) and K (3.96  $\pm$  0.13 mEq/l) indicated marginal hypomagnesemia, hypophosphatemia, hyponatraemia and hypokalaemia which simulates the findings of Samanta *et al.* (1995). Plasma Fe level was lower (< 130  $\mu$ g/dl) in 5 animals. Lower levels of copper (0.79  $\pm$  0.04 ppm) in parasitized animals indicated hypocupurosis. These observations agree with those of Sarode *et al.* (1999) who observed significantly lower values of iron, copper and manganese in parasitized animals. Also Underwood and Suttle (1999) described parasitism as one of the reasons for iron deficiency. Blood gas and acid base status did not show much alterations (Kaneko, 1997).

After the anthelmintic therapy in parasitized animals the faecal egg count was nil on day 4 and 8 indicating 100% efficacy of Oxyclozanide and Fenbendazole against trematodal (*Fasciola sp.* and Amphistomes) and nematodal (Strongyle and *Trichuris sp.*) infection respectively (Sreedevi and Hafez, 2001 and Pal *et al.* 2003).

The haematological parameters showed improvement on day 4 and 8 (Table 2), however the values were markedly lower than those of healthy

animals indicating slow recovery rate. The mean values of plasma macro and microminerals were within normal range on day 8 post treatment. The plasma level of copper was significantly ( $P < 0.05$ ) lower (0.79  $\pm$  0.04 ppm) and restored towards normalcy on day 4 post treatment. Similarly mean plasma level of iron and zinc also showed improvement. The present findings are more or less similar to those of Sarkar *et al.* (1996) and Srinivasan and Samuel (1999) who also recorded normalisation of the plasma micromineral levels after treatment with parenteral and oral haematinics.

It is inferred that endoparasitosis (fascioliasis, amphistomiasis, strongylosis and trichuriasis) is manifested by anaemia, debility, reduced rumen motility and rough hair coat with significantly ( $P < 0.05$ ) lower haemogram values, plasma minerals (Mg, P, Na, K, and Cu) and unaltered acid base status. The specific anthelmintic therapy with oral and/or parenteral haematinics alongwith multimineral and vitamins were effective with slow recovery rate.

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## Incidence of subclinical mastitis in lactating goats of Jabalpur

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### Abstract

A total of 213 (426 halves) lactating goats in and around Jabalpur area were screened for subclinical mastitis (SCM) using modified California mastitis test. Of these 80(94 halves) goats were found positive. Age wise highest incidence was seen in goats of 2-3 years (53.75%) while parity wise incidence was high in first parity. The overall incidence was recorded as 37.55% on animal basis and 23.61% on halves basis.

**Keywords:** Goats, Incidence, Mastitis, Subclinical.

Mastitis is the most economical important disease of goats because of high mortality in kids (Addo *et al.*, 1980). The disease is manifested in two forms (subclinical and clinical) and may be acute or chronic. Subclinical form of mastitis is responsible for major economic losses. In goats milk production is always presumed to be secondary, thus SCM never received prompt attention so remained neglected. Hence, with this view the present study was undertaken to assess and find out the incidence of SCM in goats. For this purpose lactating non-descript Jamnapari crosses and Barberi crosses of goats belonging to different private goat keepers and livestock Adhartal farm J.N.K.V.V., Jabalpur were screened for SCM. Total 213 lactating does were tested using Modified California Test (Devi, 1989). Epidemiological data of SCM in goats was studied by recording the number of animals affected, age, lactation number, stage of lactation and number of halves affected. Milk was examined for discoloration, clots or flakes, pus, blood and consistency.

The overall incidence of infected animals was found to be 37.55% (80/213) on animal basis and 23.61% (94/398) on halves basis. The incidence of SCM in does was reported to be higher in the age group of 2-3 years (53.75%) followed by 3-4 years (38.75%) and 4-5 years (7.50%). Forty (75.00%) animals showed unilateral half affection, of which 40 (66.67%) showed right half involvement and 20 (33.33%), showed left half involvement. The remaining 20 animals (25.00%) had both the halves infected. Parity wise highest incidence of SCM was 45.00% recorded in does of 1<sup>st</sup> parity which subsequently, decreased from 22.50 to 2.50% with the increase in lactation number from 2<sup>nd</sup> to 6<sup>th</sup>. Highest incidence of SCM was recorded during 1<sup>st</sup> month of lactation (52.50%) followed by 22.50% in 2<sup>nd</sup> month of lactation, 16.25% in 3<sup>rd</sup> month of lactation

and 8.75 in 4<sup>th</sup> month of lactation

The overall incidence of SCM was found to be 23.61% on halves basis which was closely approximated with the reports of Sanchez *et al.* (1999) and Hole *et al.* (2006). On the contrary, Bhujwal *et al.* (1999) and Siti zubaidah *et al.* (2005) stated the incidence of SCM to be 40.96% and 61.00% on halves basis respectively.

The incidence of SCM in goats on the basis of unilateral and bilateral involvement of udder halves during the present study was found to be 75.00% and 25.00%, respectively. These findings are in agreement with the findings of Ameh and Tari (2000) and Dadhich *et al.* (2007), who have reported 75.51% and 68.62% unilateral halves involvement and 24.49% and 31.38% bilateral halves involvement. However the findings of the present study are not in agreement with the reports of Siti Zubaidah *et al.* (2005) who reported 50% bilateral halves involvement and 8% unilateral halves involvement.

Lactation wise incidence from first to sixth lactation decreased gradually i.e., 45.00, 22.50, 13.75, 11.25, 5.00 and 2.50 percent. These findings are similar to the findings of Dadhich *et al.* (2007) who reported the maximum susceptibility to intramammary infection in the first lactation. Further, most of the cases in first parity does was due to teat injury. This finding agrees with the observations of Ameh and Tari (2000).

Further, the lactation stage wise incidence in SCM was found to be highest during 1<sup>st</sup> month of lactation which may due to the increase in the somatic cell count (SCC) in initial stage of lactation, as also reported by Scott *et al.* (2002). Age wise incidence for SCM in lactating does was highest (53.75%) in 2-3 years age group followed by that of 3-4 years (38.75%) and

4-5 years (7.50%) respectively. These findings are similar with the earlier reports of El-idrissi *et al.* (1994).

Epidemiological studies on mastitis in goats are rare from our country due to importance of this animal for meat production mainly. High incidence in age of 2-3 years and in first parity may be due to short reproductive span (as the animal is used for meat after this period).

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## Seasonal variations in plasma trace mineral profile and their subclinical deficiencies in cattle of Punjab

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### Abstract

A base line survey on plasma trace mineral (zinc, copper, iron, manganese, and iodine) status of apparently healthy dairy cows (n=136) and their fodder in relation to season (summer and winter) was conducted in 67 dairy units of 29 villages of Punjab, India. Seasonal variations revealed higher plasma zinc and copper levels during winter season. Plasma zinc levels were significantly ( $P<0.05$ ) higher while other minerals showed non significant variations. Changes in the fodder mineral concentrations corresponded to the plasma mineral variations, indicating a direct plant-animal relationship. Zn deficiency among cows was 24.3 and 47.6 per cent in winter and summer, respectively whereas the overall prevalence of hypocuprosis was 40.40 and 46.9 per cent in winter and summer, respectively. Iodine deficiency was the most common, prevailing in 73.5 percent cows during summer and 93.4 percent cows during winter season.

**Keywords:** Cows, Fodder, Inorganic elements, Trace minerals, Plant-animal relationship.

Farm animals derive most of their mineral requirements from their feed and fodder. Therefore, all the factors that influence mineral content of the fodder determine the mineral intake of animals, especially the agro-climatic and environmental factors like climate, soil type, species and stage of maturity (Underwood and Suttle, 1999). Trace minerals are very essential in animal nutrition for effective production and reproduction. Trace mineral imbalances have been established in many parts of Punjab (Singh, 2002), where intensive agricultural practices having been practiced for over decades. Present investigation was carried out to assess the trace mineral profile (Cu, Zn, Mn & I) in plasma of cows and forage consumed by these animals with respect to winter and summer season in the semi arid region of Punjab, India.

### Materials and Methods

A base line survey on mineral (Zn, Cu, Fe, Mn and I) status of cows and fodder was conducted in a total of 67 dairy units of 29 villages representing semi arid region of Punjab during months of June in summer and January in winter. Average temperature during the experimental year was  $38 \pm 5^\circ\text{C}$  during summer and  $14 \pm 5^\circ\text{C}$  during winter. A total of 136 apparently healthy cows were selected randomly.

### Chemical Analyses

**Blood :** Blood samples from the selected cows

were collected in sterile heparinised test tubes and centrifuged at 3000 rpm for 30 minutes at room temperature to separate plasma. The plasma samples were stored in small aliquots in mineral free glass vials at  $-10^\circ\text{C}$  until analysis. Concentrations of various plasma minerals viz. Zn, Cu, Fe and Mn were measured by Atomic Absorption Spectrophotometer (SpectraAA 20 plus, Varian, Melbourne, Australia). Plasma inorganic iodine (PII) was determined by the method of Aumont and Tressol (1987).

**Fodder:** Fodder which was being fed to the selected animals was collected, oven dried (overnight at  $65^\circ\text{C}$ ) and ground fodder samples were digested on hot plate with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982) and their mineral contents (Cu, Mn, Zn, Fe) were estimated by Atomic Absorption Spectrophotometer.

### Statistical analysis

Statistical analysis of the data was done by method described by Singh *et al.* (1998).

### Results and Discussion

Mean plasma Zinc (Zn) level of the cows was significantly higher in winter (Table 1) and was above the critical level of  $12.2 \mu\text{mol/l}$  as cited by Radostits *et al.* (2000). Goswami *et al.* (1993) recorded significantly higher winter plasma Zn levels in crossbred cow bulls. However, Mehta and Gangwar (1984) observed non-significant seasonal changes in plasma Zn levels of buffaloes. In the present study, significantly higher

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plasma Zn levels in winter compared to summer levels were ascribed to overall significantly higher fodder levels during winter compared to summer (Table 2). Although zinc contents of fodder were below the critical limit of 30 ppm (NRC 1996), plasma levels of Zn did not indicate deficiency. The present findings were in agreement with those of Singh (2002) and Valle *et al.* (2003) who recorded similar findings in Punjab and Brazil, respectively.

The prevalence rate of Zn deficiency among cows was 24.3 and 47.6 per cent in winter and summer, respectively (Table 1). These findings could be correlated to significantly higher plasma Zn levels in winter season. Yadav *et al.* (2002) had also reported lower incidence of Zn deficiency among buffaloes during winter.

Plasma iron levels varied non-significantly among both the seasons. Shrikhande *et al.* (1998) had also recorded non-significant seasonal variations in the mean plasma Fe levels of cattle. The mean plasma Fe values in the present study were above the normal range of 17.9 - 35.8  $\mu\text{mol/l}$  (Radostits *et al.*, 2000). High Fe contents of fodder (Table 2) against the dietary requirement of 50 ppm (NRC, 1996) appeared as the main factor behind elevated plasma Fe concentrations.

**Table 1:** Plasma trace minerals concentrations (mean  $\pm$  SE) and deficiency rate during summer and winter seasons in cows

Element	Summer	Winter
Zn ( $\mu\text{mol/l}$ )	13.41 $\pm$ 0.69(47.6)	18.51* $\pm$ 1.01(24.3)
Fe ( $\mu\text{mol/l}$ )	45.53 $\pm$ 3.14(6.2)	39.80 $\pm$ 3.09(16.2)
Cu ( $\mu\text{mol/l}$ )	10.33 $\pm$ 0.31(46.9)	10.87 $\pm$ 0.39(40.4)
Mn ( $\mu\text{mol/l}$ )	0.93 $\pm$ 0.04(10.9)	0.70 $\pm$ 0.11(15.4)
PII (ng/ml)	66.15 $\pm$ 5.40(73.5)	62.31 $\pm$ 3.34(93.4)

-Figures in parenthesis show prevalence rate of deficiency  
\* = Winter v/s summer difference ( $P < 0.05$ )

**Table 2:** Trace minerals concentrations (mean  $\pm$  SE) in fodders of summer and winter seasons

Element	CL <sup>a</sup>	Summer	Winter
		Mean <sup>b</sup> $\pm$ SE <sup>c</sup>	Mean <sup>b</sup> $\pm$ SE <sup>c</sup>
Cu (ppm)	<10.0	3.03 $\pm$ 0.17	3.83 $\pm$ 0.29
Mn (ppm)	<30.0	19.25 $\pm$ 1.19	15.60 $\pm$ 1.03
Zn (ppm)	<30.0	7.93 $\pm$ 0.61	17.39* $\pm$ 1.77
Fe (ppm)	<30.0	239.18 $\pm$ 12.88	294.81 $\pm$ 40.48

a=Critical level (McDowell, 2003), b=Least square mean from samples from all the districts in both the seasons, c= SE of least square means, \* = Winter v/s summer difference ( $P < 0.05$ )

The prevalence of Fe deficiency in the cows was 16.2 and 6.2 per cent in winter and summer, respectively (Table 1).

Overall mean plasma Cu levels during both seasons were within the normal physiological range of 9.5-23.6 mmol/l (McDowell 1992). Baruah and Baruah (1997) also recorded no seasonal variation in mean plasma Cu levels of Jersey heifers. Overall prevalence of Cu deficiency in cows during summer was 46.9 per cent compared to 40.4 per cent in winter (Table 1).

Pooled plasma Mn level in summer was non-significantly higher as compared to that of winter (Table 1) and were above the critical level of 0.37  $\mu\text{mol/l}$  (Hidiroglou, 1979). The overall deficiency percentage during winter (15.44%) was higher than that in summer (10.94%) and was supported by lower plasma Mn levels in that season.

Overall mean plasma inorganic iodine (PII) level of winter season was higher than that of summer season (Table 1). However, both the values were well below the critical mark of 104.9 ng/ml as suggested by Rogers (1992). These findings were in agreement with those of Kaur (2002) who recorded similar PII levels (63.60  $\pm$  4.82 ng/ml) in Ludhiana and Sangrur districts of Punjab. On the contrary, Singh (1999) and Singh (2002) recorded higher mean PII levels of 180.1 and 92.78 ng/ml, respectively. Lundgren and Johnson (1964) reported that the low levels of PII could be due to higher environmental temperature. Mee *et al.* (1994) also recorded seasonal variation in the plasma iodine levels. The reports of Jain (1990) found low iodine content of soil and presence of endemic goiter among the children in Punjab and Sethi (1988) documenting low iodine levels in water (1.07  $\mu\text{g/ml}$ ) further corroborated the results of the present study. High percentage of sub-clinical iodine deficiency was observed during summer (73.5) as well winter (93.4) seasons.

It was concluded that plasma Zn levels of cows varied significantly in summer and winter seasons and had a direct correlation with fodder Zn levels. Further, iodine deficiency was the most common subclinical mineral deficiency in the cows of the surveyed semi arid region.

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## **Livestock health care management practices in the dryland areas of Tamil Nadu**

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### **Abstract**

The present study was carried out in Dharmapuri district which is dryland areas of north-western zone of Tamil Nadu. The data were collected through personal interview schedule applied on 100 livestock owners from four categories i.e., large, small, marginal and landless farmers who were selected through proportionate random sampling method. The data thus generated contained 63 large animal farmers and 37 small animal farmers. Data were analyzed by frequency and percentage analysis. Majority of the respondents; both large and small animal farmers treated their animals by local healers. Deworming was practised by all the respondents in small animals and 22 percent in large animals, but practice of vaccination was less. Major diseases found for large animals were foot and mouth disease, haemorrhagic septicaemia and black quarter. The small ruminants mainly suffered from sheep pox, enterotoxaemia, orf and goat pox.

**Keywords:** Deworming, Dryland areas, Health Management, Local healers, Vaccination

India accounts for amongst the largest livestock population in the world. In the year 2010, India has approximately 210.2 million cattle, 111.3 million buffalo, 154 million goat, 73.99 million sheep and 9.63 million pig (FAOSTAT, 2012). Livestock plays a major role as subsidiary occupation in such dryland tracts. The farmers of dryland areas generally belong to small and marginal categories having meagre resources, low management skill and low risk taking capacity. Past researches have shown that the living standard of the dryland farmers could be improved by adopting different intensive farming system such as crop husbandry integrated with animals, poultry components and silvi-pastoral systems (Ganeche et al. 2000). Understanding the animal husbandry and health management system has been practiced in dryland areas and various dimensions of these are necessary which will throw light on the various intrinsic factors of production system.

### **Materials and Methods**

The study was conducted in purposively selected Dharmapuri district of Tamil Nadu where livestock population was high. Undivided district of Dharmapuri ranked first among district that fall under the north-western zone of dryland areas. Pennagram block was purposively selected, as the block is one of the two blocks that receives lowest rainfall in the district. Pikkili village was selected randomly from the Pennagram block. A total of 100 livestock owners from four categories on the basis of land holding i.e., large, small, marginal and landless farmers were selected through proportionate random sampling method. For analysis of data, statistical tools such as frequency and percentage were used.

### **Result and Discussion**

The study shows that, out of 100 randomly selected livestock owners 63 were found to be rearing large animals and rest 37 were rearing small ruminants. Among 37 respondents rearing small ruminants, all were found to be rearing sheep while 25 respondents were also rearing goat along with sheep. Among the respondents who were rearing large animals, majority of them (69.84%) get their animal treated by the local healers (treating with indigenous traditional knowledge) while 30 percent were consulting the veterinarians. The veterinary dispensary in the study area was located far away. However, large farmers (80%) and small farmers (33.33%) were found to consult qualified private or government veterinary doctors for treating their animals by paying them. In case of vaccination, only 36 percent of the respondents had their animals vaccinated against diseases such as foot and mouth disease, haemorrhagic septicaemia and black quarter. Regarding deworming, only 22 percent of the respondents deworm their animals regularly and almost all the respondents took measures for the control of ectoparasites by consulting the veterinarian for the type of drug and method of application, but only after the infection. None of them were found taking preventive measures for the control of the same. (Table- 1)

Table 2 reveals that majority of the sheep farmers (60%) were found to treat their animals by local healers while only 40 percent of the sheep farmers were found to treat their animals by veterinarians. Further, it was found that mostly the marginal (73.33%) and landless (78.57%) farmers got their animals treated by



the local healers, since these farmers could not take their animals to the government veterinary hospital which was far away and moreover they could not bear the heavy fees of the qualified veterinary doctors when they gave treatment at doorstep. Regarding vaccination, only 29.72 percent of the sheep owners were found to follow the practice of prophylactic vaccination against diseases such as FMD, sheep pox and enterotoxaemia. It was found that in case of any outbreak in the nearby area majority of the farmers got their animals vaccinated against these epidemic diseases. A comparison between the different categories of farmers indicated that majority of the marginal (80%) and landless (85.71%) farmers were not vaccinating their animals and the major reason was that they were unaware of the preventive measures and had lack of knowledge. All the respondents were found to practice deworming of animals, but none of the respondents were found to adopt prophylactic measure for control of ectoparasites. Major diseases of

sheep reported in the study area were sheep pox and enterotoxaemia as reported by 29 and 27 percent of the respondents respectively and the major reason for the disease is the lack of prophylactic vaccination against these diseases.

Table 3 depicts that majority of the goat farmers (56%) were found to treat their animals by local healers while only 36 percent of the goat farmers were found to treat their animals by veterinarians. Further, majority of the marginal (70%) and landless (78.57%) farmers got their animals treated by the local healers, and major reason is high fees charged by the qualified veterinary doctors when they treat at their doorstep. Tudu (2003) found in his study that the tribal goat owners were not provided by veterinary aids, they treated their goats by kabiraj/ojha. In case of vaccination, only 32 percent of the goat owners were found to follow the practice of prophylactic vaccination. Ghokhale *et al.* (2002)

**Table 1:** Distribution of the respondents according to the health care management practices followed for their animals

Health Management		Category of farmers				TotalN=63
		Large(n=5)	Small (n=21)	Marginal (n=35)	Landless (n=2)	
1.	Treatment of sick animals by					
	i) Veterinarian	4 (80.00)	7 (33.33)	6 (17.14)	2 (100.00)	19 (30.15)
	ii) Local healers	1 (20.00)	14 (66.66)	29 (82.85)	0 (0.00)	44 (69.84)
2.	Vaccination	4 (80.00)	4 (14.04)	13(37.14)	2 (100.00)	23 (36.50)
3.	Deworming	3 (60.00)	4 (14.04)	5 (14.28)	2 (100.00)	14 (22.22)
4.	Control of ectoparasites	5(100.00)	21(100.00)	35(100.00)	2(100.00)	63(100.00)
5.	Prophylaxis against ectoparasitic infection	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Figures in parentheses indicate percentage

**Table 2:** Distribution of the respondents according to the health care management practices followed for their sheep

Health Management		Category of farmers				TotalN=37
		Large n=2	Small n=6	Marginal n=15	Landless n=14	
1.	Treatment of sick animals by					
	i) Veterinarian	2(100.00)	5 (83.33)	4 (26.66)	3 (21.42)	14 (37.83)
	ii) Local healers	0 (0.00)	1 (16.66)	11 (73.33)	11 (78.75)	23 (62.17)
2.	Vaccination	2(100.00)	4 (66.66)	3(20.00)	2(14.18)	11(29.72)
3.	Deworming	2(100.00)	6 (100.00)	15(100.00)	14(100.00)	37(100.00)
4.	Control of ectoparasites	2(100.00)	6 (100.00)	15(100.00)	14(100.00)	37(100.00)
5.	Common disease encountered at farm					
	i) FMD	0 (0.00)	0 (0.00)	2(13.33)	3(21.42)	5(13.51)
	ii) Sheep pox	0 (0.00)	2(33.33)	5(33.33)	4(28.57)	11(29.72)
	iii) Enterotoxaemia	0 (0.00)	1(16.66)	4(26.66)	5(25.71)	10(27.02)

Figures in parentheses indicate percentage

**Table 3:** Distribution of the respondents according to the health care management practices followed for their goat

Health Management		Category of farmers				TotalN=25
		Largen=1	Smalln=6	Marginal n=10	Landless n=8	
1.	Treatment of sick animals by					
	i) Veterinarian	1(100.00)	3 (50.00)	3 (30.00)	2 (25.00)	9 (36.00)
	ii) Local healers	0 (0.00)	3 (50.00)	7 (70.00)	6 (78.75)	14 (54.00)
2.	Vaccination	1(100.00)	2 (33.33)	3 (30.00)	2(25.00)	8(32.00)
3.	Deworming	1(100.00)	6 (100.00)	10(100.00)	8(100.00)	25(100.00)
4.	Control of ectoparasites	1(100.00)	6 (100.00)	10(100.00)	8(100.00)	25(100.00)
5.	Common disease encountered at farm					
	i) Tetanus	0 (0.00)	3 (50.00)	3 (30.00)	2(25.00)	8(32.00)
	ii) Orf	0 (0.00)	2(33.33)	4(40.00)	3(37.5)	9(36.00)
	iii) Goat pox	1(100.00)	2(33.33)	3 (30.00)	2(25.00)	8(32.00)

Figures in parentheses indicate percentage

reported that the proportion of goat holders who followed vaccination increase with increase in flock size while, reverse trend was observed for those not adopting vaccination practices. All the respondents were found to practice deworming of animal regularly by procuring the medicine after consulting the veterinary officers. Sharma (2005) reported that among the large flock holder, 63.33% goat rearers practiced deworming twice in a year while 9.17% of medium goat flock holder practiced deworming once in a year. Balakrishnan (1994) in his study on goat farming practices in Ramnad district of Tamil nadu found that 48 percent and 22 percent of the respondents followed deworming and deticking practices. Major diseases of goat reported in the study area were orf, and goat pox as reported by 36 and 32 percent of the respondents respectively.

### Conclusion

The study revealed that livestock owners in the dryland areas were unaware of the preventive measures and had lack of knowledge about vaccinating their animals against major diseases prevailing in the area.

Therefore, proper measures should be taken to increase awareness about vaccinating the animals.

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## A study on clinico biochemical, endoscopic and duodenal aspirate culture of small intestinal bacterial overgrowth affected dogs

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### Abstract

The aim of the study was to diagnosis of small intestinal bacterial overgrowth (SIBO) through gastroduodenoscopic aspiration of duodenal content. Clinical signs suggestive of chronic diarrhea, vomiting and weight loss were selected. Duodenal juice content, biopsy and brush cytology were collected through gastroduodenoscopy. Highly significant increased in the bacterial count ( $2.01 \pm 0.27 \times 10^5$  cfu/ml) and *Escherichia coli*, *Staphylococcus* sp. and *Pseudomonas* sp was reported in SIBO affected dogs.

**Keyword:** Dog, Duodenal aspiration, Gastroduodenoscopy, SIBO.

Small intestinal bacterial overgrowth (SIBO) is a syndrome where increased/excessive numbers of bacteria (more than  $10^5$  organisms per millilitre of intestinal contents) in the duodenum and jejunum during a fasting state were reported (Tams, 2003). Dramatic increase in the luminal bacteria lead to damage of the brush border of enterocytes directly or indirectly (Hall and German, 2000). This leads to chronic intermittent diarrhea, weight loss and or failure to gain weight (Willard *et al.*, 1994). Quantitative bacteriological culture of duodenal juice is considered to be a golden standard test for SIBO (Steiner, 2005). Thus the present study was undertaken for the diagnosis of small intestinal bacterial overgrowth through gastroduodenoscopic aspiration of duodenal contents.

### Materials and Methods

The study was conducted at the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, Chennai over a period of two semesters (2007-2008). This study consisted of apparently healthy dogs and clinical cases. Sick dogs that were brought with chronic/intermittent diarrhoea, vomiting and weight loss were screened by detailed physical examination, haematology, serum biochemistry, faecal and gastroduodenoscopy examination. During endoscopy duodenal juice, duodenal biopsy and brush cytology samples were obtained for further analysis.

Gastroduodenoscopy was conducted by Video endoscope, Karl Storz type No. 60914 PKS1, with an outer diameter of 9.8 mm and biopsy channel diameter of 2.8 mm. The procedure was performed as per Tams (1999). The dogs were premeditated with atropine sulfate (@ 0.04 mg/kg SC) and xylazine hydrochloride

(@ 1mg/kg IM). Anaesthesia was induced and maintained by ketamine (@ 5.5 mg/kg IM) and diazepam (@ 0.25 mg/kg IV). Biopsy samples were taken by pinch biopsy forceps and interpretation was done (Day *et al.*, 2008) and also brush cytology and duodenal juice was collected. About 0.25 ml to 1.0 ml of duodenal contents were collected in a sterile test tube by a sterile plastic catheter inserted through an endoscope operating channel (Strombeck, 1996). The collected duodenal contents (0.1ml) were serially diluted up to  $10^6$  for aerobic culture and 0.1ml of each dilution transferred to nutrient agar plates followed by incubated at 35°C for 24 hrs and bacterial colonies were counted with colony counter (Lynch *et al.* 1999). The collected duodenal contents were cultured for bacteria were isolated and identified (Koneman *et al.*, 1979). Hi- motility biochemical ready kit was used for *E.coli* identification (KBM 001). A thin film of growth from culture was taken and smeared on a clean glass slide and stained with Gram's stain. Faecal samples were also collected and examined (Broussard, 2003). The data were statistically analyzed by independent t test (Snedecor and Cochram, 1994).

### Results and Discussion

During the study period 22240 dogs were brought to the Madras Veterinary College. Out of this 3610 (16.8 %) dogs had gastro intestinal disorders and duodenal juice samples were obtained endoscopically in 29 dogs. SIBO was diagnosed by quantitative bacterial culture of duodenal juice samples in 6 out of 29 (20.6%) dogs. German Shepherd was found to be most commonly affected (66 %) dogs followed by mongrel and Spitz (17% each). Willard *et al.* (1994) stated that SIBO predominantly affects young animals, and the German Shepherd dog is predisposed. The reason

for such a predisposition has not clear, but may be related to the concurrence of IgA deficiency in this breed (Rinkinen *et al.*, 2003). A study of GSD colony associated with reduced serum and faecal IgA concentrations, increased serum IgG concentrations, and colonization of the gut by enteropathogenic *Escherichia coli* (Littler *et al.*, 2000). Rutgers *et al.* (1995) reported that the dogs affected with SIBO were 6 months to 14 years of age. These observations were similar to the present study, in which there was no specific age of occurrence of disease. Female dog had higher incidence. Dogs were admitted with vomiting and weight loss (100% each), followed by anorexia (67%), abdominal pain (50%) and lethargy, diarrhoea, dehydration (32% each) and smell in faeces (16%). These findings were also observed by Rutgers *et al.* (1995) and Batt (2002). In SIBO an increased numbers of luminal bacteria in the small intestine could damage the brush border of enterocytes directly or indirectly by attracting polymorphonucleocytes, deconjugating bile acids, producing hydroxylated fatty acid and alcohols, metabolizing dietary nutrients which alter the absorptive function of the cells (Hall and German, 2000).

In the present study, the endoscopic appearance of duodenum was normal, which were correlated with findings of German *et al.* (2003) who stated that the duodenoscopic findings in SIBO were unremarkable or

non specific. Donaldson *et al.* (1993) and Lamb (1999) stated that the gastro duodenoscopy was an important tool for examination of a dog with chronic vomiting and diarrhoea and also suggested that visualization of duodenum, aspiration of duodenal contents and microscopic examination of mucosal samples could be useful in diagnosis. Histologically, duodenal biopsy specimens did not revealed abnormalities, which concurred with Willard *et al.* (1994) findings who stated that the SIBO was not always associated with severe mucosal lesions. Duodenal cytology revealed epithelium cells and bacteria and this may be due to increased luminal bacterial activity.

No significant changes in haemato-biochemical parameters were observed (Table 1). A highly significant increased ( $p < 0.01$ ) in the bacterial count was observed in SIBO affected dogs ( $2.01 \pm 0.27 \times 10^5$  cfu/ml) when compared to healthy animals ( $0.92 \pm 0.03 \times 10^5$  cfu/ml) (Table 1). Culture of duodenal juice revealed *Escherichia coli*, *Staphylococcus* sp. and *Pseudomonas* sp. Microbiological culture of duodenal juice obtained endoscopically was need to confirm the diagnosis of SIBO with more than  $10^5$  colony forming units per ml (Delles *et al.*, 1994). Rutgers (1996) reported that the most frequent isolates typically including enterococci and *Escherichia coli*, *Staphylococcus* sp and *Pseudomonas* sp. in dogs. Culture of duodenal juice

**Table 1:** Haemato-biochemical and bacterial counts ( mean  $\pm$  SE) in duodenal juice of dog

Parameters	Healthy (n=6)	SIBO (n=6)
Hb (g/dl)	12.33 $\pm$ 0.61	10.20 $\pm$ 0.28
PCV (%)	39.83 $\pm$ 0.53	33.17 $\pm$ 3.71
RBC (mill/c.mm)	6.48 $\pm$ 0.26	5.58 $\pm$ 0.79
WBC (X10 <sup>3</sup> cells/c.mm)	12.18 $\pm$ 1.46	7.68 $\pm$ 1.85
MCV (fl )	61.29 $\pm$ 2.31	60.87 $\pm$ 3.29
MCH (pg)	17.90 $\pm$ 1.89	18.20 $\pm$ 0.62
MCHC (%)	32.29 $\pm$ 1.61	30.89 $\pm$ 1.29
Platelets X 10 <sup>5</sup> / $\mu$ l	1.984 $\pm$ 0.09	1.68 $\pm$ 0.01
BUN (mg/dl)	20.68 $\pm$ 1.82	20.85 $\pm$ 1.97
Creatinine (mg/dl)	0.823 $\pm$ 0.06	0.95 $\pm$ 0.56
ALT (IU/L)	44.973 $\pm$ 6.92	42.77 $\pm$ 6.84
ALP (IU/L)	64.24 $\pm$ 7.57	68.18 $\pm$ 6.42
Total Protein (g/dl)	6.17 $\pm$ 0.26	6.26 $\pm$ 0.29
Albumin (g/dl)	3.00 $\pm$ 0.21	2.40 $\pm$ 0.28
Globulin (g/dl)	3.17 $\pm$ 0.11	3.86 $\pm$ 0.57
Bacterial counts x 10 <sup>5</sup> cfu/ml	0.92 $\pm$ 0.03	2.01 $\pm$ 0.27**

Means showing the same superscript in the column do not differ significantly, \*\* - Highly Significant (P < 0.01)

had been regarded as the gold standard for diagnosis of SIBO as also opined by Johnston (1999), German *et al.* (2003) and Marks (2007).

### Conclusions

Bacterial colony count of duodenal juice samples collected through duodenoscopy was found useful for confirmatory diagnosis of SIBO.

### Acknowledgement

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Table 1. haemato-biochemical and bacterial counts ( mean  $\pm$  SE) in duodenal juice of dog

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## Outbreak of anthrax in Deccani sheep

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### Abstract

An atypical form of anthrax outbreak was recorded in Deccani sheep which are of migratory type. In this outbreak a total of 26 sheep (21 sheep adult and pregnant, 5 lambs) died within 15 to 20 days and haematuria was evident in all the cases. Upon laboratory examination the disease was confirmed to be anthrax. Immediately rest of the sheep were treated with antibiotic streptopenicillin (Inj. Dicrystacin®) for five days and vaccinated with live anthrax spore vaccine (IAH and VB). The sheep were monitored for a month, with no mortality being reported later.

**Keywords:** Deccani sheep, *Bacillus anthracis*, Haematuria.

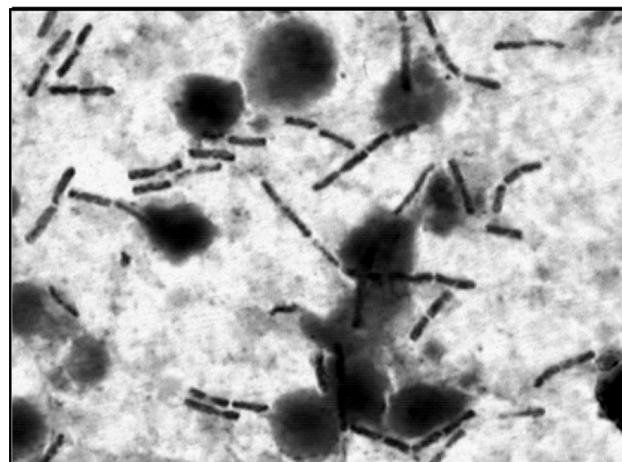
Anthrax is one of the devastating diseases of homoeothermic animals including man. The bacilli discharged from infected animals or from opened carcass contaminate the soil. Infection gains entrance to body by ingestion, inhalation, or through skin. Spores can be picked up directly from soil or from fodder grown on infected soil (Radostits *et al.*, 2007). In India the anthrax occurs sporadically in cattle, buffaloes, sheep and goats leading to severe loss to the farmers (Mathur and Dubey, 1994; Venkatesh *et al.*, 2006; Suchitra *et al.*, 2010). It can also occur in humans when they are exposed to the bacterium, usually through handling infected animals or animal hides or by consuming meat (Ichhpujani *et al.*, 2004; Prejit *et al.*, 2006; Radostits *et al.*, 2007). The losses may result from death of the animals, cost of immunization, treatment of animals and dead animal disposal. Additional losses are incurred on treatment of people who come in contact with anthrax positive animals. Anthrax may occur as per acute, acute, sub acute and rarely chronic forms. In the per acute form of anthrax the clinical signs are not so prominent and death of animal occurs within an hour. Therefore, it increases the risk of exposure to anthrax positive animals. In view of this the present paper reports an atypical form of anthrax outbreak among Deccani sheep of migratory type.

### Case history and clinical observation

An atypical form of anthrax outbreak among migratory Deccani sheep in Aurad taluka of Bidar district in Karnataka state was reported during the month of December. These Sheep had earlier been to different places during migration and then were congregated at one place. It has been suggested that anthrax cycle

commences with mingling of sheep with each other (Radostits *et al.*, 2007). The incursion of disease leads to an outbreak in Deccani sheep, resulting in the death of 26 sheep (21 sheep adult and pregnant, 5 lambs) within 15 to 20 days. Initially the affected sheep were treated with Berenil® and Tonophosphon® but no response was achieved. The cases were then referred Veterinary College, Bidar.

The investigation revealed that the disease was characterized by sudden death and before that the affected animals appeared dull, depressed, had high fever with congested mucous membranes, sudden stoppage of grazing, intermittent convulsions, rapid respiration, voiding of urine in an arched back condition, howling and haematuria. After voiding urine, the sheep fell down and succumbed to death within an hour. Passing of red colored urine (Haematuria) was evident in all the cases and the course of disease lasted for 1-2 hour (Jensen and Swift, 1982). In the dead animals little quantity of blood mixed frothy discharge was voided from nares



**Fig:** The presence of short chain *Bacillus anthracis* in blood smear

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and rectum. Based on the clinical signs observed, the outbreak was suspected to be per acute form of anthrax (Jensen and Swift, 1982; Radostits *et al.*, 2007). The samples were collected from both live and dead animals and brought to laboratory for further investigation. The smears prepared from the discharge from natural orifices and the peripheral blood smears were subjected to polychrome methylene blue staining technique. The presence of square ended bacilli in short chains showing the Mc Fadyean's reaction (Fig.), confirmed the outbreak as an atypical per acute form of anthrax in Deccani sheep.

The death of sheep one after the other as noticed in this outbreak can be attributed to improper disposal of infected carcasses. The economic loss due to mortality itself raised to the tune of approximately one lakh rupees. In addition to this, shepherds themselves had to undergo medication as they were exposed to the infected animals. To stop further loss, the rest of the sheep were treated with antibiotic streptopenicillin (Inj. Dicrysticin®- 2.5g, Zydus AHL) @ 10,000 IU/kg bwt I/M for five days and vaccinated with live anthrax spore vaccine (IAH & VB, Bangalore) @ 0.2 ml S/C. The sheep were monitored for a month, with no mortality being reported later.

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R.S. Pura, SKUAST-Jammu, J&K.

## Ventricular septal defect in a Cocker Spaniel - A case report

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### ABSTRACT

An eight month old male cocker spaniel was brought to madras veterinary college teaching hospital with the history of mild exercise intolerance and respiratory distress. The major clinical findings were restlessness, mildly abducted forelimbs and holosystolic murmur of grade III / VI over right cardiac apex. By echocardiography moderate sized defect with left to right shunting was observed in the high septal membrane of interventricular septum. Based on these findings the diagnosis of Ventricular Septal Defect was made and the case was successfully managed with Enalapril Maleate.

**Keywords:** Cocker Spaniel, Echocardiography, Ventricular septal defect,

Congenital heart diseases include morphologic and functional abnormalities of the heart and adjacent great vessels that are present at birth (Marco *et al.*, 2008). Congenital heart defects arise from altered or arrested embryonic development of the heart. The eventual consequences of such defects include gross anatomic alterations and inability of heart to perform normal functions. These abnormalities may be discovered early or much later in life. Although the malformation of heart and great vessels constitute a relatively small percentage of cardiovascular disorders in dogs, they are clinically important.

Congenital heart diseases like subaortic stenosis, patent ductus arteriosus and pulmonic stenosis are common than other defects in dogs. Ventricular septal defect, which is a deficiency of the interventricular septum that creates a communication between the ventricles, is the fourth most common congenital malformation in dogs (Johnson, 2006). Failure of the embryonic septal components to fuse or hypoplasia/agenesis of septal components is the presumed cause of ventricular septal defect. The exact incidence of congenital heart disease is not known because of asymptomatic nature of some defects and some congenital heart defects result in neonatal death which remain unreported. Incidence of congenital heart disease was assumed to be 6 to 8 per cent (Bussadori *et al.*, 2001).

### Case history and Observations:

An eight month old male cocker spaniel, weighing 12 kgs, was suffering with mild exercise intolerance and respiratory distress since a month. The pup was treated by a veterinarian with antibiotics and was referred to madras veterinary college teaching

hospital for further investigation. The major clinical findings were inappetence, restlessness, mild abduction of forelimbs, holosystolic murmur of grade III / VI over right cardiac apex and soft murmur over left cardiac apex.

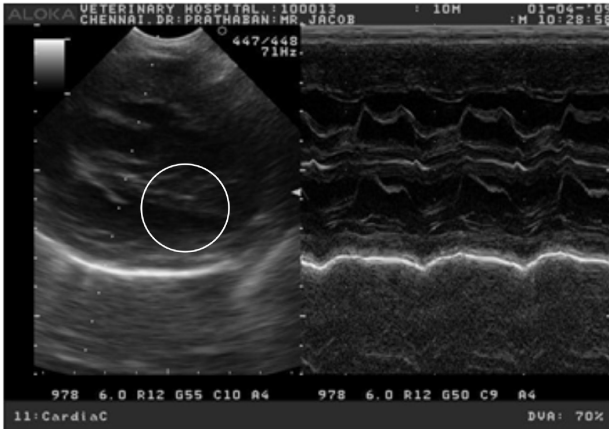
Hematology and serum biochemical observations were within normal range. Thoracic radiography revealed pulmonary vessel congestion and mild cardiomegaly. 2-D echocardiographic examination (Right parasternal long axis view) revealed the presence of a moderate sized visible defect in the high septal membrane of interventricular septum. During systole, left to right shunting through interventricular septum was noticed on color doppler echocardiography. Fractional shortening and ejection fraction were within normal range. Based on these findings the case was diagnosed as ventricular septal defect.

### Treatment and Discussion:

The dog was managed with enalapril maleate, an Angiotensin Converting Enzyme (ACE) inhibitor @ dose of 0.5 mg / kg B.W, b. i. d, PO. Clinical improvement was noticed after two weeks of treatment and echocardiographically evident reduction in the percentage of shunting was noticed after three weeks of treatment. The owner was advised to continue the medication and report for regular review.

Ventricular septal defect is one of the main congenital heart defects causing systemic to pulmonary shunting thereby resulting in volume overload. Ventricular septal defect may occur as an isolated defect or may coexist with concurrent defects e.g., patent ductus arteriosus, atrial septal defect etc (Tidholm, 1997). The present case of ventricular septal defect was





**Fig1.** 2-D echocardiograph showing the presence of a moderate sized visible defect in the high septal membrane of interventricular septum (Right parasternal long axis view)



**Fig2.** Color doppler echocardiograph showing left to right shunting during systole (Right parasternal long axis view)

an isolated defect, other defects being ruled out by echocardiographic examination. Medical management alone was sufficient to successfully manage the case because of small sized defect and absence of pulmonary hypertension. Enalapril maleate an ACE inhibitor decreases the percentage of shunting by reducing the left ventricular pressure (Bonagura *et al.*, 1999) and is therefore used for medical management of small to medium sized ventricular septal defects.

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## Delayed hypersensitivity reaction to oral Ivermectin in a dog and its management

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### Abstract

A delayed hypersensitivity reaction to 2<sup>nd</sup> dose of oral ivermectin was recorded in a Labrador dog aged one year. The clinical manifestations included vomiting, incoordination, frequent and involuntary urination and change in barking tone. The dog was also unable to recognize the owner and the surroundings. Treatment with inj. dexamethasone, inj. avil and dextrose saline (5%) proved successful.

**Keywords:** Dog, Hypersensitivity reaction, Oral ivermectin, Treatment

Ivermectin is a semi synthetic derivative of one of the avermectins having broad spectrum of action, unprecedented potency and good margin of safety and thus making it the drug of choice for treatment of nematodes and arthropods parasitism in all animal species of livestock. The present communication reports a case of hypersensitivity reaction to oral ivermectin in a Labrador dog.

A one year old Labrador dog was presented in the College Veterinary Clinics with the history of incoordination of limbs, anorexia and involuntary urination. Detailed history revealed that the dog was given ivermectin orally (6 mg total dose) about 4 hours before and that was the 2<sup>nd</sup> dose of ivermectin after an interval of a week as routine deworming by the owner. Just within few minutes of the oral dose, there was vomiting. Afterwards, change in the barking tone, incoordination and frequent & involuntary urination were observed by the owner and animal was not taking any food & water. The dog was also unable to recognize the owner and the surroundings. Clinical examination revealed hyperesthesia, ataxia, mydriasis, low temperature (99.8<sup>o</sup> F), heart rate of 38/min and body extremities were cold. Based on the history and clinical manifestations, the case was diagnosed as a hypersensitivity reaction to oral ivermectin.

The dog was treated with inj. Dexamethasone @ 0.25 mg/kg b.wt. (total dose 8 mg), inj. Avil 1.5 ml im along with dextrose saline (5%) 500 ml intravenously. On 2<sup>nd</sup> day, animal was alert and apparently normal except hind limb weakness. Also there was constipation and accumulation of gas in the stomach and treatment was repeated. Additionally, liquid paraffin (20 ml bid) for one day and suspension Gelusil-MPS 2 tsf twice daily for 2 days was given. The dog was normal in all aspects on 3<sup>rd</sup> day.

Upendra *et al.* (1995) reported ivermectin toxicity in German shepherd dog within 90 minutes of its administration and Wadhwa and Prasad (2000) reported acute hypersensitivity reaction which occurred immediately after second exposure to the drug given subcutaneously. However in the present case, it was Labrador breed in which toxicity has not been reported. Moreover, it was a delayed hypersensitivity reaction after oral administration of ivermectin. Adverse reactions due to oral ivermectin in dogs are poorly reported (Paul and Tranquilli, 1989). Wadhwa and Prasad (2000) successfully treated a case of acute hypersensitivity reaction of ivermectin in dog with glucocorticoid and fluid therapy. PicROTOXIN, a potent GABA antagonist and physostigmine, a reversible AChE inhibitor may also be of some use (Yadav and Srivastava, 2005) while treating ivermectin toxicity.

### Acknowledgement

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## Therapeutic management of hypogalactia in crossbred cows

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### Abstract

Field trials in crossbred cows belonging to dairy farmers, both in the organized and unorganized sectors in and around Mhow, M.P. revealed that serum macro-mineral: total calcium, inorganic phosphorus, and magnesium titres were positively correlated with milk production. Further, marked narrowing of serum Ca: Pi ratio from the optimal value of 2.13: 1, or of Ca: Mg ratio < 6: 1 was associated with hypogalactia. Intravenous infusion of sterile calcium borogluconate solution @ 450 ml animal<sup>-1</sup> in combination with magnesium hyposulphite solution (Lactomag<sup>®</sup>) @ 0.1 g kg<sup>-1</sup> body weight effectively countered hypogalactia, as evidenced by the overall improvement in lactation performance. Follow-up oral supplementation of calcium- phosphorus liquid feed supplement (e-Cal<sup>®</sup>) @ 1 dl animal<sup>-1</sup>d<sup>-1</sup> in combination with magnesium sulphate @ 0.1 g kg<sup>-1</sup> body weight produced a sustained lactogenic response.

**Keywords:** Crossbred cows hypogalactia, Mineral deficiency, Therapy

Lactation, a state of physiological stress, challenges the homeostatic mechanisms and tissue reserves. Cows at freshening are more susceptible because of the sudden spurt in the turnover of fluids, minerals and organic constituents, required in milk synthesis (Radostits *et al.*, 2007). Besides genetic factors, nutritional deficiencies also affect animal productivity markedly in different geo-climatic zones across the world. Hypocalcaemia, hypophosphataemia, hypomagnesaemia and hypoglycaemia are mainly implicated in functional hypogalactia: less than the expected milk production (Underwood and Suttle, 1999; Guff, 2000). Poor quality crop residues and restricted availability of green fodder/ concentrates contribute to prolonged energy deficit: blood glucose concentration remaining well below 50 mg dl<sup>-1</sup> *cf.* the normal range of 65-70 mg dl<sup>-1</sup> under well-fed conditions (McDonald *et al.*, 2002). Continuous leaching of the grazing lands by heavy downpour leading to mineral deficiency (Pfander, 1971) adversely influences the soil-plant-animal interrelation (Prasad *et al.*, 1999). Chronic phosphorus deficiency in paddy straw, the mainstay of roughage-based cattle feed remains a serious managerial concern in the entire rice belt. This communication reports on the response to remedial therapy in hypogalactic crossbred dairy cows: initial i.v. loading dose of calcium borogluconate + magnesium hyposulphite solution *per se*, or in combination with continuous daily oral supplementation of calcium, phosphorus and magnesium salts in the feed.

### Materials and Methods

Total 20 lactating crossbred dairy cows with a

history of decreased milk production (hypogalactia) were included in the present study after preliminary screening of 60 apparently healthy animals in and around Mhow, district Indore in west Madhya Pradesh. Unhaemolyzed serum samples were collected in labelled vials for the estimation of control values of macro-elements: total Ca, Pi and Mg on d 0 (before the start of treatment). These hypogalactic cows were randomized into two equal groups comprising 10 animals each. In treatment 1 (T<sub>1</sub>), each cow was administered i.v. 450 ml sterile calcium borogluconate + magnesium hyposulphite solution (Lactomag<sup>®</sup>), and serum samples were collected on d 4 and d 10 post-treatment. In treatment 2 (T<sub>2</sub>), following the specified initial i.v. therapy, starting from d 1, calcium and phosphorus (e-Cal<sup>®</sup>) @ 1.0 dl ) + magnesium (magnesium sulphate @ 0.1 g) supplementation in the daily feed was continued during the entire 28-d trial, and serum samples were collected at the two specified intervals, d 4 and d 10 post-treatment. Standard procedures were used in the colorimetric estimation of serum total Ca, Pi and Mg. The experimental data were statistically analyzed with the completely randomized block design (Snedecor and Cochran, 2004).

### Results and Discussion

Serum macro-minerals: Ca, Pi and Mg are variously involved in maintenance of normal body conformation, modulation of immune responses, bioenergetics, enzyme activity and brain function. The incidence of subclinical hypocalcaemia in buffaloes in the Kokan region of Maharashtra ranged from 27.5 to 40% (Waghmare *et al.*, 2000). Significant cumulative

**Table 1.** Effect of remedial therapy on the serum macro- mineral profile and daily milk production performance (Av.  $\pm$  SE) in hypogalactic cross-bred cows

Parameter	Treatment	Pre-treatment d0	Post-treatment	
			d4	d 10
Ca (mg dl <sup>-1</sup> )	T <sub>1</sub>	8.0 0 $\pm$ 0.12 <sup>a</sup>	8.49 $\pm$ 0.17 <sup>a</sup>	9.89 $\pm$ 0.17 <sup>b</sup>
	T <sub>2</sub>	7.89 $\pm$ 0.11 <sup>a</sup>	8.50 $\pm$ 0.05 <sup>b</sup>	10.50 $\pm$ 0.20 <sup>b</sup>
Pi (mg dl <sup>-1</sup> )	T <sub>1</sub>	3.34 $\pm$ 0.13 <sup>a</sup>	3.72 $\pm$ 0.11 <sup>a</sup>	5.09 $\pm$ 0.16 <sup>b</sup>
	T <sub>2</sub>	3.25 $\pm$ 0.12 <sup>a</sup>	3.78 $\pm$ 0.14 <sup>b</sup>	5.00 $\pm$ 0.16 <sup>b</sup>
Mg (mg dl <sup>-1</sup> )	T <sub>1</sub>	1.60 $\pm$ 0.05 <sup>a</sup>	1.63 $\pm$ 0.04 <sup>a</sup>	1.78 $\pm$ 0.05 <sup>a</sup>
	T <sub>2</sub>	1.69 $\pm$ 0.02 <sup>a</sup>	1.72 $\pm$ 0.20 <sup>a</sup>	2.10 $\pm$ 0.41 <sup>b</sup>
Ca: Pi ratio	T <sub>1</sub>	2.20 $\pm$ 0.02 <sup>a</sup>	2.15 $\pm$ 0.04 <sup>a</sup>	2.06 $\pm$ 0.41 <sup>a</sup>
	T <sub>2</sub>	2.40 $\pm$ 0.13 <sup>a</sup>	2.20 $\pm$ 0.16 <sup>b</sup>	2.10 $\pm$ 0.08 <sup>b</sup>
Ca: Mg ratio	T <sub>1</sub>	5.00 $\pm$ 0.55 <sup>a</sup>	5.20 $\pm$ 0.06 <sup>a</sup>	5.50 $\pm$ 0.04 <sup>b</sup>
	T <sub>2</sub>	4.61 $\pm$ 0.40 <sup>a</sup>	4.95 $\pm$ 0.80 <sup>a</sup>	5.50 $\pm$ 0.20 <sup>a</sup>
Milk yield (kg) (animal <sup>-1</sup> d <sup>-1</sup> )	T <sub>1</sub>	9.70 $\pm$ 0.76 <sup>a</sup>	10.96 $\pm$ 0.68 <sup>a</sup>	13.00 $\pm$ 0.82 <sup>b</sup>
	T <sub>2</sub>	10.25 $\pm$ 0.50 <sup>a</sup>	10.61 $\pm$ 0.44 <sup>a</sup>	12.15 $\pm$ 0.60 <sup>b</sup>

economic losses from hypogalactia, resulting from latent hypocalcaemia in buffaloes are on record (Patel and Jadhav, 2003). In hypogalactic cows, the value of serum total Ca decreased markedly to 5 to 9 mg dl<sup>-1</sup> *cf.* the normal range of 9 - 12 mg dl<sup>-1</sup> (Vetcare, 2004). Marked narrowing of Ca: Pi ratio from the optimum value of 2.13: 1 (Chakravarti, 2000), and Ca: Mg ratio < 6:1 (present study) appear to be associated with persistent hypogalactia.

In grazing cattle, macro- and micro-mineral supplementation elicited a noteworthy clinical response in terms of improved general body condition, concurrent with enhanced reproductive efficiency and milk production capacity (Samant *et al.*, 1995). In the present study (Table 1), whereas both serum total Ca and Pi titres in crossbred dairy cows had increased significantly ( $P < 0.05$ ) towards the corresponding normal value on d 10 in treatment 1, a similar response was discernible early on d 4 post-treatment in treatment 2. These findings are in agreement with the earlier observations (Queen *et al.*, 1993). Significant ( $P < 0.05$ ) reversal of the declining trend in circulatory Ca: Pi ratio towards the normal value at both sampling intervals: d 4 and d 10 in treatment T2 *cf.* the d 0 pre-treatment value attests to sustained lactogenic response, elicited by the combination therapeutic regimen.

Significantly ( $P < 0.05$ ) increased daily milk production in both treatments T<sub>1</sub> and T<sub>2</sub> on d 10, compared to the d 0 (pre-treatment) control value is

noteworthy. This favourable biological response appears to be primarily a consequence of improved macro-mineral profile: enhanced circulatory titres of Ca, Pi and Mg with optimized Ca: Pi and Ca: Mg ratios (present study), favourable influence on pH of rumen contents with increased starch digestibility (Clark *et al.*, 1989), and augmented rumen motility leading to improved absorption/ assimilation of food nutrients (Radostits *et al.*, 2007). Restoration of normal microbial balance in the rumen with enhanced feed intake appears to be the key factor in overcoming functional hypogalactia in dairy cows. In a recent critical review of the hepatic oxidation theory of metabolic control of feed intake (Allen *et al.*, 2009), the role of markedly enhanced hepatic propionate pool in overcoming hypogalactia in the ruminants has been emphasized with convincing experimental evidences. Propionate is the major gluconeogenic precursor contributing to as much as 80% of the normal blood sugar level in lactating cows (McDonald *et al.*, 2002). This unique metabolic feature appears to be crucial in maintenance of the delicately poised energy balance in ruminants, faithfully reflected in the blood sugar status.

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## Electrocution in an Indian rat snake (*Ptyas mucosus*) - A case report

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### Abstract

Electrocution may be an important factor of mortality in arboreal population, but incidence of electrocution in snakes is generally rare. Such an electrocuted male Indian rat snake was presented in the Teaching Veterinary Clinical Complex. Emergency treatment like fluid therapy, steroid, cardiopulmonary resuscitation and antibiotic therapy were carried out as soon as possible. But, as the rescue process was too late and snake was in severe shock, recovery was not possible.

**Keywords:** Burn, Electrocution, Emergency, Shock, Snake.

High level of urbanization and the consequent result of destruction and loss of natural habitat has led to an increasing number of confrontation between wild lives and human being. For this reason, many a times, we find the wild lives especially the snakes both poisonous and non-poisonous in our locality. The present paper reports the therapeutic management of electrocuted burn in a snake.

One electrocuted male Indian rat snake (*Ptyas mucosus*) was rescued by snake help line and presented at Teaching Veterinary Clinical Complex, Bhubaneswar. In an attempt to move from a branch of tree to another, the snake fell on a high voltage tension electric power lines (Fig. 1) and then rescued in shock condition (Fig. 2). On physical examination, it was found that pupils were fully dilated keeping mouth open, dull,

lethargic and was unable to move. The hallmark of electrocution was burn marks over the body. Burns were confined to the specific sites on body coming in contact with the electrical current. There was cooked meat smell emanating from the affected parts. The measurement of anterior burning point was 4cm x 2.5cm (Fig. 3) and posterior burning point was 2.5 x 2cm (Fig. 4). Haemorrhages in the subcutaneous tissue and scales were observed which were suggestive of cardiovascular damage (Fig. 5). Both the points of electric contact were burnt and the mid area between both points was contracted including muscles and belly scales (Fig. 6). Total length of the snake was 175cm i.e measurement taken from snout to vent region length (SVL) was 127cm and from vent to tip of tail length (VTL) was 48cm. The portion of body in between two electrocutions was



Fig 1. Snake hanging over electric wire.



Fig 2. Snake after rescue.



Fig 3. Anterior wound (4cm x 2.5cm).

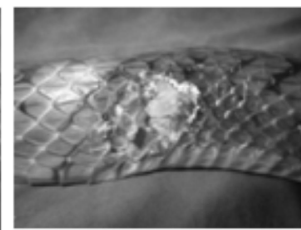


Fig 4. Posterior wound (2.5cm x 2cm).

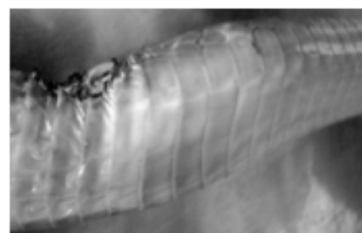


Fig 5. Haemorrhage in subcutaneous tissue.



Fig 6. Comparison of normal scale (1) with affected scale (2) between electrocuted burn spots.

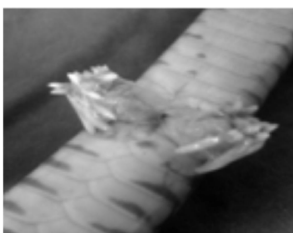


Fig 7. Everted hemipenis.

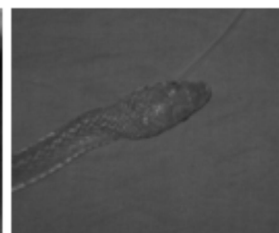


Fig 8. Resuscitation through a tube.

33cm as seen in contracted state but on straightening the body, it was 36cm. So, the burning contraction after electrocution was 3 cm. On squeezing at vent region, the everted hemipenis came out with many spikes, which measured 1 cm each (Fig. 7). The snake showed arching of body segments at injured sites indicating pain and discomfort due to irritation.

Treatment was carried out without giving anaesthesia as it was in lethargic and inactive state. The head was mechanically restrained by snake handler throughout the period of treatment. Normal saline and 5% dextrose solution, (20 ml) was given subcutaneously. Inj. dexamethasone @ 0.2mg/kg bodyweight along with amikacin @ 0.2 mg/kg bodyweight was given intramuscularly (Chaudhary and Deshmukh, 2008). The site was cleaned with normal saline solution to remove debris and dead tissue. Gentian violet was applied over burnt area along with topical application of silver sulphadiazine. As the snake was very sluggish and inactive, it was kept in open space on a dressing table. Cardiopulmonary resuscitation was done by

administration of oxygen through scalp vein tube to the nostril and orally through a tube (Fig.8). But, in spite of all remedial procedures, it was not possible to save the life of snake. The cause of death was suspected to be delay in rescue process, which is particularly seen in snake cases. So, by taking immediate step both in rescue and treatment procedures this type of incident could be avoided. Then it was handed over to the snake helpline people.

#### **Acknowledgement**

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## Management of acute nitrate poisoning in organized dairy farm

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### Abstract

Total 20 animals exhibited sudden onset of clinical symptoms resembling to acute nitrate poisoning. Prompt diagnosis and specific antidote treatment with methylene blue resulted in rapid recovery of the animals from point of death. Field diagnosis as well as toxicological analysis of feed and water samples confirmed nitrate poisoning of feed origin.

**Keywords:** Nitrate, Nitrite, Methemoglobin, Methylene Blue, poisoning

Consumption of fodder containing high levels of accumulated nitrate which are later reduced to nitrite and ammonia (~10 times more toxic than nitrate) by ruminal flora leads to acute poisoning in ruminants and animal dies because of anoxic anoxia (Radostits *et al.*, 2006). Cases of nitrate poisoning in Punjab are frequently encountered during the months of December and January when crops are affected by extreme cold and frost.

Out of total 20 animals (18 calves and 2 cows) eleven died suddenly without any treatment. Ten of them were calves in the age group of 1- 1½ year and only 1 pregnant cow of 7 year age. Onset of symptoms was rapid in these animals which included severe abdominal pain, bluish/chocolate brown mucous membranes, rapid/difficult breathing, rapid pulse (150+/minute), salivation, bloat, tremors, ataxia, staggering, weakness, sudden falling on ground, convulsions and death.

The venous blood drawn was dark brown coloured. Instant postmortem of one calf revealed main necropsy finding as chocolate brown coloured blood while no other significant findings could be noticed. The fodder samples were initially subjected to qualitative field test with diphenylamine blue (DPB-1% in concentrated sulfuric acid) to know the presence/absence of nitrate which gave dark bluish colour as a result.

Based on the profound history, prevalent extreme cold weather which was also visited by rain for continuous 3 days, symptom exhibited by animals, post mortem and field test findings and differential diagnosis

from cyanide poisoning, diagnosis ended up with nitrate toxicity.

Remaining nine animals which were also showing terminal stage symptoms, slow iv injection of 1% methylene blue in isotonic saline @ 22 mg/kg b wt was administered. Care was exercised to avoid the escape of any of the solution into the tissues surrounding the vein, owing to its irritant properties. Along with methylene blue, dexamethasone @ 1 mg/kg iv was also administered. Animals responded to the treatment with the symptoms started disappearing and the animals which were at the point of death, started showing recovery within 10-15 min following the treatment.

Fodder and water samples after toxicological analysis at Animal Disease Research Center, of the Veterinary University revealed fodder samples positive for nitrate (3700-4750 ppm) and water samples had nitrate concentration as 46-50 ppm.

Acute nitrate poisoning resulting death in 4-6 hrs after consumption of feed containing higher accumulated nitrate content. Quick diagnosis, prompt decision making and specific antidote treatment resulted in successful and rapid recovery of succumbed animals and prevented further losses.

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## Concurrent ehrlichiosis and hepatozoonosis in a Doberman pinscher dog

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### Abstract

A three year old Doberman pinscher was brought to the Referral veterinary polyclinic, IVRI with the history of anorexia, dullness, difficulty in walking and mild respiratory distress since 10 days. Major clinical findings were high rectal temperature, pale mucous membrane, enlarged peripheral lymph nodes and hind limb weakness. Peripheral blood smear examination revealed the presence of *Hepatozoon canis* and *Ehrlichia canis*. Based on these findings diagnosis of concurrent ehrlichiosis and hepatozoonosis was made and the case was managed successfully with Doxycycline.

**Keywords:** Canine, Ehrlichiosis, Hepatozoonosis

*Ehrlichia canis* and *Hepatozoon canis* are the etiological agents of monocytic ehrlichiosis and hepatozoonosis in dogs. *E. canis* (a rickettsia) and *H. canis* (a protozoan) are vector borne diseases transmitted by the brown dog tick *Rhipicephalus sanguines* (Tsachev *et al.*, 2008). *Hepatozoon canis* and *Ehrlichia canis* are the most common tick borne pathogens found in India with a prevalence of 20.6% and 30 % respectively (Abd Rani *et al.*, 2011). Co-infection of various tick borne pathogens is common (Ramprabhu *et al.*, 2001). Present paper discusses about the concurrent infection of *E. canis* and *H. canis* in a Doberman pinscher.

### Case history and Clinical approach:

A three year old Doberman pinscher weighing 26kg was brought to the Referral veterinary polyclinic, IVRI with the history of anorexia, dullness, difficulty in walking and mild respiratory distress since 10 days. Animal was treated by local veterinarian with Intamox® and was referred for further investigation. Clinical findings like high rectal temperature (104.6°F), pale mucous membrane, enlarged popliteal and prescapular lymph nodes, tachycardia (140bpm), hind limb weakness and emaciation were noticed. Ticks were present on the skin. On abdominal palpation mild hepatomegaly and splenomegaly were noticed.

Blood smear examination revealed the presence of oval cytoplasmic bodies in neutrophils. They were identified as gametocytes of *Hepatozoon canis*. In monocytes, cytoplasmic inclusion bodies, identified as *Ehrlichia canis*, were observed. Hemato-biochemical findings were anemia, monocytosis, elevated serum alkaline phosphatase and alanine aminotransferase levels

**Table 1:** Hemato-biochemical findings before and after treatment

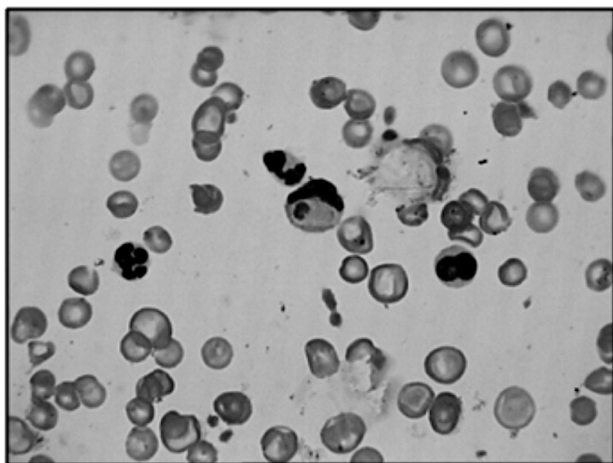
Parameters	Before treatment	One week after treatment
Hemoglobin (g/dl)	6.2	8.4
Packed Cell Volume (%)	19	26
Total Erythrocyte Count (10 <sup>6</sup> /Cmm)	3.6	4.4
Total Leukocyte Count (10 <sup>3</sup> /Cmm)	12.8	12.6
Absolute monocyte count(10 <sup>3</sup> /Cmm)	1.53	1.21
Blood Urea Nitrogen (mg/dl)	18	20
Creatinine (mg/dl)	1.2	1.2
Alanine amino transferase (U/L)	134	68
Serum Alkaline Phosphatase (U/L)	324	136
Total bilirubin (mg/dl)	0.1	0.1
Total protein (mg/dl)	6.4	6.6
Albumin (mg/dl)	3.8	3.8
Globulin (mg/dl)	2.6	2.8

(Table 1). On the basis of clinical signs and laboratory analyses, a diagnosis of monocytic ehrlichiosis and hepatozoonosis coinfection was made.

### Treatment and Discussion

Case was managed with Doxycycline @10mg/kg, PO, od for 14 days, other supportive therapy like Meloxicam @0.5 mg/kg, im, for 5 days and Haemup® syrup @5ml, PO, bid was given. 12.5% Amitraz was used to control tick infestation. Case was monitored daily and was reviewed after one week of post treatment. Clinical improvement was noticed after 5 days of treatment and significant improvement in the values of hemoglobin, packed cell volume and total erythrocyte count was noticed after one week of post treatment. Absolute monocyte count, alanine amino transferase and serum alkaline phosphatase levels decreased after one

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Peripheral blood smear showing gametocyte of *Hepatozoon canis* in neutrophil and cytoplasmic inclusion bodies of *Ehrlichia canis* in monocytes

week of treatment (Table 1). Owner was advised to continue the medication and regular review.

Tick-borne infections: ehrlichiosis, hepatozoonosis, anaplasmosis, babesiosis etc., are frequently seen as coinfections, because their

epidemiology is characterized with the same vectors (Ciaramella *et al.*, 1997). As *Rhipicephalus sanguinus* is the common vector for both ehrlichiosis and hepatozoonosis concurrent infection is possible.

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at  
Faculty of Veterinary Sciences & Animal Husbandry  
R.S. Pura, SKUAST-Jammu, J&K.

## Therapeutic efficacy of Diclazuril against coccidiosis in goats

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### Abstract

Therapeutic efficacy of Diclazuril against coccidiosis in goats was evaluated on the basis of reduction in faecal oocyst load (oocysts per gram of faeces) and improvement in haematobiochemical parameters at different time intervals. Diclazuril @ 1 mg / kg b wt was found to be 100% effective on 3<sup>rd</sup> day post treatment with faecal outcome as zero. The haemoglobin, packed cell volume and total erythrocyte count increased progressively to the level of 10.05± 0.15 g/dl, 32.13±0.39 % and 8.42 ±0.15 × 10<sup>6</sup> / μ l respectively by 14<sup>th</sup> day post treatment.

**Keywords:** Goats, OPG, Diclazuril.

Coccidiosis is one of the most economically important diseases in goats. It can be especially devastating to recently weaned kids and occasionally causes losses in other age groups. The most obvious signs of coccidiosis are scouring (occasionally with blood in the faeces) and weight loss. In milder cases there are loss of pellet formation and reduced growth rate, changes that could easily be missed. Recently, Diclazuril was introduced as a safe, effective and economic drug to overcome this problem. This paper reports the therapeutic efficacy of Diclazuril against naturally acquired infection of coccidiosis in goats.

A total of 15 goats of either sex and various age groups were showing clinical symptoms eg scouring, faeces with blood, loss of pellet formation and reduced growth rate. Coccidia was confirmed on the basis of faecal samples examination by sedimentation method (Soulsby, 1982). A single oral dose of Diclazuril @ 1

mg /kg b wt was given to each goat.

Efficacy of drug was assessed on the basis of faecal oocyst load ( Oocyst Per Gram, OPG) on '0' day (pre treatment), 3<sup>rd</sup>, 7<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day (post treatment) by modified Mc Master egg-counting technique (Sloss *et al.*, 1994). Also improvement in haematobiochemical parameters- Haemoglobin (Hb), Packed Cell Volume (PCV) and Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) as per method by Jain (1986) and Serum Total Protein (STP), Alanine Transaminase (ALT) and Aspartate Transaminase (AST) before and after treatment by using Erba diagnostic kits. The data were statistically analyzed with the completely randomized design (Snedecor and Cochran, 1994).

The OPG in treated animals was zero on 3<sup>rd</sup> day post treatment indicating 100% efficacy. However, the OPG was found to increase 7<sup>th</sup> and 14<sup>th</sup> day with a

**Table 1:** Pre and post treatment values of OPG (Mean ±SE)

Days of observations	0	3	7	14	21	28
Mean & SE	19606.67 ±3914.81	0	53.33± 13.22	750± 204.15	30053.33± 4237.24	34680± 4592.97

**Table 2:** Pre and post treatment values of haemogram (Mean ±SE)

Parameters	Intervals of observations (in days)						F value
	0	3	7	14	21	28	
Hb (g/ dl)	8.82±0.23	9.90±0.21	9.98±0.17	10.05±0.15	9.86±0.17	9.73±0.18	6.46**
PCV (%)	29.12±0.70	28.00±0.83	31.76±0.43	32.13±0.39	30.71±0.92	27.82±0.29	0.45 <sup>NS</sup>
TEC (× 10 <sup>6</sup> / μ l)	7.80±0.14	8.32±0.17	8.35±0.15	8.42±0.15	8.15±0.17	8.20±0.15	14.65**
TLC (× 10 <sup>3</sup> / μ l)	14.33±0.54	14.73±0.56	15.33±0.79	14.26±0.75	13.73±0.57	14.06±0.53	10.29**
Serum total protein (dl)	7.10±0.08	6.77±0.16	6.75±0.07	6.58±0.06	6.74±0.07	6.47±0.11	0.72 <sup>NS</sup>
ALT (U/L)	17.80±0.34	23.60±0.49	38.40±7.20	15.46±0.73	16.46±0.90	32.00±4.62	0.21 <sup>NS</sup>
AST (U/L)	62.46±1.32	62.33±1.03	58.33±6.12	56.33±1.50	46.53±1.99	40.00±3.49	0.30 <sup>NS</sup>

Values having ; \*\* =P<0.01; NS= Non Significant

very high count on 21<sup>st</sup> and 28<sup>th</sup> day post treatment (Table -1). These findings corroborate with the findings of Le Sueur *et al.* (2007) and Ruiz *et al.* (2011) wherein they recorded that Diclazuril @ 1 mg/kg b wt lowered faecal oocyst with the improvement of growth performance monitored at 2 weeks intervals for 6 consecutive weeks.

As evident from Table-2 the haematological values especially Hb, PCV and TEC increased slowly and progressively on 3<sup>rd</sup>, 7<sup>th</sup> and 14<sup>th</sup> day with a slight decline on 21<sup>st</sup> and 28<sup>th</sup> day post treatment. Similar observations were also recorded by Padmaja *et al.* (2006) and Tiwari *et al.* (2003). The mean values of TLC varied significantly at different time intervals in goats treated with Diclazuril. Similar findings were reported in sheep by Radha and Harikrishan (2006).

The total serum protein value was found to be continuously decreased till 28<sup>th</sup> day post treatment. Arora *et al.* (2003); Tiwari *et al.* (2003) and Kumar and Balsubramanium (2003) observed decreased serum total protein in coccidiosis. The values of ALT and AST varied non-significantly in goats treated with Diclazuril (Table-2). Similar observation was also recorded by Pandey *et al.* (2012).

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## REPORTS OF 31<sup>st</sup> ANNUAL CONVENTION OF ISVM HELD AT C.V.Sc. &A.H., MHOW

The 31<sup>st</sup> Annual Convention of ISVM and National Symposium were organized with theme “**Advancing Veterinary Medicine and its Specialities for Augmented Productivity and Health: Issues and Strategies in Farm and Companion Animals**” at College of Veterinary Science and Animal Husbandry, MHOW from **9-11<sup>th</sup> Jan. 2013**. The National Symposium was inaugurated in the gracious presence of **Dr Ajay Vishnoi**, Hon’ble Minister of Animal Husbandry, Government of Madhya Pradesh; **Dr. Govind Prasad Mishra**, Hon’ble Vice Chancellor, **Dr. R. P. S. Baghel**, Dean, Faculty of Vety. Sc. & A.H.; **Dr. S. N. S. Parmar**, Dean, Vet. College, Jabalpur; **Dr. S. P. Shukla**, Dean, Vet. College Rewa and **Dr. U. K. Garg**, Dean, Vet. College, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur; **Dr S. R. Srinivasan**, President, ISVM and **Dr. J. L.Singh**, General Secretary, ISVM.



During the National Symposium 11 different technical sessions, beside Inaugural and Valedictory Session were held. The technical sessions included:

1. Thematic Session
2. Animals Experimentation Vis-A-Vis Animal Welfare.
3. Animal Health with Application of Biotechnology
4. Avian and Rat Medicine
5. Non -Infectious Disease of Farm Animals
6. Infectious Diseases of Farm Animals
7. Diseases of Companion Animals
8. Equine and Pack Animal Medicine
9. Zoo and Wildlife Medicine
10. Poster Session
11. Continuing Veterinary Education Programme

Over **250 delegates/ participants** from different parts of the country attended the Symposium. A total of **375 research papers, 18 lead papers and 3 Continuing Veterinary Education deliberations** were presented during 3 day conference in different technical sessions.

The scientific sessions started with Thematic Session, in which the Key- note paper was presented by **Dr. S. R. Srinivasan**, President, ISVM, on the theme “**Advancing Veterinary Medicine and its Specialities: Where we stand?**” The speaker stressed that we need to collaborate on global scales for development to reach the unreached and ultimate advancement of animal and human welfare. Towards this goal, at least we can start the

Asian level Co-ordinations by proposing the need for establishment of an autonomous institute to cater to the needs of SAARC and Asian countries. He further, suggested that it may be started by recognizing Centers of Excellence in each country and fostering their collaboration and networking. It can be christened as The Asian Institutes of Veterinary Excellence.

In **Technical session-II** on “Animal Welfare Approaches in Animal Experimentation” a total 7 abstracts were included with lead paper on Sero-diagnosis of viral infections in companion animal.

During **Technical session-III** on “Animal Health with Application of Biotechnology”, 3 research papers were presented.

**Technical session-IV** was on “Diseases of Avian and Rat Medicine” where a total 22 research papers were presented. These papers were on different aspects of diagnosis and effect of treatment of various diseases of Poultry and Laboratory Animals like rats, etc.

**Technical session-V** was on “Non Infectious Disease of Farm Animals” where two lead papers were presented, viz. “Mineral Imbalances in Livestock and their Impact on Animal Health” by **Dr. Rajiv Singh** and “Latest trends in management of production diseases in dairy cattle” by **Dr. B. K. Bansal**. A total number of 68 research papers were also presented in this session.

**Technical session-VI** was on “**Infectious Diseases of Farm Animals**” in which two lead papers were presented on “**Scenario of Foot rot in sub-himalayan region and managemental strategies**” by Dr. V. S. Wazir and “**Recent Advances in diagnosis and management of chronic diarrhoea in dairy animals**” by Dr. Swaran Saran Randhawa. A total number of 114 research papers were presented in this session.

**The highest number of research papers was presented in the Technical session-VII on “Diseases on Companion Animals”. A total number of six lead papers were presented by various scientists, viz. Dr. A.P. Nambi, Dr. J. P. Varshney, Dr. K.M. Jadhav Dr. S. Prathaban, Dr. J. S. Soodan, & Dr. J. L. Singh along with 130 research papers on different aspects of diagnosis, case report, treatment of infectious and non-infectious diseases of dogs and cats.**

**Technical session-VIII** was on “**Equine and Pack Animal Medicine**”. In this session a lead paper on “**Common emerging ailments of pack animals and their management approach**” was presented by Dr. Mahesh Kumar. A Total 30 research papers were also presented during the session.

**Technical session-IX** was on “Zoo and Wildlife Medicine”. During this technical session, a lead paper was presented by **Dr. R. G. Jani** on the topic, “Present status and future strategies of EX-SITU and IN-SITU Conservation and health management of big cats in India”. A total number of 13 research papers were also presented during this session.

**Technical session-X** consisted of “Poster session” in which a total 27 posters were displayed by different scientists on the diagnosis and treatment of diseases of companion animals and small and large ruminants.

Each Technical session was conducted by a Chairman, Co-chairman and a Rapporteur. For the best paper presentation, awards were given to the presenters.

A **Continuing Veterinary Education programme** was also held as a part of this National Symposium and ISVM Convention. In this session, classroom lectures and practical demonstration on various techniques of diagnosis and treatment were included. The session was highly beneficial for the young faculties and post-graduate students of clinical subjects. They got the opportunity to learn the new skills and techniques for diagnosis and treatment. **Dr. G. Vijayakumar, Dr. J. P. Varshney and Dr. V. V. Rao** conducted this important technical education session in a befitting manner.

**The Intas Young Scientist Award Session** was held on the **10<sup>th</sup> January, 2013**. There were a total 12 participants / contestants in this prestigious session for young Scientists. Three contestants were adjudged as

winner with 1<sup>st</sup> and 2nd Runner. Awards of this Session were sponsored by the M/s Intas Pharmaceuticals Ltd., Ahmadabad

In the Plenary Session, the technical report of each and every session was presented by the rapporteurs of the respective sessions to evolve the Scientific Recommendation of the National Symposium.

**The following are the ultimate recommendations of National symposium and 31<sup>st</sup> Annual Convention.**

1. There is an urgent need for creation of a National body at par with ICAR/CSIR/ICMR for providing funds and guidelines on Veterinary and Animal Husbandry Education and Research in India, as well as providing service conditions for the Veterinary Professional.
2. Indian Society for Veterinary Medicine should take up Continuing Veterinary Medicine Education Programme.
3. Ethno- Veterinary Practices needs to be validated and popularized for their better use by the farming community
4. Laboratory and wild animal healthcare should be prioritized and the courses on these topics should be included in the syllabus of U.G. and P.G. teaching.
5. Experimentation on animals for research needs support and funding. CPCSEA should approve the projects within 3 months for the large animal experimentation, so that any proposed protocol of the research could be completed in time bound period.
6. Continuous Surveillance of the important Infectious Diseases of the livestock should be carried out to control the diseases incidence.
7. All the Teaching Veterinary Clinical Complex should be well equipped with the latest diagnostic facilities and there should be well equipped Critical Care Unit with specialists. Establishment of I.C.U. and C.C.U. in State Veterinary Polyclinics was strongly recommended
8. Veterinarians should be encouraged to develop skill and knowledge on equine Clinical practice in India. Training of faculty at NRC Equine, Hisser needs to be grade up urgently.
9. Strengthening of the academic /scientific staffs in Veterinary Colleges & Institutions in India should be made as a regular feature for catering the need of the quality based education.
10. Establishment /Provision for the veterinary laboratory technician training centre should be made in every states with similar training curriculum/contents
11. There should be uniformity in the set up of Veterinary Medicine Departments in all Veterinary Colleges of the Country.
12. National Research centre on Companion Animals should be established at national level to cater to the changing needs of the society.
13. Wildlife information network and data based on disease should be planned out. All India network project on Zoo Animals and Free Ranged wild animals needs to be initiated.
14. The Glanders & Farcy Act -1899 should be revised with a view to compensate charges and punishment.

At the end of the hectic Scientific Sessions of each day, an attractive, diversified, fully entertaining cultural program was organised by the students of the College of Veterinary Science and Animal Husbandry, MHOW, which was highly appreciated by all the delegates.

The valedictory function was organised at the last day of the Symposium i.e. 11<sup>th</sup> January 2013, in which **Dr. U. K. Mishra**, Hon'ble Vice Chancellor, C.G. Kamdhenu Veterinary University, was witnessed as Chief Guest of the function. The award ceremony was performed to facilitate various scientists on their outstanding work.

### ISVM AWARDS -2012

The following awards of the ISVM for the year 2012 were conferred in the Inaugural/ Valedictory function of the National Symposium at the College of Veterinary Sciences and A.H., MHOW, M.P.

S.N.	Award Category	Award Winner
1	Fellow of ISVM	Dr. A. P. Nambi, Prof. & Head, VCM, Chennai
2	Shri Ram Lal Agarwal Gold Medal	Dr. N. P. Dakshinkar, Prof.& Head , VCM, Nagpur
3	Dr. D. C. Blood Gold Medal	Dr. R. Ramprabhu, Prof. & Head, TVCC, Tirunelveli
4	Smt. P. Z. Sharma Gold Medal for Canine Medicine	Dr. C. N. Galdhar, Asstt. Prof, Bumbai
5	Dr. G. N. Dutta Memorial Gold Medal	Dr. R.G. Jani, Assoc. Prof., Anand
6	Dr. P. K. Das Gold Medal	Dr. V. V. Rao, Prof& Head, Gannavaram
7	Smt. Ava Roy Gold Medal	Dr. Usha Narayan Pillai, Assoc.Prof. VCM, Kerala
8	Dr. P.L.Narayana Rao Gold Medal	Dr. R. C. Patra, Prof. & Head Bhubaneshwar
9	Intas Young Scientist Awards	Winners: Dr. Jyothi Jatavath , Hyderabad1 <sup>st</sup> Runner: Dr. Latesh Bhagat, Pantnagar2 <sup>nd</sup> Runner: Dr. C.G. Umesh, Kerala
10	ISVM Merit Award for for P.G. Research (M.V.Sc.)	Dr. Sonal Srivastava, Jabalpur
11	ISVM Appreciation Award	Dr. K. S. Misraulia, Mhow Dr. Dr. K.G. Umesh, Banglore Dr. N. A. Tufani, Pantnagar Dr. Akhilesh Kumar, Izatnagar Dr. H. Vijay Kumar, Izatnagar Dr. Amit Prasad, Pantnagar /Junagadh
12	IJVM Best Clinical Article Award	“Efficacy of ayurvedic liniment againststicks of Sheep & Goats” Dr. K. Muraleedharan, Thrissur Dr. A. Sahadev, Tiptur, Karnataka
13	IJVM Best Research Article Award	“Serotyping of FMD virus from bovine tongue epithelium and Virus visualization using T.E.M.” Dr. Anand Mohan, Pantnagar Dr. Rajeev Kumar Dr. A.K. Upadhyay Dr. Mahesh Kumar Dr. Sumit Mahajan Dr. Arbind Singh Dr. Vipul Thakur



## **MINUTES OF ISVM ANNUAL GENERAL BODY MEETING HELD AT MHOW ON 10-01-2013 AT 10.00 AM IN THE C.V.Sc. & A.H. AUDITORIUM**

The 31<sup>st</sup> ISVM Annual General body meeting (AGM) was convened at 10.00 AM on 10-01-2013 in the auditorium of C.V.Sc. & A.H., Mhow. Total 96 life members of the ISVM participated in this AGM. As the quorum was not fulfilled, it was adjourned and again reconvened at 10.20 AM as per Clause no 2.7.1, Chapter 2 of Constitution and by-laws of the ISVM, to discuss the various agenda of the Society. The following ISVM life members (list enclosed) attended the meeting.

Dr. S. R. Srinivasan, President chaired the meeting. Dr. K. M. Jadhav, Vice-President and Dr. B. K. Bansal, Vice-President and Dr. J. L. Singh, General Secretary shared the dais.

At the outset, the president welcomed the life members present at the General body Meeting and discussed the following agenda items in detail and accordingly necessary decisions were taken.

### **A. Address by the President, ISVM:**

The president welcomed the life members and congratulated the ISVM Award winners. The AGM joined the president in the appreciation of award winners.

### **B. Report from the General Secretary, ISVM:**

General Secretary gave action taken report on the decisions of the last GBM held on 2/2/2012 at Aizawal and narrated the round the year activities of the Society, transition of the files and accounts from previous General Secretary. The AGM approved this report.

### **C. Report from Treasurer, ISVM:**

The Treasurer presented the financial status of the society and the AGM approved this report.

### **AGENDA ITEMS RECOMMENDED BY EXECUTIVE COMMITTEE**

### **D. Certain changes/Modification in the rules for the award of Dr. P.L. Narayana Gold Medal:**

The committee recommended that if the immediate past president has already received this Award, the Society may consider bestowing the award to the immediate past G.S. in the first year of the tenure of the Executive body. In such a situation, in the second year the candidature of the Editor/Assoc.Editor/ Treasurer shall be considered. In case the above recommendation is accepted by the A.G.M., Dr. R.C. Patra, Immediate past General Secretary may be awarded this award in this year.

**Minutes:** Approved the above decision along with inclusion of Vice-Presidents also in the second year of the award.

### **E. Deciding the venue of the next I.S.V.M. Annual Convention and National Symposium:**

It was informed by the president that an email from Dr. P. C. Alex, HOD, Deptt. of Veterinary Clinical Medicine, C.V.Sc. , Mannuthy, Kerala Veterinary and Animal Science University was received, conveying their interest to hold the next I.S.V.M. Annual Convention. The email conveyed the message that Vice-Chancellor, K.V.A.S.U. has approved his proposal

**Minutes:** As there were also requests from Jammu, Jabalpur and Ludhiana, the president sought the opinion of AGM and decided with majority support (with voice vote) that the College of Veterinary Science, Jammu would be the next venue.

It was also decided that from next time, the length of time lapse from the past Convention of ISVM at the place of request will be taken into consideration.

#### **F. Creation of State chapters of ISVM:**

President gave an account of the importance of allowing the creation of state chapters to emerge and informed that EC had accepted the proposal.

**Minutes:** Approved.

A committee was constituted comprising **Dr. J. P. Varshaney, Dr. S. Prathaban, Dr. R. C. Patra** and **Dr. H. U. Mallik** to come out with modus operandi for establishing such state chapters. **Dr. J. P. Varshaney** was nominated as the chairman and **Dr. R. C. Patra** as Convenor of this committee. The committee was requested to develop the blue print and its implementation strategies.

#### **G. Financial strengthening of the Society**

Appeal to HODs for advertisement in I.J.V.M and enrolment of the P.G. students as life member was suggested.

**Minutes:** Approved

#### **H. To decide honouring of the past Organising Secretary of 30<sup>th</sup> Annual Convention and National Symposium:**

It was resolved by the E.C. that the contribution of the 10% or more of the registration fee shall remain the criteria of the honouring of the past Organizing Secretary as per by-laws. However they said criteria may be relaxed by the E.C. in special cases like Organization of the Convention in some remote area or some untoward happening at the place of the organization of the Convention.

**Minutes:** Approved

#### **I. ISVM executive body election process:**

It was recommended that the ballot papers should be despatched by registered / speed post and election processing fee may be levied as follows:

**Presidential candidate, General Secretary & Treasurer:      Rs 2000 .00**

**For others:      Rs. 1000.00.**

**Minutes:**

It was resolved by the G.B. to levy the election processing fee to materialize the election process through registered post along with the following levy structure

**President, Vice-President and General Secretary:      Rs. 2000.00**

**For others:      Rs. 1000.00**

Moreover, General body also suggested for online / electronic voting system. Towards this goal, a committee on election reforms for ISVM was constituted comprising **Dr. Rajiv Singh, Dr. Rahul Srivastava, Dr. Prakash Bhatt, Dr. S. L. Ali and Dr. K. Vijayakumar** to come out with modus operandi for the future election process.

**Dr. K. Vijayakumar** was nominated as the Chairman and **Dr. Rajiv Singh** as the convenor.

#### **J. Institution of the Life Time Achievement Award to the distinguished services rendered by ISVM life members.**

President proposed institution of life time achievement award to distinguished services rendered by ISVM life member. EC discussed and recommend the proposal to GB. If accepted by GB, a committee may be constituted comprising **Dr. S. K. Misra, Dr. B. B. Verma, Dr. D. Swarup, Dr. P. Dhanapalan and Dr. S. Yathiraj** to work out the modalities. **Dr. S.K.Misra**, Founder President ISVM and **Dr. D. Swarup** will be,

respectively the Chairman and Convenor of this committee.

**Minutes:** Approved.

**K. Quarterly publication of the IJVM and quality improvement for increased journal rating and online journal release:**

The Editorial team expressed their view that there is paucity of good quality articles received in sufficient numbers and so that the decision of quarterly publication can be considered in future. Online publishing of ISVM Journal shall be made available to reduce the number of copies and related printing costs. The expenditure related to online publication of the journal shall be incurred by the society. The publication fee IJVM journal is to be charged as follow:

<b>Research article:</b>	<b>Rs. 1000.00</b>
<b>Short communication:</b>	<b>Rs. 800.00</b>
<b>Clinical Article:</b>	<b>Rs. 600.00</b>

**Minutes:** Approved

**L. Professional recognition to the members of ISVM through the award of “Diplomat, ICVIM” and establishment of Indian College of Veterinary Internal Medicine for this purpose.**

President has briefed about the international recognition of clinical excellence in the form of awarding the status of Diplomat of ACVIM / ECVIM and speciality practice boards throughout the world. The ISVM president underscored the necessity for similar one in our country and our society via the establishment of Indian College of Veterinary Internal Medicine (ICVIM) and Indian Boards of Speciality Practices (IBSP).

The EC had accepted and recommend the proposal to AGM.

If approved by AGM, a core group may be constituted with **Dr. S. R. Srinivasan, Dr. B. B. Verma, Dr. D. Swarup, Dr. A. Samad, Dr .K. G. Umesh , Dr. H. P. Dwivedi and Dr. P. Selvaraj** for formulation of the modalities.

**Minutes:** Approved. **Dr. S. R. Srinivasan**, President ISVM was nominated as the Chairman **and Dr. P. Selvaraj** as the convener. The committee was requested to come out with rules, regulations, processes, procedures and implementation strategies. The EC was advised to implement the same at the earliest

**M. Financial approval to meet the legal expenses on the Court Case over the Society:**

G.S. briefed that five ISVM life members from Ludhiana filed a suit in the **Court of the Addl. Civil Judge Senior Division at Ludhiana challenging the decisions of the ISVM GBM taken on 2/2/2012 at Aizwal and requested the approval of G.B.** to bear legal expenses connected with fighting the case. A sub committee comprising present and past President and G.S. and incumbent Vice -President **Dr. K. M. Jadhav**, was recommended to guide and monitor the legal matter.

**Minutes:** AGM approved the same as proposed, along with the remarks of the condemning this anti society activity, in the form of the Court case.

**N. Nomination of Overseas Secretary:**

EC recommended inducting **Dr. H. P. Dwivedi** as Overseas Secretary.

**Minutes:** Approved

**O. More weightage for the research relevant to the award:**

It was suggested that while forwarding applications for ISVM awards, the relevance to the award applies and

quality of the research work should be verified by the competent authorities / Head of Veterinary Medicine department in the serving organisation. Also, EC requested G.S. to verify the weightage and take necessary action to modify the same if necessary.

While discussing the agenda, the members came to know that in the previous GB, a committee comprised of **Dr. A. K. Gahlot** as Chairman, **Dr. S. N. S .Randhawa and Dr. S. Prathaban** as members was constituted to consider the similar agenda and hence pursuing the results of the above committee may resolve the issue raised in this agenda.

**Minutes:** Approved

**P. Continuing Veterinary Medicine Education (CVME):**

Society may permit the members to conduct CVME in collaboration with educational institutes / Govt. Agencies / private firms to arrange workshop / training / CVME etc. A registration fee can be fixed and the balance money if any in the account to be transferred to a dedicated CVME development account of ISVM. However, there should not be any financial commitment to the society.

**Minutes:** Approved

**Any other items:**

**Q. Increasing ISVM Fellowship numbers**

Many members expressed that there may be more than one award for ISVM Fellowship.

**Minutes:** It was resolved that two persons may be awarded if applications received are up to five and three persons may be awarded if the application received are more than five with a maximum of three per year.

**R. Honouring of the senior life members before their retirement.**

Members desired that senior life members should be honoured before their retirement.

**Minutes:** It was resolved that any retiring person should come forward 1 year in advance to intimate his / her retirement to the President or General Secretary, so that they will be honoured in the forthcoming ISVM Convention, before their retirement.

All the honourable members of the different committees are requested to submit the consolidated report within 3 months i.e. before 30th April, 2013, so that necessary action can be initiated at the Society level

**(J.L. Singh)**

## **ISVM Awards & Rules**

The members of the ISVM are requested to send their applications in quadruplicate (4 copies) for the below mentioned awards with all relevant supportive documents including photocopy for proof of age, date of enrollment as life members etc., as required for specific award, through their controlling officer/Head of the department verifying their credentials/contributions to the General Secretary, ISVM.

### **General rules applicable to all the awards:-**

1. Only members of ISVM are eligible to apply for any of the awards
2. Incumbent executive committee members of ISVM are not eligible for consideration of any of these awards, exception being FISVM.
3. Recipient of any ISVM award during last two years is not eligible to apply or to be considered for any of ISVM award
4. Persons applying for more than one award should indicate the preference order.
5. The minimum quorum for all the awards except FISVM is two.
6. The General Secretary, in consultation with the President, may reject application for any award for want of required quorum or incomplete application.
7. The General Secretary and President reserve all rights to accept/reject any application without assigning any reason for the same.
8. All the applicants will be required to submit half page write-up for the citation along with their original applications.
9. A sum of Rs. 250/- will be payable by the applicants as demand draft in favour of Indian Society for Veterinary Medicine, for each award applied as the processing fee to the ISVM.
10. The award may not be given for a year if an applicant does not get the qualifying minimum score.

### **1. SHRI RAM LAL AGRAWAL GOLD MEDAL**

The Gold Medal has been instituted by M/s Indian Herbs Research & Supply Co. Saharanpur (Uttar Pradesh) in the memory of its Founder, Late Shri Ram Lal Agrawal. The award shall be conferred annually upon a scientist who is a life member of the Indian Society for Veterinary Medicine in recognition of his/her distinguished work/contribution in the field of Veterinary Medicine. In every third year, the award shall be given to a scientist who has contributed significantly in the field of Indigenous Veterinary Medicine. The individual competing for this Gold Medal, should be of minimum 45 years of age as on 1st January of the year of evaluation.

### **2. INTAS YOUNG SCIENTIST AWARD**

The ISVM award silver trophy to a young scientist (life member of ISVM) below 32 years of age on 1st of January of the year of presenting his/her own research findings at the time of convention of ISVM. Besides the award, citation will also be given. Entries in duplicate of full length research paper typed in double space on bond paper with zerox copy of the proof of age should be sent on or before the last date prescribed to the General Secretary, ISVM along with the certificate from the Head of the Department, verifying the place of research work and year during which work was done. The paper to be presented for this award shall not find a place as Abstract in the proceedings of the convention. Only the author's name will be given who will present the paper himself/herself. The award in the form of trophy and a certificate to the selected young scientist shall be given at the Annual Convention in the same year. If the research work presented by the young scientist is the part of his/her post-graduate thesis, the same has to be mentioned clearly along with a certificate from the Advisor (Guide). The name of the guide will also figure in the award certificate. The executive committee may reject incomplete application/award for want of required quorum (minimum of 2 candidates). They also reserve all rights to accept/reject the application without assigning any reasons for the same.

### **3. DR. D.C. BLOOD GOLD MEDAL**

Dr. D.C. Blood Gold Medal will be awarded every year from the interest accruing on the corpus fund of Rs. 10,000/- deposited for the said purpose out of the savings of ISVM convention held at A.P.A.U., Hyderabad,

1990. The award will comprise a gold plated medal and a citation. The award is open for the life members of ISVM of the age above 32 and below 45 years as on 1st January of the year of evaluation.

**4. SMT. P.Z. SHARMA GOLD MEDAL FOR CANINE MEDICINE**

The Gold Medal will be awarded from the interest accrued on the corpus funds of Rs. 10,000/- deposited for the said purpose by Dr. S.Z. Sharma, Veterinary Clinic Sukhmani, TVPD Scheme, 10th Road, Mumbai-49 (Maharashtra). The medal will be awarded every alternate year from 1994 onwards (the date of commencement of award) to a life member of the ISVM in recognition to his/her outstanding contributions in the field of Canine Medicine during the last 5 years in the form of quality research and clinical publications, generating innovative ideas and development of newer techniques.

**5. S.K. MYLSAMY GOUNDER GOLD MEDAL FOR POULTRY MEDICINE**

The award is open for life members of ISVM. The applications indicating teaching, research and extension and other activities in the field of Poultry Medicine will be invited by the General Secretary every year and must be submitted by aspirants through respective Heads of Department/Office, verifying their credentials/contributions on or before the prescribed date.

**6. DR. G.N. DUTTA MEMORIAL AWARD**

This award will be conferred to an applicant who has completed 5 years as life member of ISVM and is of minimum 45 years of age as on 1st January of corresponding year. The award will be given in recognition of his/her meritorious research contributions during the last 10 years to the Veterinary Medicine especially in area of management of infectious diseases in animals.

**7. P. K. DAS GOLD MEDAL**

This award will be conferred to life member of ISVM in recognition of his/her outstanding contributions in the field of Clinical Toxicology during the last 5 years in the form of quality research and clinical publications, generating innovative ideas and development of newer techniques.

**8. AWARD OF FELLOWSHIP OF ISVM (FISVM)**

The life members of ISVM for the past 10 years are only eligible for the award of FISVM. ISVM Fellowship is awarded only to the members having master's degree in Veterinary Medicine/Preventive Medicine/Clinical Medicine and their designation should not be lesser than the rank of Professor. The fellowship is awarded to a person of high professional standing, who has rendered commendable service for the cause of Veterinary Medicine. The application for FISVM is required to be submitted along with comprehensive information about academic and professional achievements, distinguished published work/contributions in the field of Veterinary Medicine. A demand draft of Rs. 2000/- in favour of "Indian Society for Veterinary Medicine" must be enclosed with the application as the required fee for FISVM. The eligible ISVM members are requested to submit their application with detailed information under the following subheads: Academic and research achievements. Total particulars of employment; National and ISVM awards received; Total number of research and clinical publications (not popular articles) in peer reviewed Indian and Foreign Journals (enclose the list); List of published reviews in Journals and international proceedings/books; Books/Monographs published; Number of research and clinical publications relevant for specific award (enclose list); Contributions to the advancement of Veterinary Medicine; List of papers presented in international conferences/symposium held abroad and any other scientific achievements not covered in above cited information.

**9. FIELD VETERINARIAN AWARD**

- i) The Field Veterinarian Award shall be given to a field veterinarian who is a life member of ISVM.
- ii) The award shall be given in the form a plaque and certificate at ISVM convention based on the oral presentation by the applicant in National Symposium.
- iii) The presentation shall be judged by a committee of three members nominated by the executive committee for this purpose.

**10. ISVM MERIT AWARD FOR POST GRADUATE RESEARCH:**

There shall be two ISVM Merit Awards annually – one for a student pursuing PhD .degree in the discipline of

Veterinary Medicine and one for a student pursuing MVSc. degree in the discipline of Veterinary Medicine (Veterinary Clinical / Preventive Medicine). The value of award for a Ph.D. and M.V.Sc student shall be Rs.5000/-andRs.4000/-respectively to be given once during the degree programme of the student. The applications for the award must reach the office of ISVM by (stipulated date) duly recommended by the guide and forwarded by the Head of the Department concerned.

#### **11. BEST CLINICAL ARTICLE AWARD**

It will be awarded to the best clinical article published in the Indian Journal of Veterinary Medicine during the year immediately preceding the year of annual conference of ISVM. The articles will be sent to three Judges by the Editor and based on the score; the best article will be selected and placed before the executive committee members for approval. The award will be in the form of a plaque and certificate to the first author, and certificates to co-authors to be given every year at the ensuing ISVM convention. 4. The criteria for scoring marks shall be as follows: Title 5 marks; Materials & Methods 10 marks; Results & Discussion 10 marks; Contribution to Science 5 marks

#### **12. BEST RESEARCH ARTICLE AWARD**

It will be awarded to the best full length research article published in the Indian Journal of Veterinary Medicine during the year immediately preceding the year of annual conference of ISVM. The research articles will be sent to three Judges by the Editor and based on the score; the best research article will be selected and placed before the executive committee members for approval. The best research article award will be in the form of a plaque and certificate to the first author, and certificates to co-authors to be given every year at the ensuing ISVM convention. The criteria for scoring marks shall be as follows: Title 5 marks; Materials & Methods 10 marks; Results & Discussion 10 marks; Contribution to Science 5 marks

#### **13. ISVM APPRECIATION AWARD**

The ISVM Appreciation Award will be given to a life member of the Society. It will carry a citation and plaque of honour. The proposal for the award with justification will be made by the President, General Secretary, Organizing Secretary and Editor/Associate Editor during the executive committee meeting at the time of annual convention on the basis of services rendered by a life member for the betterment of ISVM/IJVM affairs. The executive committee will approve a maximum of 4 persons each year for this award from the names proposed as above.

#### **Award Application procedure**

The eligible members are requested to submit application for the awards/fellowship (SerialNo.1 to 10) with detailed information under the following subheads with detail description on specific need of the individual award: Academic and Research Achievements; Total particulars of employment; National / international and ISVM awards received Total number of research and clinical publications(not popular articles); in peer reviewed Indian and Foreign Journals(Enclose the list); List of published reviews in journals and international proceedings/books; Books/monographs published; Number of research and clinical publications relevant for the specific award(Enclose the list); Contribution to the advancement of Veterinary Medicine; List of papers presented in the international Conference & Symposia

#### **Remark Note:**

- (i) Members of the incumbent executive committee of ISVM are not eligible for consideration of any of these awards except for Fellow ISVM.
- (ii) Recipient of any of the ISVM awards during last 2 years is not eligible to apply/or be considered, for example, any person getting the award in 2005 shall be eligible to apply in 2008 and so on.
- (iii) Person applying for more than one award should indicate preference order.
- (iv) Person competing for any of these awards cannot become member of any of the Judging Committee

## GENERAL GUIDELINES FOR CONTRIBUTORS

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.

The manuscripts are accepted on the basis of scientific importance and suitability for publication on the understanding that they have not been published, submitted or accepted for publication elsewhere wholly or partly in any language. All authors are jointly and severally responsible to the various authorities for the contents of the articles. The Editorial board shall in any case not be held responsible in any manner whatsoever to the contents of the article and the views and interpretations expressed by the authors in the articles.

In case the research work includes experimentation on animals, authors has to submit a certificate that the work carried out is with the approval of the **Institutional Ethics Committee or as per the laws in force in the country in which it has been conducted.** A certificate to this effect should be signed by corresponding author on behalf of all the authors. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

**For a article to be published in IJVM it is mandatory that atleast one of the author should be a life member of the Indian Society for Veterinary Medicine.** In case none of the author (s) is permanent member of the Indian Society for Veterinary Medicine he/she may apply for the permanent membership to “The General Secretary, Indian Society for Veterinary Medicine” in a prescribed format given at end of the journal.

The official language of journal is English. The articles should be sent to “**The Associate Editor, Indian Journal of Veterinary Medicine, Division of Medicine, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, U.P. (India)**” along with a self addressed envelope of A4 size having postage tickets of Rs 30 for further correspondence. The manuscript should be typewritten in A4 size paper in Times New Roman, font size 12 on one side of the paper with wide margins (2.5 cm all around the page) and double spacing throughout the article except in abstracts, footnotes and references which should be in single spacing. **It should be sent in duplicate.** Each page of the manuscript should be numbered on the top corner including title page, references, tables, etc. All the pages should contain running title of the paper at the top.

Article once received will be allotted a registration number and will be send to reviews and on acceptance/rejection will be send back to the corresponding author for modification if any. The author(s) should revise and modified the article in light of the recommendation of the reviewer and the editorial board and should adhere to the format of the journal (follow instruction to authors as given below). The revised article (one hard copy) along with a soft copy in CD or as an attachment to email id [ijvmisvm@gmail.com](mailto:ijvmisvm@gmail.com) should be submit to “The Associate Editor, Indian Journal of Veterinary Medicine, Division of Medicine, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, UP (India).

Authors are requested also to return the original version along with original comments of the reviewer to the editorial office for reference and records. The modified articles should be submitted to editorial office within 30 days of receipt, failing which the publication of article may be delayed. A demand letter will be sent to the corresponding author for payments of processing and publication fee of the article. Only on receipt of full payments, the article will be taken up for publication and the author will be informed accordingly.



**The manuscript should be organized in the following order in general:**

1. **Title Page:** Should be typed on separate page contain full title of the article, name of the author(s) along with their affiliation, name of the place (Department, College, University etc.) where work was done. Name of the corresponding author, complete postal address including Pin-code along with phone number and the email address at the bottom of the page.
2. **Manuscript:** In general should be arranged as follows: (Contributors should take care that name of the author(s), their affiliation and the institution name should not be included in this section and only be mentioned in the title page only.)
  - A. **Title:** Title of the article should be clear, self descriptive in nature and should not contain abbreviation or symbols
  - B. **Abstract:** Abstract should not exceed 300 words and should outline briefly the purpose of the study, important findings and conclusions. Repetition and generally known information should be avoided.
  - C. **Key words:** 4 to 5 Key word.
  - D. **Introduction:** No subtitle should be given and briefly state the nature and purpose of the work together with the important findings of previous workers.
  - E. **Materials and Methods:** The author(s) should describe materials, methods, apparatus, experimental procedure and statistical methods in detail to allow other workers to reproduce the results. Sub-heading may be used in this part.
  - F. **Results:** The experimental data should be presented clearly and concisely. Information presented in tables and figures should not be repeated
  - G. **Discussion:** This should focus the interpretation of experimental findings along with reasoning. Do not repeat data presented in the introduction or information given in the result. References in this part should be cited as follows.....as observed by Kumar *et al.* (1984) or in parentheses..... were found (Dwivedi *et al.*, 1983; Singh and Singh, 1984). At last each article should have definite interpretation with research findings.
  - H. **Acknowledgement(s):** This should be short. Grants and technical helps provided should be acknowledged.
  - I. **References:** All publications cited in the text should be presented in the form of a list of references arranged alphabetically according to authors' surnames. Don't give serial numbers. Use the following system for arranging the references.
    - 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 numerical. 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	l
Calory	cal	Meter	m
Centimeter	cm	Microlitre	μl
Counts per minute	cpm	Milligram	mg
Cubic centimeter	cm <sup>3</sup>	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 <sup>2</sup>
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 important clinical observations of the case, tentative diagnosis and its confirmation, line of treatment used and fate of the case. At last, it should have a brief discussion on the line of 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.

### **Processing and publication fee**

*Indian J. Vet. Med.* charges article processing and publication fee per accepted article as following:

<b>Research article</b>	<b>:</b>	<b>Rs. 1000 per accepted article</b>
<b>Short communication</b>	<b>:</b>	<b>Rs. 800 per accepted article</b>
<b>Clinical article</b>	<b>:</b>	<b>Rs. 600 per accepted article</b>

**Note:** The decision of the Editor is final in all matters pertaining to the publication of the articles. No reason shall be given for the non-acceptance of the article. Editor/editorial board has the right to do final editorial revision of the accepted articles, restriction of number of pages, tables and figures.



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**MEMBERSHIP FORM**

**THE INDIAN SOCIETY FOR VETERINARY MEDICINE (ISVM)**

**(Registered under Society Act 21 of 1860)**

**OFFICE: Department of Veterinary Medicine, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar- 263 145, U. S. Nagar, Uttarakhand (INDIA)**

**Ph. (0) 05944-233066, (R) 05944-234348, Fax. 05944-233473, Mob-09410119561, Email:**

**drjlsingh@rediffmail.com**

I, Dr. \_\_\_\_\_ wish to enroll myself as a Life Member of ISVM by paying the prescribed membership fee of Rs.1500.00 (Fifteen hundred rupees only) to the Society and declare that I would not indulge in any activity subversive to ISVM. Following are my brief particulars which are true to the best of my knowledge.

1. Name: \_\_\_\_\_

(Print in BLOCK Letters, SURNAME first)

2. Date of birth \_\_\_\_\_

3. Educational Qualification \_\_\_\_\_

4. Home address \_\_\_\_\_  
\_\_\_\_\_

Pin \_\_\_\_\_ Telephone (with STD code) \_\_\_\_\_ Send one spare copy of photographs.

5. Mailing address \_\_\_\_\_  
\_\_\_\_\_

City \_\_\_\_\_ Pin Code \_\_\_\_\_ State \_\_\_\_\_

Telephone (with STD Code) \_\_\_\_\_ Fax \_\_\_\_\_ Email: \_\_\_\_\_

6. Official designation: Post \_\_\_\_\_ Organization \_\_\_\_\_

7. Professional accomplishment:

a) Service experience (beginning with current position) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

b) Honours (Citations, Awards, fellowship: give any best four)

i. \_\_\_\_\_

ii. \_\_\_\_\_

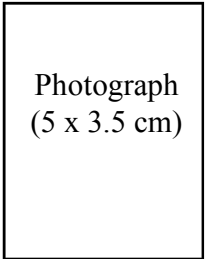
iii. \_\_\_\_\_

iv. \_\_\_\_\_

c) Membership in professional organisations (give position if any)

i. \_\_\_\_\_

ii. \_\_\_\_\_



iii. \_\_\_\_\_

iv. \_\_\_\_\_

(d) Scientific publications (Give No. only)

i. Research \_\_\_\_\_ (Indian J.) \_\_\_\_\_ (Foreign J.) \_\_\_\_\_

ii. Popular \_\_\_\_\_ iii) Books/Monographs \_\_\_\_\_

e) No. of post graduate students guided

i. M.Sc./MVSc. \_\_\_\_\_

ii. PhD/DSc. \_\_\_\_\_

f) Any other relevant information (s) \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

I am enclosing a demand Draft of Rs. 1500.00 in favour of Indian Society for Veterinary Medicine, payable at Pantnagar, Branch of SBI (Code No. 01133) for the above purpose. (Strike out if not required)

Date: \_\_\_\_\_

Signature of Applicant: \_\_\_\_\_

Name: \_\_\_\_\_

Address: \_\_\_\_\_

**Recommendation by a life member of ISVM**

I am recommending the name of Dr. \_\_\_\_\_  
for consideration as a life member of the society.

Date: \_\_\_\_\_

Signature of recommending life member of ISVM: \_\_\_\_\_

Name: \_\_\_\_\_

Address: \_\_\_\_\_

**(For Secretariat records)**

Membership of Dr. \_\_\_\_\_ is accepted/could not accepted  
because \_\_\_\_\_ and his/her name has been enlisted  
in the state of \_\_\_\_\_ at serial  
no. \_\_\_\_\_.

**General Secretary**

**Treasurer**

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