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Blue  
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Book*

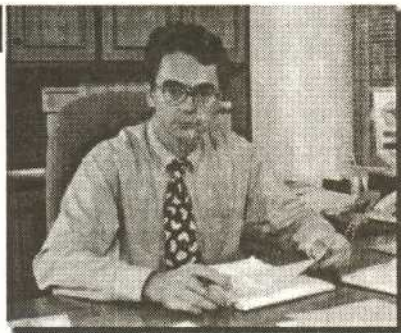
for the Veterinary Profession



**Hoechst Roussel Vet**

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## PREFACE



**W. J. Bader**  
Managing Director  
Hoechst Roussel Vet Pvt. Ltd.

Dear Sirs,


It gives me great pleasure to hand over another issue of *"The Blue Cross Book"* to you. I hope, you will like it. Your comments / suggestions shall help us to consolidate the status of this journal which is mainly dedicated for the veterinary professional in the country.

Please note, we have started a new column entitled, "The Pioneer Veterinary Institute in India". In this issue, we shall highlight the contribution of "Bombay Veterinary College", in the field of veterinary sciences. This will be followed by other pioneer institutes in the country. Your comments on the column will be highly appreciated.

Lastly, I would request you to send your FIELD REPORT / CLINICAL OR REVIEW ARTICLES which may kindly be addressed to :

**Dr. A. K. Datta**  
Editor,  
*"The Blue Cross Book"*  
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Best wishes,



**Jochen Bader**

## GUIDELINES TO CONTRIBUTORS

*"The Blue Cross Book"* is published biannually. The contributions to the journal are accepted in the form of invited review articles, research articles (clinical / field studies), case reports, other information pertaining to animal health and production. The decision of the Editorial Board members will be final regarding acceptance of the article for publication. The manuscript should be typed on one side of the paper with double spacing except for footnotes and references for which single spacing be used. The style of reference citing should be stickly followed as shown below. The words to be printed in Italics should be underlined. The manuscript should be arranged in the following order :

- Title** : Note on outbreak of Pox in Sheep
- Name/s of author/s** : K. Kumari, P. C. Chowdhri and P. K. Das
- Place of work** : Department of Pharmacology  
Bombay Veterinary College, Parel, Mumbai
- Materials and Methods** : In details.
- Results and Discussions** : With the help of tables / figures etc.
- References** : **For Periodicals** : Name and initials of author/s, year of publication in parenthesis, abbreviated title of journal, volume number, first and last page number/s.  
e.g. Chhabra, D., Moghe, M. N. and Tiwari, S. K. (1996). *Ind. Vet. J.*, **82** : 1-3.
- : **For Books** : Name/s of author, year of publication in parenthesis, title of the book, edition, name of publishers and page number/s.  
Radostitis, O. M., Blood, D. C. and Gray, C. C. (1994). *Veterinary Medicine*, **8th Edn.**, *ELBS*, London
- Tables and Figures** : Tables are to be numbered in Roman numerals (I & II so on). each table should have a clear title. Figures should be good quality and numbered in Arabic numbers (1, 2, 3 so on).

Abstracts and sub-headings are not necessary for clinical articles and short communications. These should not exceed three typed pages. For case reports, history, observation, tentative and confirmatory diagnosis, line of treatment and follow up on the case should be given.

**Authors are requested to indicate that the paper has not been published elsewhere.**

All manuscripts should be mailed to the following address :

**DR. A. K. DATTA**, Editor, *"The Blue Cross Book"*, Hoechst Roussel Vet Pvt. Ltd., Neeta Park, Airport Road, Yerwada, Pune : 411 006. Tel.: +91-212-686623/71/95, 686704 Fax : 91-212-686593/ 98



## THE PIONEER VETERINARY INSTITUTE IN INDIA

### Bombay Veterinary College

The year 1886 had a great significance for the Animal Health development and advancing veterinary science in India and also in Asia. On the 2nd of August, 1886, an institution devoted to animal health and welfare, advancing veterinary sciences was established in the name of Bombay Veterinary College at Parel village, Bombay (Mumbai). Prof. J. H. Steel, B.Sc.,



**Prof. J. H. Steel,**  
B.Sc., F.R.C.V.S., F.Z.S., J.P.  
First Principal,  
1886-1891

and Rangoon Veterinary Colleges started afterwards and began to impart high education in English. Other veterinary colleges were also opened in Calcutta, Madras and Patna. The Bombay Veterinary College can therefore, rightly be called the pioneer of Veterinary Education in India.

and Rangoon Veterinary Colleges started afterwards and began to impart high education in English. Other veterinary colleges were also opened in Calcutta, Madras and Patna. The Bombay Veterinary College can therefore, rightly be called the pioneer of Veterinary Education in India.

#### Education

In the initial stage, the college had three year diploma course (G.B.V.C.) in Veterinary Science after matriculation. In the year 1945, the college was affiliated to Bombay University for the award of B.Sc. (Vet.) degree. In 1958, the degree was changed from B.Sc. (Vet.) to B.V.Sc. In 1960, the post-graduate courses leading to Master's degree (M.V.Sc.) were introduced. The post-graduate teaching programme was further strengthened by introduction of Doctoral degree (Ph.D.) by research. The undergraduate syllabus underwent further modifications in 1963 and the degree of B.V.Sc. was again changed to B.V.Sc. & A.H. Since 1996 the common syllabus formed by the Veterinary Council of India has been accepted and with this the course is further extended to a total period of five years including six months' Internship training programme.

For some years Bombay Veterinary College continued to supply fully trained veterinary practitioners for all parts of India, Lahore

## Research

The college has the best tradition of excellent need-based and practical oriented research work. Extensive work in relation to the detection of subclinical mastitis in farm animals and practical-oriented work pertaining to fluoresis was carried out. Research on several viral and bacterial diseases like Mucosal Disease Complex, Rinderpest, Foot and Mouth Diseases, Gumboro, have also been carried out. A separate Radio Isotope Laboratory was established and studies pertaining to diagnosis of fertility and infertility including hormonal estimations are being undertaken to help farming community. The college was the first to identify the Gumboro disease virus in poultry. Studies on management aspects particularly in relation to the heat stress, salt toxicity, housing system and effect of various energy levels on economic traits in growing and laying birds were also undertaken.

Development of technology in the manufacture of insulin and heparin from buffalo pancreas and lungs was successfully completed and the scientists earned the Best Team Research Award by I.C.A.R. Extensive research work on slaughterhouse by-products, project was undertaken. There is also much work on the nutritive evaluation of feeding of animals. The financial assistance of World Bank and the I.C.A.R. under the NARP on "Complete Ration" in cattle is in progress under the Network programme. Work on basic aspects pertaining to the embryo transfer technology is also being undertaken and is in progress under the Network programme. From the financial help of different agencies, work was undertaken on the establishment of Microbial standards for meat and meat products, microbiological quality of milk and milk products, meat speciation and

detection of adulteration of meats, preservation of meats and by-products development

## Extension

The Bombay Veterinary College is carrying out extensive extension programmes. Transfer of technology, technical inputs, lab-to-land programmes are undertaken on the three-tier basis. The three main beneficiaries are farmers, field veterinarians and industry concerning with animal husbandry. Short term courses are organised for women, tribal people, beneficiaries of IRDP in adopted villages. Regular demonstrations are organised on farmer's field to demonstrate the utility of improved technology.

The college is organising orientation courses, refresher courses on poultry farming, goat farming, dairy farming, artificial insemination, disease diagnosis, feeding of animals, etc. for the farmers, field veterinarians with the recent advances in the field of animal sciences.

The college was started as a pioneer institution of its kind and it can be said without any fear of contradiction that even today it occupies a unique position in India in regards to high standard of education which is made possible by the qualified and trained staff members. The institute has excellent hospital facilities for animals, provided by S.P.C.A. and Laboratory support from some of the biggest medical institutions which exist in its close proximity.

# Comparison of Electrocardiographic Abnormalities in Xylazine / Detomidine Premedicated and Ketamine Anaesthetised Goats

D. Dilip Kumar, A.K. Sharma and O.P. Gupta

Division of Surgery, IVRI, Izatnagar, U.P.

## Introduction :

Xylazine sedation was associated with premature ventricular contraction (Clark *et al.*, 1982) and varying degree of A.V. block in dogs (Haskins *et al.*, 1986). This paper reports ECG abnormalities in xylazine / detomidine premedicated and Ketamine anaesthetised goats.

## Materials and Methods

Twelve goats of either sex, aged 18-24 months and weighing 15-20kg were randomly divided into two groups of six animals each. All animals were dewormed with Fenbendazole (Panacur, Hoechst Roussel Vet) @ 5mg /kg b.w. orally, one month prior to experiment. The animals of both the groups were kept off feed and off water for 24 and 12 hours respectively before administration of test drugs.

**Group I :** Atropine sulphate (Hoechst Roussel Vet) was given @ 0.66mg/kg b.w. subcutaneously. It was immediately followed by Detomidine hydrochloride (Domosedan-Formas Group Ltd., Finland) @ 0.22mg / kg b.w. intramuscularly). Ten minutes after Atropine and Detomidine administration, Ketaminehydrochloride (Parke-Davis) was injected @ (11mg/kg b.w. intramuscularly)

**Group II :** Atropine Sulphate (Hoechst Roussel Vet) was given @ 0.66 mg/kg b.w. subcutaneously. It was immediately followed by xylazine hydrochloride (Bayer, Germany) injection @ 0.22mg/kg b.w. intramuscularly. Ten minutes later Ketamine hydrochloride (Parke Davis) was given as in Group I.

Electrocardiograms were recorded with the help of cardiart 308 (BPL India) on base apex, at different intervals viz. just before administration of drugs and at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150 and 180 minutes after administration of drugs.

## Results and Discussion :

There was no ECG abnormality in the animals where atropine-xylazine-ketamine combination was given whereas, in atropine-detomidine-ketamine administered animals, one animal showed ECG abnormality viz. Ventricular trigeminy (for every two normal heart beat there was one premature ventricular beat).

Xylazine or detomidine when used alone produce ECG abnormalities (Fischer, 1986). The ECG abnormalities of these drugs can be modulated by combining with drugs like atropine (Kumar and Thurmon, 1979) and Ketamine (Kumar *et al.*, 1979). In the present study atropine and Ketamine were able to modulate the ill effects of xylazine completely. However, these drugs failed to modulate ECG abnormality completely in detomidine administered animals. Indicating detomidine is more powerful arrhythmogenic agent and this observation confirms the findings of Dyson *et al.* (1987)

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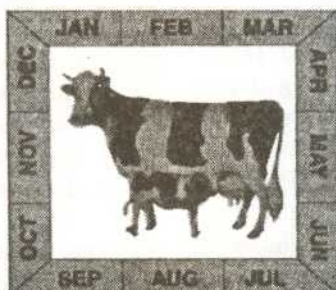
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# Pre-treatment with GnRH Analogue (Receptal®) Enhances Synchronization Efficiency of Prostaglandin F<sub>2α</sub> in Ewes

N. Prakash, D. Deshpande\*, M. M. Appannavar\*\* and V. B. Shettar\*\*

Department of Pharmacology and Toxicology, Veterinary College, Bidar

## Introduction :

Oestrus synchronization is one of the preliminary steps in any kind of reproductive manoeuvre, especially in sheep where herd mating is in vogue. Several methods have been tried using various pharmacological agents viz. progesterone implants (Selvaraju, *et al.*, 1997) and prostaglandins (Deshpande, 1997). The most commonly used method is double dose PGF<sub>2α</sub> administered 11 days apart. Prostaglandins, being luteolytic agents do not have any influence on follicular activity. Hence, the present study was conducted to test the effect of GnRH administration prior to PG (Prostaglandin) induced luteolysis on the synchronization response in ewes.

## Materials & Methods :

A total of thirty ewes were divided into two groups of fifteen each. Ewes in group I were administered a synthetic PG (prostaglandin) analogue, cloprostenol (Estrumate® Coopers, UK) at a dose of 100 µg per animal intramuscularly. Two injections were given 11 days apart. Ewes in group II were administered synthetic GnRH, Buserelin acetate (Receptal®, Hoechst Roussel Vet Pvt. Ltd.) at a dose of 4 µg per animal intramuscularly. Seven days after the GnRH treatment they were administered PG (Prostaglandin) analogue, Cloprostenol (Estrumate®) at a dose of 100 µg per animal intramuscularly. All the animals were observed for oestrus using aproned rams and

by clinico gynaecological examination. They were confirmed in oestrus by plasma progesterone assay. The oestrus response was grouped as : <24, 48, 72, and 96hrs and above.

Blood samples were drawn from all the animals before PG (Prostaglandin) administration and on the day of oestrus by jugular venipuncture. Plasma samples were separated and stored at -20°C till further use.

The plasma samples were extracted in hexane and subjected for progesterone assay in duplicate by radio immunoassay (Prakash, 1989 and Deshpande, 1997) and validated as per Prakash, (1989).

The concentrations were calculated by extrapolation from the standard curve.

The plasma samples were extracted in hexane and subjected for 17β oestradiol assay by radio immunoassay (Prakash, 1989).

## Results :

The number of animals exhibiting oestrus in group I and group II have been presented in Table 1. In group I, 2 (13%) ewes exhibited oestrus at 48 hrs. while 9 ewes (60%) at 72 hrs. and 2 (13%) took 96 hrs. and above. The minimum time taken was 48 hrs.

In group II, 3 (20%) ewes exhibited oestrus before 24hrs. and 7 (47%) before 48 hrs. while 3 (20%) showed oestrus at 72 hrs. and 2 (13%) took more than 96 hrs.

\* Department of Physiology, Veterinary College, Bihar

\*\* Department of Animal Genetics and Breeding, Veterinary College, Bihar

The total percentage of ewes which came into oestrus was 87% in group I and 100% in group II.

#### Plasma progesterone and 17 $\beta$ oestradiol concentration

The mean plasma progesterone and 17 $\beta$  oestradiol concentrations in group I and II have been presented in Table II.

Mean plasma progesterone concentration was  $0.80 \pm 0.02$  ng/ml in group I and  $1.13 \pm 0.04$  ng/ml in group II before PG injection. The progesterone declined to  $0.035 \pm 0.02$  ng/ml and  $0.360 \pm 0.23$  ng/ml respectively on oestrus day. The decline was significant ( $p < 0.05$ ) in both the groups.

The mean plasma oestradiol concentration was  $3.0 \pm 0.50$  pg/ml in group I and  $2.75 \pm 0.25$  pg/ml in group II before PG (Prostaglandin) injection. On the day of oestrus the concentration was  $14.54 \pm 1.03$  pg/ml in group I and  $15.38 \pm 1.48$  pg/ml in group II. Within the groups the concentration was significantly ( $p < 0.05$ ) higher on the day of oestrus and the values did not differ significantly ( $p > 0.05$ ) between the groups.

#### Discussions :

GnRH (Receptal<sup>®</sup>) administration has induced oestrus early compared to double

dose PG (Prostaglandin) treatment. This could be attributed to follicular stimulating action of the GnRH which would have been enhanced before PG administration. Gonadotrophins are the key hormones in the control of recruitment, selection and dominance during folliculogenesis (Driancourt, 1991). The endogenous GnRH having short half-life is being removed rapidly from circulation and follicles lose their support.

In the present study, the GnRH administered would have initiated the recruitment of the follicles. Buserelin acetate (Receptal<sup>®</sup>) being synthetic product, would sustain release of the gonadotrophins. Once recruited the preantral follicles are dependent on FSH support for continued development (Richards, 1980). Alternatively, the exogenous GnRH would have enhanced the growth of already recruited follicles and would have supported their development.

Ovulatory follicle in ewes is derived from a pool of follicles greater than two millimeters in diameter at the time of luteolysis (Driancourt and Cahill, 1984) and ovulatory follicles emerge as growing, oestrogenic follicles within 10 hrs. of luteolysis (Mc Natty *et al.*, 1982 and Webb *et al.*, 1989). Hence, GnRH appears to have increased the pool from which the ovulatory follicle could be selected early at the time of luteolysis

**Table I : Shows Number and percentage of ewes exhibiting oestrus at different intervals after PG (Prostaglandin) administration in group I and II.**

Treatment	<24hrs.	48hrs.	72hrs.	96hrs. & above	Total
Group I (n=15)	0 (0.0)	2 (13%)	9 (60%)	2 (13%)	13 (87%)
Group II (n = 15)	3 (20%)	7 (47%)	3 (20%)	2 (13%)	15 (100%)

n : number of animals.

**Table II : Shows mean plasma progesterone (ng / ml) and 17 $\beta$  oestradiol (pg/ml) concentration in ewes of group I and II before and after PG administration.**

Animal Group	Progesterone (ng / ml)		17 $\beta$ oestradiol (pg/ml)	
	Pre-injection	Day 0	Pre-injection	Day 0
Group I (n=15)	0.80 $\pm$ 0.02*	0.035 $\pm$ 0.02	3.00 $\pm$ 0.50*	14.54 $\pm$ 1.03
Group II (n=15)	1.13 $\pm$ 0.04*	0.360 $\pm$ 0.23	2.75 $\pm$ 0.25*	15.38 $\pm$ 1.45

n : number of animals  
ng/ml : nanogram per ml

\* = (P<0.05) within rows.  
pg/ml : Picogram per ml.

compared to double PG (Prostaglandin) regimen.

FSH induces aromatization in-vivo and increases estrogen synthesis (Driancourt *et al.*, 1985). It appears that GnRH administered would have augmented rate of oestrogen synthesis in the follicles thereby reducing the time required for follicular maturation resulting in early onset of oestrus.

Ovulatory response is linked to relative age of the follicle at luteolysis (Houghton *et al.*, 1995) and changes in granulosa cells, concentration of steroids in follicular fluid and specific binding of FSH for oestrogen active and oestrogen inactive follicles are the characteristics of changes in ovulatory and non ovulatory follicles respectively (Ireland and Roche, 1982). This indicated that GnRH would have increased oestrogen in follicular fluid and the follicles would have been made sufficiently old enough at the time of luteolysis.

Luteolysis is the critical factor for complete maturation of follicles (Baird and Mc Neilly, 1981). Removal of corpus luteum (Dufour *et al.*, 1971) or luteal regression (Deaver *et al.*, 1986) at any stage of oestrus cycle results in the induction of oestrus in ewes.

Partly matured follicles would enter final maturation if GnRH is administered before luteolysis and hence, it would take less time to induce oestrus in ewes. The difference in the onset of oestrus in different animals is attributed to the difference in the day of oestrous cycle of different animals at the time of PG administration (Acritopoulou and Haresing, 1981).

Similarly, stimulatory effects of GnRH have been reported in other species. GnRH administration during anestrus has induced oestrus in buffaloes (Nasr *et al.*, 1983; Nautiyal *et al.*, 1997) and "Receptal<sup>®</sup>" has been used in management of reproductive problems in bovines (Mujumdar, 1989). GnRH given along with PMSG (Pregnant Mare Serum Gonadotropin) has improved the ovulation rate and incidence of anovulatory follicles in buffaloes. (Sodhi *et al.*, 1997).

### Conclusion :

GnRH (Receptal<sup>®</sup>) administration before PG (Prostaglandin) injection enhances the synchronization efficiency by early onset of oestrus in ewes. This ascertains the follicular activity along with synchronization which will benefit fertility in ewes.

### Acknowledgement :

Authors are thankful to SGR and HNK, Primate Research Lab, IISc, Bangalore for providing RIA facility. Constant encouragement from the Director (Vety.) and Director of Research (UAS, Dharwad) is gratefully acknowledged.

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If you can't make a mistake,  
you can't make anything

- Marva Collins

# Efficacy of Floxidin (Enrofloxacin) in the Prevention of Chronic Respiratory Disease (CRD) in Broiler

V. S. Narsapur\* and A.K. Datta\*\*

\* DOLCOS, Veterinary Pathology Laboratories, Jogeshwari, Mumbai

## Introduction :

The third generation quinolone, Enrofloxacin is available worldwide as oral solution for poultry use. A major claim of this product is the therapy of *Mycoplasma* infection and secondary respiratory colibacilliosis in poultry. Kempf *et al.*, (1989) showed that Enrofloxacin is highly effective in reducing the level of egg transmission of *Mycoplasma gallisepticum* in layer breeder chicken. Stipkovits (1988) reported that experimentally induced infection with *M.gallisepticum* and *Escherichia coli* in chicks, treated with Enrofloxacin (Baytril), Tiamulin and Tylosin showed that Enrofloxacin is a much better antibiotic than Tiamulin and Tylosin in reference to mortality, pathomorphological lesions, reisolation rate and live weight gain.

Enrofloxacin (Floxidin oral solution from Hoechst Roussel Vet) is also reported to be effective in controlling chick mortality when administered orally via drinking water (Narsapur and Mulbagal, 1996). The combination of the treatment i.e. simultaneous uses of Floxidin injectible (intramuscular route in day old chick) and followed by Floxidin oral solution via drinking water was reported efficacious in Chronic Respiratory Disease (CRD) infection (Narsapur and Mulbagal, 1997).

It was therefore, decided to use Floxidin as a regular anti-micoplasmal drug as well as an antibiotic to prevent Complicated Chronic

Respiratory Disease (CCRD) on commercial broiler farm. After using Floxidin in 24 consecutive batches, an analysis was undertaken to find out its continued efficacy to combat CCRD. The same findings are presented in this paper.

## Materials & Methods :

The experiments are based on a commercial multi-batch deep litter farm with a weekly intake of about 8000 chicks. Mortality pattern and lesion scores of total 32 batches (total of 2,61,000 chicks) were considered for analysis. The period of observations was divided into four phases viz.

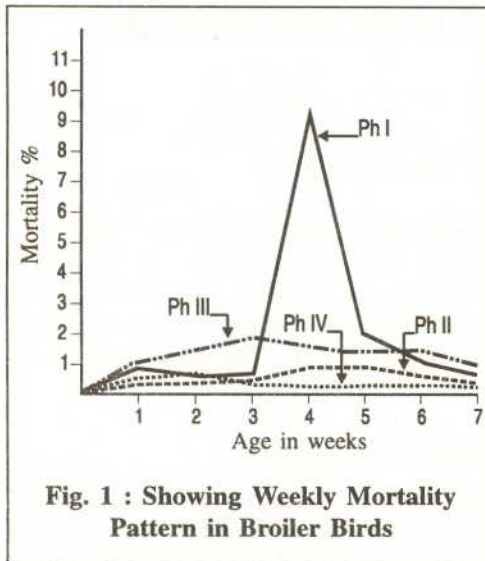
**Phase I (Control Batches):** 8 batches without Floxidin treatment.

**Phase II, III & IV (Enrofloxacin dose ensured, was 10 mg/ kg b.w.) :** all phases were with 8 batches each, received Floxidin treatment schedule as mentioned below :

- 1) Floxidin 10% Injection @ 5ml/1000 chick (day old) through sub-cutaneous route.
- 2) Followed by Floxidin 5% Oral solution @ 10 ml/1000 chicks daily for 5 days (Day 1 to day 5) via drinking water.

No other antibiotics or anti-CRD drugs were given to flocks in Phase II, III & IV. All batches were maintained under identical practices of vaccination and other farm management schedule.

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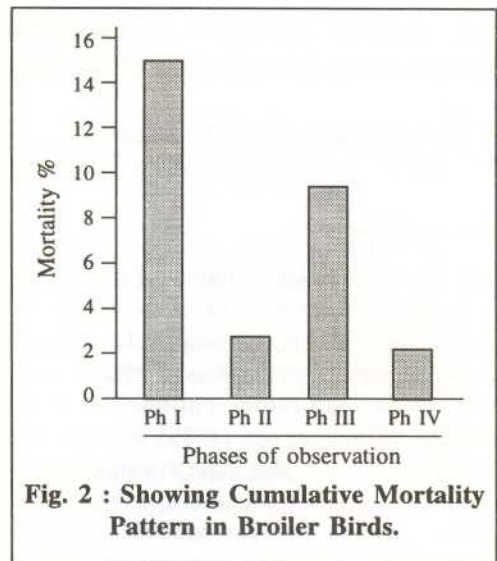
Average weekly mortality of 8 batches in each phase was calculated and compared. Similarly, lesion scores were noted during postmortem of dead birds, once in a fortnight.

After liquidation of broiler birds of the last batch of Phase I, the farm was disinfected and closed for further commercial production of broiler birds for about four weeks.

#### Observations :

In batches of Phase I (control batches) the total mortality was very high (15.08%) which was due to a peak in the 4<sup>th</sup> week (Fig. 1). Severe lesions (+++ to +++) of Infectious Bursal Disease (IBD), Ranikhet Disease (RD) and Complicated Chronic Respiratory Disease (CCRD) were noticed in these batches (Table).

In phase II, III and IV mortality was greatly reduced. Mild lesions of sub-clinical IBD, RD and IBD (in few cases) were noted. However, lesions of CCRD were either mild (+ in phase III) or absent (phase II & IV).



In phase III mortality was higher than Phases II & IV (Fig.2) and feed toxicity lesions (+ to ++ ) were noticed in five of the eight batches.

#### Discussion :

In control batches (phase I) high mortality was due to combined infections of IBD, RD and CCRD / colibacillosis. After cleaning and rest to the farm, mortality percentage came down greatly. However, the two viral diseases (IBD & RD) were not eradicated from the farm and continued to affect the chicks after restoring the farm. The batches continued to suffer immunosuppression. In this situation, it is significant that CCRD lesions receded greatly and were not found in Floxidin treated flocks.

Even after continuous use in 24 batches, over period of 6 months, the Floxidin continued to be effective as evidenced by very low mortality (2.15%) and absence of CCRD lesions in the batches of Phase IV.

**Table : Showing mortality pattern and lesion scores in broiler birds**

Observ. Phase	Total No. of Batches	Total No. of Chicks	Av.Chicks Per Batch	Weekly Mortality Percent							Total Mortality Percent	Lesions Scores
				1st	2nd	3rd	4th	5th	6th	7th		
I Control	8	66,200	8,275	0.83	0.66	0.64	9.13	2.0	0.96	0.84	15.08	IBD RD CCRD } (++)to(++++)
II treated	8	63,840	7,980	0.5	0.39	0.3	0.27	0.25	0.43	0.25	2.73	RD (+) CCRD 0 to (+) IBD (+)
III treated	8	65,680	8,210	0.86	1.5	1.85	1.54	1.42	1.28	0.97	9.44	Fatty liver (++)to(+++) RD (+) IBD 0 to (+)
IV treated	8	66,008	8,251	0.49	0.61	0.4	0.19	0.19	0.13	0.14	2.15	RD } 0 to (+) IBD } Occasional

IBD : Infectious Bursal Disease  
 CCRD : Complicated Chronic Respirating Disease  
 RD : Ranikhet Disease

### Conclusions :

1. Simultaneous use of Floxidin injections and oral administration during early life of chick is very effective in prevention of CCRD and other bacterial infections.
2. Floxidin resistance was not seen to develop even after continuous use for six months.
3. Floxidin is effective in immunosuppressed flock also.

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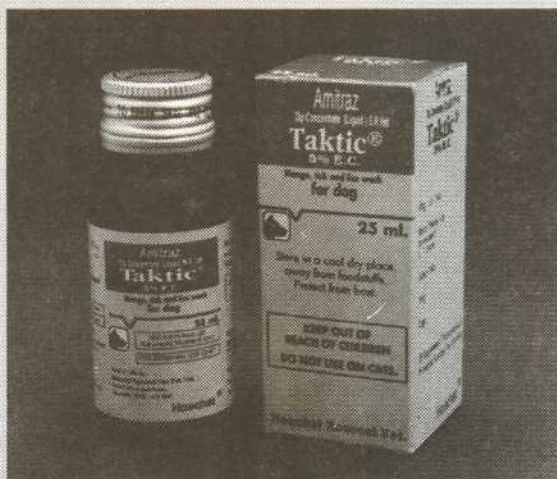
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- William Rotsur

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## ***In-vitro* Antimycotic Sensitivity of Fungi, Isolated from Mastitis Milk**

**S. K. Das and G. P. Patgiri**

Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati

### **Introduction :**

Mastitis is a global problem of economic importance. Although bacteria are the predominant causes of bovine mastitis, sporadic cases and occasional outbreaks of the disease have been attributed to infections by various fungi. In most cases of mycotic infection of udder, yeasts are recovered in higher frequency than molds. In India, fungal pathogens are held responsible for about 6 - 29% of mastitis (Jand and Dhillon, 1975; Sharma *et al.*, 1977; Rahman and Baxi, 1983; Simaria and Dholakia, 1986 and Chhabra *et al.*, 1996). Indiscriminate use of anti-bacterial agents for the treatment of bovine mastitis has given rise the chances of many fungi to grow in the mammary glands. It is also stated that fungal infection had almost always followed antibiotic therapy for bovine mastitis (Monga and Kalra, 1971).

Sensitivity patterns of many conventional and newer antifungal drugs, namely-Spermine, Undecylenic Acid, Flucytosine, 5-Fluorocytosine, Miconazole, Ketoconazole, Griseofulvin, Cycloheximide, Mixin, Nystatin, Clotrimazole, Tolnaftate, Amphotericin-B against a huge number of fungal pathogens have been investigated (Razin *et al.*, 1958; Abu-Gabal and Graham, 1978; Mc Donald *et al.*, 1980; Yeo and Choi 1982; Shan *et al.*, 1986; Pal *et al.*, 1987 and El-Kholy and Hosein, 1990). The present study reports on the antimycotic sensitivity patterns of Nystatin, Clotrimazole and Amphotericin - B against different fungal cultures, recovered from different cases of bovine mastitis.

### **Materials and Methods :**

#### **Fungal cultures :**

A total of 17 representative fungal isolates (9 yeasts and 8 molds) recently recovered from cases of clinical and subclinical bovine mastitis based on positive California Mastitis Test (CMT) were subjected to antibiogram. Purified cultures were maintained on Sabouraud's dextrose agar (SDA) slants.

#### **Antifungal compounds :**

Three antifungal drugs - Nystatin, Clotrimazole and Amphotericin - B (Hi-Media Laboratories Pvt. Ltd.) were tested against the fungal isolates.

#### **Sensitivity test :**

The drug sensitivity test was performed using disc diffusion technique as per the method described by Shan *et al.*, (1986).

Pure culture of each isolate was inoculated into 10 ml Sabouraud's dextrose broth tubes and incubated for 48 hours at 25°C. The SDA plates were poured with the broth culture by flooding the agar surface and kept for 30 minutes for absorption of the inoculated culture. The excess of broth was pipetted out. The sensitivity disc was placed centrally on the inoculated plate and incubated at 25°C for yeast cultures and upto 7 days for molds.

The zone of inhibition measuring 15 mm and above was marked as highly sensitive, 10 - 15 mm as moderately sensitive, 5-10 mm as least sensitive and below 5 mm as resistant.

### **Results and Discussion :**

The results of the antibiogram are presented

**Table I : Shows antibiogram of yeasts, isolated from clinical and sub-clinical cases of mastitis**

Name of the Organisms	No. of isolates tested	Sensitivity to different antibiotics								
		Nystatin			Clotrimazole			Amphotericin-B		
		HS	MS	R	HS	MS	R	HS	MS	R
<i>Candida albicans</i>	6	3 (50)	2 (33)	1 (17)	4 (67)	2 (33)	--	--	--	6 (100)
<i>C.krusei</i>	8	6 (75)	2 (25)	--	8 (100)	--	--	--	2 (25)	6 (75)
<i>C.tropicalis</i>	4	--	--	4 (100)	4 (100)	--	--	--	--	4 (100)
<i>C.pseudotropicalis</i>	3	2 (67)	1 (33)	--	3 (100)	--	--	--	--	3 (100)
<i>C.stellatoidea</i>	1	1 (100)	--	--	1 (100)	--	--	--	--	1 (100)
<i>C.guilliermondii</i>	4	3 (75)	1 (25)	--	4 (100)	--	--	--	1 (25)	3 (75)
<i>C.vini</i>	1	1 (100)	--	--	--	--	1 (100)	--	--	1 (100)
<i>Saccharomyces cerevisiae</i>	1	1 (100)	--	--	1 (100)	--	--	--	--	1 (100)
<i>Rhodotorula rubra</i>	1	--	1 (100)	--	1 (100)	--	--	--	--	1 (100)

Figures in parenthesis indicate percentage.

HS = Highly sensitive , MS = Moderately sensitive, R = Resistant

in the Table I and II.

It is evident from the Table I that Clotrimazole was the most effective drug followed by Nystatin against the yeast cultures tested.

Amphotericin - B was found to be ineffective. *Candida krusei*, *C. tropicalis*, *C. pseudotropicalis*, *C. stellatoidea*, *C.*

*guilliermondii*, *Saccharomyces cerevisiae* and *Rhodotorula rubra* and 67% of *C. albicans* were highly sensitive to Clotrimazole. Only *C. vini* showed resistant to this drug but was highly sensitive to Nystatin. Nystatin was equally effective (100%) against. *C. stellatoidea*, *C. vini* and *S. cerevisiae* and 67% to 75% against *C. krusei* *C. guilliermondii* and *C. pseudotropicalis*.

**Table II : Shows antibiogram of molds, isolated from clinical and sub-clinical cases of mastitis**

Name of the Organisms	No. of isolates tested	Sensitivity to different antibiotics								
		Nystatin			Clotrimazole			Amphotericin-B		
		HS	MS	R	HS	MS	R	HS	MS	R
<i>Aspergillus fumigatus</i>	2	--	--	2 (100)	2 (100)	--	--	--	--	2 (100)
<i>A.niger</i>	3	2 (67)	1 (33)	--	--	3 (100)	--	--	--	3 (100)
<i>A.flavus</i>	2	--	2 (100)	--	2 (100)	--	--	--	--	2 (100)
<i>Sporotrichum schenckii</i>	3	2 (67)	1 (33)	--	3 (100)	--	--	--	--	3 (100)
<i>Penicillium sp.</i>	2	--	--	2 (100)	2 (100)	--	--	--	1 (50)	1 (50)
<i>Mucor sp.</i>	1	--	1 (100)	--	1 (100)	--	--	--	1 (100)	--
<i>Curvularia verruculosa</i>	1	--	1 (100)	--	1 (100)	--	--	--	--	1 (100)
Unidentified sp.	1	1 (100)	--	--	1 (100)	--	--	--	1 (100)	--

Figures in parenthesis indicate percentage.

HS = Highly sensitive , MS = Moderately sensitive, R = Resistant

Similarly, Clotrimazole was found to be a very useful drug against the mold cultures viz. *Aspergillus fumigatus*, *A. flavus* *Sporotrichum schenckii*, *Penicillium sp*, *Mucor sp* and *Curvularia verruculosa*. Nystatin was effective (67%) against *A. niger* and *S. schenckii*, whereas, Amphotericin-B had almost no effect on the fungi under test.

Clotrimazole as one of the effective antifungal agents against variety of fungal agents encountered in bovine mastitis has been earlier reported by EL-Kholy and

Hosein (1990). Flucystosine followed by Nystatin and Miconazole was also found to be equally effective. However, over 75% of fungal isolates comprising mostly of yeasts recovered from mastitis milk samples were reported to be highly sensitive to nystatin (Mc Donald *et al.*, 1980; Yeo and Choi, 1982; Shan *et al.*, 1986 and Pal *et al.*, 1987). Moderate to significant sensitivity was also exhibited by Ketoconazole, Clotrimazole, Miconazole and 5- fluorocystosine against the many fungal isolates, the reports said.

Mycotic mastitis, other than cryptococcal mastitis is clinically almost indistinguishable from bacterial mastitis. Majority of the cases of actual mycotic mastitis are either ignored or not adequately investigated and in almost all cases the ailment is treated with various antibacterial antibiotics. This practice not only enhances the growth of fungi in many cases but also makes the ailment more troublesome to control.

In the recent years, the incidence of mycotic mastitis is gradually increasing (Chhabra *et al.*, 1996). It is, therefore, very essential to examine the milk samples culturally and antibiogram is always recommended, whenever cases of mycotic mastitis are established. However, antimycotic preparations of choice for intramammary infusion are not readily available in the market.

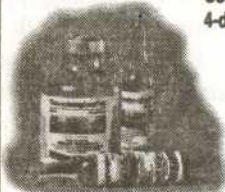
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# ***Ancylostoma ceylanicum* and *Plasmodium berghei* : Correlation of Infectivity of Adult Ingested Hookworm in Swiss Albino Mice (*Mus musculus*) and Immunodepression due to Malaria Infection**

**A. K. Datta and V. S. Narsapur\***

Hoechst Roussel Vet Pvt. Ltd., Neeta Park, Yerwada, Pune

## **Introduction :**

A study of immunodepression in the aspect of pathogenesis of parasites infections is of importance because of the fact that immunodepression is used in certain therapeutic procedures and also could be a side effect of therapeutic regimes used in different diseases. So far, mice have been found normally resistant for the complete development of human and canine hookworm infections. Several workers (Kerr, 1935; Lindquist, 1952; Soh, 1958; Kono and Sawada, 1961) have studied *Ancylostoma canium* in mice, but none have reported any development of the parasite in that host. Mathies (1962) showed that the use of cortisone increased the worm burden of mice with *Aspicularis tetraoptera*. It is obvious that both parasite and host factors play an important role in determining the outcome of infection. But it is a fact that if the immune response, one of the major host factors, is in a depressed conditions, parasites may get a path to establish themselves in the host body. It is well known that the immune response to other antigens becomes depressed during malaria infection. McGregor and Barr (1962) observed that children suffering from malaria had diminished responses to tetanus toxoid. The relations between Burkitt's Lymphoma and malaria have already received considerable amount of attention (Ziegler *et al.*, 1972). Ray *et al.*, (1975) have shown the complete development of dog, cat and human helminth parasite,

*A. ceylanicum*, in mice using hydrocortisone acetate to induce immunodepression. Previously, Sen *et al.*, (1965) studied the effect of hydrocortisone acetate on the development of *A. caninum* in mice.

The present work deals with the correlation of infectivity of the adult worm *A. ceylanicum* by ingestion under the influence of immunodepression, caused by rodent malaria parasite, *Plasmodium berghei*, which may have a role in determining the outcome of infection in swiss albino mice.

## **Materials and Methods :**

**Host animal :** Albino mice (*Mus musculus*) of the Swiss strain, weighing 35-40 grams were used throughout the experiment. Mice were maintained in polypropylene cages and were fed with vitamin added case in pellets and water given *ad libitum*. Room temperature was maintained at 22°C.

**Parasite :** *P. berghei* (NICD strain), used in all experiments, was maintained in this laboratory by biweekly syringe passage of heparinised blood.

**Determination of parasitaemia :** Thin smears were prepared from tail blood of infected mice and were stained with Giemsa stain. Quantification of parasitaemia to within 10% probable error was done according to the method described by Hartman (1927).

\* DOLCOS, Veterinary Pathology Laboratories, Jogeshwari, Mumbai

**Infection of host animals :** Blood from an infected donor mouse of known parasitaemia was obtained by cardiac puncture using a heparinized syringe (Datta, 1984) and under anaesthesia with chloroform. Blood infection by syringe passage was carried out by the intraperitoneal route (i.p.) using dilutions of infected blood in cold phosphate buffer (pH 7), giving approximately  $10^7$  infected red blood cells per 0.5 ml.

**Adult *Ancylostoma ceylanicum* (Hamster strain) :** 12 - 14 days old *A. ceylanicum* worms were collected from hamsters after performing autopsy (Ray and Shrivastava, 1982). The worms were washed repeatedly with physiological saline to free them from intestinal masses and kept in sterile saline for

immediate use.

**Infection of experimental animals :** The experimental animals were divided into two groups each having 25 animals of either sex in a series of five individual experiments.

In group A, *P. berghei* infected mice were drenched *per os* with mixed adult *A. ceylanicum* worms by a specially made feeding needle. In experiment I, mice were drenched orally with 20 mixed adults on the day (DO) of *P. berghei* infection. In experiment II, 20 mixed *A. ceylanicum* adults were administered orally on the 6th day (D+5) post *P. berghei* infection. Proper infected control mice of comparable weight group, without *P. berghei* infection were always maintained.

**Table I :** Recovery of adult worms of *Ancylostoma ceylanicum* from small intestine of mice, uninfected with *Plasmodium berghei*, on the 7th day post administration with 20 mixed adult worms per mouse by the oral route.

Series of expt.	<i>P. berghei</i> infected mice (DO and D+5) & control	Mice examined	Worms recovered		
			Range	Mean	Mean (%)
1	DO	5	0	0	0
	D+5	5	4-15	8'6	43
	Control	5	0	0	0
2	DO	5	0	0	0
	D+5	5	3-12	9'6	48
	Control	5	0	0	0
3	DO	5	0	0	0
	D+5	5	7-14	9'6	48
	Control	5	0	0	0
4	DO	5	0	0	0
	D+5	5	8-13	10'2	51
	Control	5	0	0	0
5	DO	5	0	0	0
	D+5	5	5-14	9'8	49
	Control	5	0	0	0

**Table II :** Recovery of adult worms of *Ancylostoma ceylanicum* from small intestine of mice, treated with **hydrocortisone acetate**, on the 7th day post administration with 20 mixed adult worms per mouse by the oral route.

Series of expt.	Hydrocortisone acetate (Treated) & Control	Mice examined	Worms recovered		
			Range	Mean	Mean (%)
1	Treated	5	7-12	8'8	44
	Control	5	0	0	0
1	Treated	5	6-11	8'6	43
	Control	5	0	0	0
1	Treated	5	9-15	10'2	51
	Control	5	0	0	0
1	Treated	5	7-14	10	50
	Control	5	0	0	0
1	Treated	5	4-15	8'6	43
	Control	5	0	0	0

In group B, Hydrocortisone acetate treated mice were drenched *per os* with mixed adult *A. ceylanicum* worms. Hydrocortisone acetate (EFCORLIN, GLAXO), in freshly prepared sterile normal saline suspension, was injected subcutaneously at 10 mg/kg b.w. (0.1 ml/10g b.w.) into each mouse. This dosage was continued daily until they were killed. On the 6th day of this hydrocortisone acetate treatment, mice received 20 mixed adult *A. ceylanicum* worms *per os*. The control group received placebos of 0.89% sterile normal saline daily at the rate of 0.1 ml/10g b.w.

### Results :

The animals were necropsied on the 7th day post administration of adult worms and the results are summarised in Tables I and II.

In group A, experiment I where *A. ceylanicum* worms were administered on

DO of *P. berghei* infection, no worms could be recovered on necropsy, though the blood smears were individually positive for malaria parasites. In experiment II where *A. ceylanicum* worms were administered on D+5 of *P. berghei* infection, the evidence of helminth infection and recovery of adult worms was observed (Table I). The same results of adult worms recovery were also observed in group B, hydrocortisone acetate treated mice (Table II). The control mice of both groups were free from this helminth infection.

In most of the infected mice, the intestine was full of clotted blood, the worms were found copulating and faeces of these mice was positive for helminth ova. Viability of these ova was tested by tube filter paper culture procedure as described by Harada and Mori (1955) and found positive.

## Discussion

This is the first time, it has been demonstrated that adult worms of *A.ceylanicum* (Hamster strain) can produce infection in malaria infected mice and also hydrocortisone acetate treated mice.

It is well known that the mouse malaria model has provided considerable information on immunodepression during malaria infection. The mechanisms of immunodepression in protozoal disease are not clearly known. Antigenic variation might be one of the probable factors. The possibility of certain functions of T cells has also been suggested (Bamford and Wedderburn, 1973).

When producing helminth infection in experimental animals, success depends on the pathogenesis caused by the parasites in the intestine and passing of viable ova through the faeces of the experimental animals. The latter is also necessary in order to determine whether or not an animal was infected.

In group A experiment I, no mouse was found infected by the ingestion of adult parasites. This is due to *P.berghei* malaria infection was yet to settle in mice and worms were expelled due to inherent resistant similar to that observed in the control group of mice. In experiment II the *P.berghei* infection became patent on the D+5 when parasitaemia rose to 30-40% and all the mice showed higher infectivity rate both in ova checking by flotation method and also by autopsy.

In the group B experiment, where hydrocortisone acetate was used as an immunosuppressant, the average recovery of adult worms was high. This suggested that cortisone suppresses the resistance and thus assists the adult worms to remain for a longer

period in the host system. However, the exact mechanism of the action of hydrocortisone acetate in the host system remains at best speculative (Weinstein, 1953, Coker; 1955 and Nicol *et al.*, 1956).

In order to determine the degree of pathogenicity of *A.ceylanicum* in mice it was compared with *A.ceylanicum* infected hamsters (Ray and Shrivastava, 1982). No difference was noted in mice in the depth or extent of pathogenesis caused by adult worm ingestion.

It is, therefore, reasonable to assume that there is an inherent resistance in many animals which prevents the spread of parasitic diseases and which, in this study, has been broken down by the *P.berghei* infection in mice. In group A experiment II, *P.berghei* was an immunosuppressive agent which predominantly depressed the immune response assuring that *A.ceylanicum* could establish themselves in the host body. How far this finding is applicable to humans is uncertain.

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## Bionomics of Psoroptic Mange in Rabbits

D.K. Maske

Department of Parasitology, Nagpur Veterinary College, Nagpur

Psoroptic mange (ear-canker) caused by *Psoroptes cuniculi*, keeps the rabbit restless as the animal rubs the ear with its feet and shakes the head continuously to allay itching. The restlessness adversely affects the health of animals. Few reports on ear mange among rabbits indicate that the prevalence is very high in monsoon season (Chandra and Ghosh, 1990) in Meghalaya region of India. The present investigation deals with the prevalence and bionomics of rabbit psoroptic mange at Nagpur.

### Materials and Methods :

Observations on the prevalence of psoroptic mange in 396 rabbits of various breeds, different age groups and both sexes were made during March 1995 to February 1996. These animals were belonged to Rabbit Breeding Farm of Nagpur Veterinary College, Raman Science Centre, Medical College and Maharaj Bag Zoo, Nagpur. The waxy material inside the ears was obtained with a cotton swab. Scrappings from the ear skin of area 4 sq.cm. were taken in petri dishes with a blunt scalpel. The specimen bottle containing cotton swab was warmed to about 38°C for presence of living mites and later-on thoroughly washed and squeezed in xylene and examined microscopically. Petridishes containing scrappings were warmed and later examined under microscope to see the motility of living mites. The scrappings found negative for mites were treated with 10% KOH, cooled, centrifuged and sediments were examined microscopically.

### Results and Discussion :

Percentage of infected rabbits, range of

temperature and relative humidity for the months under study are presented in Table I.

The percentage of infection recorded at Raman Science Centre, Medical College, Maharaj Bag Zoo and Rabbit Breeding Farm of Veterinary College, Nagpur was 40%, 35%, 27% and 22% respectively.

The prevalence of psoroptic mange in respect of local, Albino, Soviet Chinchilla, Grey Giant, New Zealand white, Angora and White Giant breeds was 47%, 31%, 27%, 17%, 16%, 14% and 10% respectively.

The rate of infection in summer, monsoon and winter season was 15%, 31% and 15% respectively.

The occurrence of psoroptic mange in female rabbits 20% while in male it was 27%.

The prevalence of infection in below six months and above six months age of rabbits was 10% and 28% respectively.

Of the 396 ear-crusts, 55 were positive (14%) for psoroptic mange on direct examination. Out of remaining 341 crusts, 39 (11%) were positive for mange when treated with 10% KOH solution. In most cases (43) dry crusty lesion were confined to external ears, skin of forehead, skin of nostrils and periorbital areas followed by auditory canal (39) causing severe otitis. In some cases (8) lesions were extended towards perianal and genital opening and in few cases (4) the crusty lesions were also observed at interdigital space of paws and on scapular region.

The prevalence of infection with reference to climatic factors, locations, breeds, seasons, sex and age showed that the percentage of

**Table : Showing the prevalence of psoroptic mange in rabbits at Nagpur during the year 1995-96**

Month	No. of Rabbits Examined	No. of Rabbits Positive	Percentage of infection	Temperature °C		Relative humidity %	
				Av. min.	Av. Max.	Min.	Max.
March	13	02	15	16	30	34	90
April	06	01	17	21	38	30	70
May	10	01	10	26	44	22	56
June	32	05	16	25	38	66	86
July	92	45	49	25	34	62	90
Aug.	42	09	21	24.6	32	64	92
Sept.	46	08	17	24	33	76	84
Oct.	40	06	15	18	30	62	90
Nov.	45	05	11	20	26	60	80
Dec.	32	05	22	10	26	45	85
Jan.	23	05	22	10	24	56	70
Feb.	15	02	13	7	24	54	70
<b>Total</b>	<b>396</b>	<b>94</b>	<b>24</b>				

infection varied from month to month with overall picture 24%. (Table) These findings correlated to Chandra and Ghosh (1990) who recorded 23% psoroptic infection during January to March in Meghalaya. About a quarter of the population acted as carriers. January and August showed more than 20% infection, while March, April, June, September, October, November, December and February had less than 20%. May recorded the least whereas July the highest percentage of infection.

The variability from farm to farm recorded revealed more infection at Raman Science Centre, Nagpur as compared to Rabbit Breeding Farm of College. The climatic factors being identical, the probable cause of variation could be different methods of management adopted. None of the selected places were free from infection indicating

thereby that the infection was widely prevalent.

The study encountered highest percentage of infection in local breed as compared to other breeds of rabbit. The differences were nonsignificant at 5% level, proving that different breeds were not factors favouring the prevalence of mites. These findings agreed with those of Chandra and Ghosh (1990), who stated that prevalence had no variation in susceptibility among the breeds of rabbit.

Monsoon season appeared to be more conducive to prevalence of infection than the remaining seasons. The differences were significant at 5% level. The mites preferred moist conditions which prevailed during monsoon particularly in month of July, 1995 at Nagpur.

The females showed less susceptibility of infection than the males, probably because females are taken better care than males as they are more genetic importance on account of progeny production.

Increase in prevalence of infection in above six months rabbits corroborates to the findings of Health *et al.*, (1983). The difference regarding age may also be due to the different managemental practices for adults and younger rabbits, the latter being under special care.

The treatment of ear scrapings with 10% KOH was decidedly better as 39 of the 341 cases found negative by direct examination. However this method is more time consuming and calls for its application only in cases found negative on direct examination. Chandra and Ghosh (1990), reported the confinement of psoroptic mange to one ear of rabbit and the lesion at base of ear was in pustular form. The present

study revealed that the lesion was dry crusty and confined not only to both ears but also extended towards forehead, nostrils, periorbital areas. In few cases the lesion was also extended towards scapular region, at interdigital space, skin of perianal and genital opening. Since the disease is spread by contact and its seat of predilection is the pinna, huddling of rabbits with shoulder region and sometimes with hind quarter which may encourage the flapping of skin, will increase the scapular and anal lesion. Additionally on account of scratching of ears with hind paws the rabbits will develop the interdigital lesion.

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## Some Observations on Diagnosis of Animal Schistosomiasis

**M.C. Agrawal**

Department of Parasitology, College of Veterinary Science and Animal Husbandry, J.N. Krishi Vishwa Vidyalyaya, Jabalpur, M.P.

Schistosomiasis is wide spread causing enormous losses to animal industry in India. Nevertheless, a veterinarian generally fails to confirm the disease in an animal due to non recovery of the blood-flukes or their ova. According to an estimate, hepatointestinal schistosomiasis is diagnosed only in 30% positive cases while other go undetected.

Treatment, epidemiology, survey and control may be the reasons for diagnosis of Schistosomiasis. The history of the area or recovery of Schistosome cercariae from local snails is of paramount importance as migratory positive cases may mislead the situation. For treatment purpose, one may attempt to diagnose hepatic form on clinical grounds but symptoms are non-specific (diarrhoea or dysentery or bottle jaw) hence these are to be confirmed by searching ova/miracidia in the faeces. Likewise, determining kind of animal excreting highest number of ova. will be crucial for control measures. On the other hand, epidemiological work may be carried out following immunological methods which are more sensitive but less specific. The laboratory diagnosis is of following types.

### **Parasitological Methods :**

#### **Coprological Methods -**

In the most common Coprological methods (direct smear and salt flotation), the sample/whole sediment is mixed with saline and a few drops are examined under a microscope. D'Souza *et al.*, (1988) reported 0.27 - 0.87% infection in Karnataka by this method. Agrawal (1990) was able to detect only 6.8%

and 10% positive cases in goats and cattle giving a picture of low prevalence which was not true.

This salt flotation method was compared with liver biopsy and CHR tests in ruminants; liver biopsy gave best results (100%) followed by Cercarial Hullen Reaction (CHR) (62.5%) while salt method was least effective (Agrawal and Shah, 1989). The acid-ether, hatching, routine salt concentration, sieving and aerator methods were compared on the faecal samples of naturally infeted pigs at Jabalpur. Hatching test proved most sensitive followed by acid-ether, aerator, sieving while routine salt concentration was least effective. (Kaur, 1985).

Recently this routine salt flotation method is made more sensitive in the present laboratory by discarding all the sediment except 2-5 mg at the bottom of the centrifuge tube. After diluting it with saline, the whole sediment (0.5ml) is transferred on microslide and examined (50x) without a coverslip. This method proved effective in detecting schistomes, *Fasciola* and Amphistomes ova and was five times more sensitive than the routine salt flotation test. This method has further been made cost effective and field oriented by transferring the material in a LDPE (Low Density Poly Ethylene) bag instead on a microslide. The sealing of the bag facilitates not only microscopic faecal examination but also mailing the sample to distant places for seeking expert opinion or storing the positive faecal sample for class room demonstration (Agrawal, 1997).

Banerjee (1988) compared liver biopsy, hatching and sieving methods in bovines at Jabalpur. Sensitivity of liver biopsy was 96% in the cattle and that of hatching and sieving tests was 79% and 36%. The corresponding figures for buffaloes were 100%, 67% and 22% respectively. In the sieving method, 30 gm of the faeces was treated overnight with alkali (0.4N sodium hydroxide), filtered through 30, 50 and 80 mesh brass sieves. The sediment was washed thrice with 1.7% saline and then centrifuged at 1000 rpm. Two samples of the sediment, 0.5 ml each (1ml = 1gm faeces) were examined under low magnification (without coverslip) for presence of the eggs. Thus, it was a different method than that described by Kaur (1985).

#### **Hatching Test :**

The hatching test proved more effective in pigs and bovines vis-à-vis ova detection method though less sensitive in the mouse (Panesar & Agrawal, 1986). Its sensitivity altered by host species, age, quantity of faeces, number of faecal washings, time of examination and quantity of water examined. Use of 20 gm faeces, three washings, 3-4 hour exposure, checking of 10ml water appeared ideal for hatching method. The water should be examined on the same day as overnight keeping resulted in emergence of protozoa creating problems. These were differentiated by their smaller size, slow and haphazard movement (miracidia move faster and in straight line). A drop of iodine solution kills and stains the organisms which may well be differentiated under a compound microscope (100x).

Hatching test was conducted on stored pig faeces and the test remained positive for 20 days. Therefore, it is better to call unpreserved faeces from the field as hatching method is more sensitive than ova detection method.

For recovery of blood-flukes from animals, the following methods were developed in the present laboratory.

1. Hepatic portal vein is cut, liver and mesentery are flushed with 2% citrated normal saline using a perfusion technique. The blood is filtered through a black cloth and the cloth is inverted in a petri dish containing normal saline. The blood-flukes may be observed directly or under stereoscopic microscope.

Blood clots are also collected, dissolved by placing in a petridish with water or acid water for 2-3 hrs. Later the petri dish is examined under stereoscopic microscope for presence of flukes.

2. Liver may be perfused with citrated saline with the help of a large syringe or any other device so that saline may go with pressure. Quantity of the saline may vary according to size of the liver from 200 to 1000 ml. It is advantageous to cut periphery of the liver which facilitates its perfusion. The saline is filtered through a cloth as above and examined for presence of blood-flukes.
3. Liver is examined for schistosome ova. as it is simpler than recovery of the blood-flukes. If liver is soft (sheep, goat, laboratory animals), pieces of liver (2-10 gm) are minced and pressed in between the two slides to be tied with a rubber band. These slides are examined under low magnification (50x) for presence of blood-fluke ova. If liver is hard (eg : pig) or EPG is to be made, 10-50 gm liver may be minced in 5% KOH or pepsin/trypsin solution and left 6-12 hrs. The sediment is examined for ova after centrifugation.
4. Adult blood-flukes are present in the

mesentery of the animals, hence mesentery is collected and cut into small pieces in normal saline to be left over for 4-8 hrs. Later, saline is filtered through a cloth and the material is examined for presence of blood-flukes as mentioned above.

5. Another simple method of detecting schistosomiasis is to collect rectal or intestinal scrappings from the suspected animal. These scrappings are pressed in between the two slides and examined for presence of blood-fluke ova.

### **Immunodiagnosis :**

CHR and Miracidium Immobilization Test (MIT) have been applied in pigs (Ahluwalia, 1968) and bovines (Banerjee, 1988) owing to their simplicity. Both these tests are 70% or more sensitive and specific in diagnosing schistosomiasis in domestic animals. It was observed that normal bovine sera at a titer of 1:10 also immobilized schistosome miracidia - the criterion used for positiveness of the test. Therefore, we considered a titer 1:20 or above as positive for schistosomiasis.

Banerjee *et al.*, (1991) evaluated efficacy of serological tests like CHR, MIT and Ring Precipitation Test (RPT) in relation to a standard reference test (Parasitological method) in diagnosing hepatointestinal schistosomiasis in bovines. They observed sensitivity and specificity (indicated by J-index) of same serological test in same animal altered by varied sensitivity of reference tests which were liver biopsy, hatching and sieving methods. Accordingly, CHR was 90% sensitive and 48% specific in cattle when sieving test was reference test. These figures changed to 89% and 74% respectively when liver biopsy was the reference test.

The greatest disadvantage in using cercariae

or miracidia for immunodiagnosis is the need of continuous supply of these larval stages in a live form. To overcome this problem, Banerjee *et al.*, (1990) used ring precipitation test where homogenates, excretions and secretions of *S.spindale* were used as antigen. When a titre of 1:10 and above was taken as positive, the test proved to be 78.5% sensitive and 90.9% specific. However, production of antigen in larger quantity will again pose a problem in the field.

This problem has been solved in other countries by purifying the antigens, applying micro serological tests which are more sensitive and specific. The recent trend is detection of circulating antigens instead of antibody as the former confirms existence of the infection while the later is not. Unfortunately no work has been done on this aspect in India.

### **Nasal Schistosomiasis :**

It has been demonstrated that nasal scrappings are more efficient than nasal washings in detecting *S.nasale* egg (Muraleedharan *et al.*, 1976). Therefore nasal scrappings must be examined instead of nasal washings particularly when a new area is investigated and also symptomless carriers like buffaloes are to be examined.

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## Cysticercosis of Zoonotic Importance

Placid E. D'Souza

Centre for Advanced Studies, Department of Parasitology, Veterinary College, UAS, Bangalore

A large number of species of *Taenia* tapeworms occur in animals but only three species are known to involve human beings and animals. *Taenia saginata* and *Taenia solium* both human tapeworms involve bovine and porcine intermediate hosts respectively. In the recent years a new species commonly known as *Taiwan Taenia* has been encountered in man and animals with increasing evidence to show that a new species is evolving due to selection pressure.

*T.saginata* and *T.solium* have a world wide distribution but are more common in areas where the sanitation is poor. Adult worms of the two species are found in the intestines of man and the metacestodes *Cysticercus bovis* and *Cysticercus cellulosae* commonly occur in the muscles and organs of cattle and pigs respectively. Cysticercosis causes significant economic losses due to downgrading of cattle and pig carcasses. Cysticercosis in pigs has been estimated to cause a loss of half a million dollars from 6 abattoirs in Central America and Panama (Acha and Aguilar, 1964) and up to 81 percent from Mexico (Antonio, 1982 & Cruz *et al.*, 1993).

In India, a loss of 64,600 rupees due to 3.8 percent infection in pigs in U.P. (Pathak and Gaur, 1989) and 2,61,661 out of 61,86,102 rupees due to 4.22% infection in an organised abattoir in Andhra Pradesh (D'Souza, 1996) has been reported. In Africa losses of 1000 to 2000 million US dollars have been attributed to bovine cysticercosis. Cysticercosis is generally underdiagnosed and under reported. The WHO has estimated that these infections

occur in 50 million people with mortality of 50,000 people in a year due to this zoonotic infection.

*T.saginata* - The beef tapeworm has a cosmopolitan distribution and occurs in developing countries due to poor hygienic habits and consumption of raw or insufficiently cooked meat. It is also a great problem in developed nations due to difficulty in detection and also due to consumption of certain preparations involving improper cooking of beef even through the standard of meat inspection is high (Steaks). The ova of this tapeworm have been shown (Table I) to survive almost all stages of sewage treatment (Burger, 1984).

In Great Britain and Australia 'Cysticercosis storms' have been reported to occur in cattle on some farms after grazing on pastures where human sewage was used as fertiliser in the forms of a sludge. *T.saginata* ova can survive for more than 200 days in sludge. The other causes for prevalence of cysticercosis include access of cattle to water contaminated with sewage effluents and dispersal of *T.saginata* ova by birds which frequent sewage works or feed on effluent discharged into the river or sea and to occasional contamination of pasture by infected individuals.

As infected individual is able to excrete daily into the environment upto 5 million oncospheres and the average number of excreted oncospheres constitutes 1.5 million. Therefore, intensive environmental contamination can occur. In Great Britain

and the USA cattle are the normal intermediate hosts but in tropical countries other ruminants such as goats, sheep, Llama, giraffes etc. may also be involved.

and muscle stiffness. Experimentally calves given massive doses of *T.saginata* ova developed severe myocarditis and heart failure. Cysticerci remain viable up to 8 months in cattle whereas the worm can

**Table I : *Taenia saginata* : Survival of ova in sewage and sludge.**

Medium	Conditions	Survival (Days)	Infectivity (Remaining)
Sewage	Laboratory, 18°C	16	Yes
Sewage	Exp. Plant, trackling filter	42	No
Sewage	Septic tank	40	Not tested
Sewage	Plant, raw waste water	Yes	Yes
Sludge	Lab, anaerobic digestion, 25-29°C	200	Not tested
Sludge	Lab, anaerobic digestion, 35°C	<5	No
Sludge	Plant, anaerobic digestion 26-28°C	56	Not tested
Sludge	Exp. plant, activated sludge	42	Not tested
Effluent	Plant, activated sludge	Yes	Yes
Effluent	Plant, trickling filter + lagoon	No ?	No ?
Effluent	Plant, lagoons	No	No

Source : Burger, 1984.

In human beings, in a single worm infection, the worm develops to 3 metres in length and can produce 600 proglottides. The effect on human health is indicated by diarrhoea, abdominal pain, headache and increased appetite and sometimes may be asymptomatic and mainly objectionable on aesthetic grounds. The gravid proglottides are spontaneously discharged and are usually motile. They may creep out of the anus onto the perianal skin and may migrate over the clothes or on the ground shedding eggs as they go. This has a psychological effect on the patients. In cattle the metacystode *Cysticercus bovis* which is about 1.0 cm in diameter filled with fluid and a clearly visible scolex is located in the striated muscles especially the tongue, masseter, intercostal muscles and heart. Symptoms are not serious but heavy infections with eggs lead to fever, anorexia, debilitation and muscle stiffness. Experimentally infections with eggs lead to fever, anorexia, debilitation

survive in human beings for many years.

***T.solium*** - Pork tapeworm infection is regarded as an obligatory cyclozoonosis, an anthrozoönotic helminthiasis or euzoonosis. These terms imply that man is one of the essential or usual host involved in the life cycle. So far, apart from man the adult tapeworm has been experimentally found to develop in the lar gibbon and golden hamsters and to some extent in the dog (Varma and Ahluwalia, 1985). This zoonotic parasite is of special interest and is unique because man can also act as an intermediate host in which case the manifestations can have serious implications to health. The other intermediate hosts include domestic and wild pigs which are very commonly infected and act as an important source of infection to human beings and other animals as dogs, monkeys, sheep, goats and rodents.

*T.solium* is commonly found in Central and Southern Latin American, American and Asian countries and occurs in the hyperendemic form. It is also reported from the USA and some European countries probably spread to these developed nations by infected immigrants. Environmental conditions common to most communities where taeniasis and cysticercosis are endemic include inadequate mechanisms for disposing of human faeces together with swine husbandry practices which give the animals access to faeces. Pigs reared on free range system with easy access to human faecal material are commonly infected. Mature proglottids are frequently shed in nonmotile ribbons and hence massive infections are common in pigs. Each proglottid may contain as many as 100,000 ova. Crossbred animals are more susceptible to infection. Flisser *et al.*, (1979) reported low efficacy of establishment of *T.solium* in normal and immune host. *Cysticercus cellulosae* the metacestode of this tapeworm develop in about 10 weeks in pigs. The

Cysticerci measure 5-20x5-10mm are milky white in colour and contain fluid rich in albumin and has an invaginated scolex. They infect muscular tissue and are common in the lingual, masseter, diaphragmatic, cardiac muscles and muscles of thigh, shoulder and neck. All the parenchymatous organs, especially brain and eye are commonly infected. Clinical symptoms are usually absent in pigs except when cysticerci are lodged in the eye and brain, however cysticercosis or measily pork infection in pigs has been found to significantly affect growth, weight gain and is the cause of economic loss and the disease is of immense public health importance.

In human being usually a single adult worm 2-7 meters long is found in the intestine and when cysticercosis occurs it is of serious consequence when the eye or nervous system is affected. The intestinal infection is acquired by eating undercooked infected pork or infected dog meat in some places. Cysticercosis, however is acquired by

**Table II : Some characteristics for differentiation amongst the three *Taenia* species**

Characteristic	<i>Taenia saginata</i>	<i>T.solium</i>	<i>Taiwan Taenia</i>
Intermediate hosts	Cattle, reindeer	Pig, wild boar	Pig, cattle, goat, wild bear
Site of development	muscle, viscera	brain, skin, muscle	Liver (exclusively)
Scolex : adult worm	no hooks	hooks present	No hooks
Scolex : cysticercus	no rostellum	rostellum + hooks	rostellum + hooks
Proglottis : uterine branches	23 (14-32)*	8 (7-11)*	20 (11-32)*
Passing of proglottides	Single, Spontaneously	in groups passively	single, spontaneously
Ovary	2 lobes	3 lobes	2 lobes
Vagina : sphincter	present	absent	present

\*There is no universal agreement on the numbers of uterine branches in these two species. WHO (1983) gives a figure of 7-16 for *T.solium* which means that the number for the two species overlap and specimens with 10-16 branches cannot be accurately diagnosed. As a rough guide, specimens with over 16 branches are likely to be *T.saginata* and those with fewer than 10 branches are of *T. solium*.

Source : WHO (1983) & Fan (1988).

ingesting taenia ova shed in the faeces of a human carrier of the tapeworm through contamination of food and water or through autoinfection.

**Tawain Taenia** - a new species of *Taenia* was discovered to exist in some Asian Countries such as Taiwan, Phillipines, Korea and Indonesia. It was first discovered from the native aboriginal population from Taiwan. The differentiating characters of this species from *T.saginata* and *T.solium* are shown in the Table II.

The evidence by Fan, 1988 & Fan *et al.*, 1990, strongly suggests the evolution of a new species by selection pressure during passage for many years through diverse intermediate host and man and since this species has also been described from Korea, Philippines and some other countries it is now referred to as Asian *Taenia*.

#### **Diagnosis & Control :**

The study of the epidemiology of *T.solium* and *T.saginata* clearly indicates that these infections can be controlled and eventually eradicated as has been possible in some of the European and Western countries.

Since bovine and porcine cysticercosis are occult infections they are usually detected on post mortem/meat inspection. However, it has been found possible to detect *C.cellulosae* infection in pigs to a certain extent in the live animal by a tongue examination. It is an old method (379 AD) and cysticerci located under the lingual mucosa can be seen by the naked eye as transparent nodules and can be palpated. This method was found to be 70% sensitive and 100% specific as compared with serological test. It has been proved beyond doubt that meat inspection is not a very reliable method of detecting *C.cellulosae* and *C.bovis* infections in animals. By routine

meat inspection methods a certain percentage of positive animals are always missed. Dissection and slicing of infected carcasses could detect 60 out of 79 (76%) Iersus 23 out of 60 (38.3%) cattle with *C.bovis* (Walther & Koske 1980) IHAT could detect more positive *C.cellulosae* infected pigs than routine meat inspection (Herbert and Carlos 1974) similarly ELISA and CIEP could detect more positive pigs (Zoli *et al.*, 1990 and D'Souza, 1996).

Serodiagnostic tests have been attempted and standardized to diagnose both *C.cellulosae* and *C.bovis* infections. Different antigens have been prepared and evaluated and tests such as Precipitation / immunodiffusion, Indirect haemagglutination, complement fixation, Immunoelectrophoresis, Counter immunoelectrophoresis, IFAT, ELISA, EITB bentonite flocculation test and Intradermal tests have been conducted (Schantz 1987 & Kumar and Gaur, 1994).

Different antigens have been prepared and tested, and it has been found that while the sensitivity with crude antigens was high the specificity was considerably low. Cross reactivity with many of the metacestodes has always been a major problem. *Cysticercus tenuicollis*, and hydatid cysts ae the common cross reactors for porcine cysticercosis and *Fasciola hepatica* for bovine cysticercosis. However, better results are being obtained with fractionated antigens such as antigen B and excretory antigens in ELISA with reliable diagnostic sensitivity and specificity. Enzyme immunoelectro transfer blot has emerged as one of the most promising and highly specific test to detect cysticercosis in pigs based on identification of four low molecular weight specific glycoprotein antigens.

A fraction of *T.hydatigena* cyst fluid was reported to be more sensitive in the detection

of *C.bovis* infection in cattle and since this cyst is very common in sheep and provides sufficient quantity of fluid it could be more useful as diagnostic antigen also in view of the relatively lower cross reactivity (Schantz 1987)

The control of cysticercosis of zoonotic importance is possible and can be made a reality with the creation of an awareness of these infections among the general public improvement of sanitation and provision of facilities to dispose human faeces in a proper manner is of prime importance. Confined rearing of pigs could effectively control *Taenia solium* cysticercosis. However, since the above methods are not easily implementable the other control measures can be based on the following :

1. Detection of infection by serodiagnostic tests in order to treat animals or after slaughter to suitably attend to infected carcasses (heat or cold) in the case of light infections or incineration of heavily infected ones.
2. Cooking of meat to more than 57° which is the thermal death point of cysticerci is one of the practicable methods of control. It is also cost effective and affordable.
3. Freezing of infected carcasses at -20°C for more than 12 hours.
4. Antemortem detection of infection by tongue examination and serodiagnostic methods.
5. Treatment of infected pigs and cattle with single dose of praziquantel 50-200mg/kg and as single dose of 30 mg/kg Albendazole or 50 mg/kg for 3 days was effective against bovine and porcine cysticercosis (Soulsby, 1982).
6. Immunization trials conducted with antigenic extract from *C.cellulose* (250 -g protein IM) was considered effective

and hence immunization after proper standardization could be included in an integrated program of eradication.

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## Case Report : Degnella Disease and Probable Treatment Schedule

**B. K. Pradhan**

District Veterinary Hospital, Saharsa, Bihar

During December 1997, there had been disease which was turned to an epidemic form in the whole of Koshi region of Bihar, covering the districts of Saharsa, Purnea, Samastipur and Darbhanga.

Veterinarians all over the region primarily diagnosed the disease as 'Degnella' although it is not confirmed. The disease affected animals mostly in low lying areas and occasionally in high land areas. The disease affected country-breed bovine, specially Buffalo of all age groups. It rarely affected cattle (both cows and bulls) and exotic breeds. The affected cattle recovered quickly after treatment within two to three days. But in buffaloes, treatment schedule extended upto 7 to 10 days and the recovery sometimes prolonged upto 15 to 20 days.

### Observations :

It was observed in the field that preventive treatment with Levamisole hydrochloride (Kalmisole injection from Karnataka Antibiotics) at the recommended dose of 10ml daily for 3 days subcutaneously minimised the chances of getting Dagnella infection. Often it was also seen that where the FMD vaccination has been done (Hoechst Roussel Vet FMD vaccine @ 5ml subcutaneously) previously, the animals of that area were almost free from this disease.

### Symptoms :

The main symptoms observed by in an affected animal are :

1. Due to lameness (one or two legs) or sometimes (all the four legs), animals were unable to move.

2. Swelling of the legs in the stifle or fetlock joints
3. Anorexia
4. Temperature was slightly elevated, but became normal after initial treatment
5. Cracking of the hooves, the entire skin near the hoof was filled with lesions. The muscles became dystropied and there were lesions, leading towards wounds.
6. There was sloughing of the pinna, the border or extremities, the ear extremities curled and there was formation of dry gangrene. The tail also became inflammated in the lower to middle parts, necrosis of the tail was a common sight specially in the switch of the tail.
7. Swelling of the muzzle was also noticed.

### Suggested Line of Treatment :

Treatment of the animals suspected with 'Degnella' disease have been carried out in different ways.

#### A) First Line of Treatment Schedule

1. Antihistamine, Pheniramine maleate injection (Hoechst Roussel Vet) 10ml intramuscularly daily for 3 days.
2. Antibiotic [Kloxamp (Karnataka Antibiotic Laboratory) 2.0 gram daily for 5 days intramuscularly]
3. Diclofenac Sodium 30ml (10ml per 100 kg bw. Daily for 5 days)
4. Milk Iodine (@ 20ml intramuscularly daily for 5 days)

5. Charmil ointment (Dabur) for topical application

**Results :** It was noticed that only 60 to 70 percent of total animals were recovered

**B) Second Line of Treatment Schedule**

1. Antihistamin, Pheniramine maleate injection (Hoechst Roussel Vet) 10ml intramuscularly, daily for 3 days.
2. Floxidin injection (Hoechst Roussel Vet) 15ml daily intramuscularly, for 5 to 7 days.
3. Prednisolone injection C.S. (Hoechst Roussel Vet) 10ml daily intramuscularly for 5 to 7 days in non-pregnant animals / Diclofenac Sodium in pregnant animals.
4. Teeburb capsules (Indian Herbs) @ 4 capsules twice daily, for 5-7 days.
5. Himax Ointment (Indian Herbs) to be applied topically.

**Results :** It was noticed that 90 to 93 percent of total animals were recovered.

**C) Third Line of Treatment Schedule**

1. Pheniramine maleate injection (Hoechst Roussel Vet) 10ml twice daily, for 3-5

days)

2. Floxidin injection (Hoechst Roussel Vet) intramuscularly 15ml daily, for 6 days
3. Prednisolone injection C.S. (Hoechst Roussel Vet) 10ml daily intramuscularly for 5 to 7 days in non-pregnant animals / Diclofenac Sodium in pregnant animals.
4. Triamcinolone acetate 5ml daily, for 6 days.
5. Teeburb capsules (@ 4 capsules daily twice, for 10 days).
6. Cofecu Plus capsules (10 tabs daily, for 10 days)
7. Himax ointment for local application.
8. Agrimin powder (Glaxo) @ 100 grams daily, for 10 days.

**Results :** The above treatment which resulted 95 - 100% recovery, was most effective with Floxidin and alongwith other supportive treatments. It is hoped that the above observations will be of some help to the veterinarian in the field, coming across such cases.

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## Case Report : An Unusual Case of Ehrlichiosis with Nervous Symptoms in Dog

D. N. Rajguru, L.G. Anantwar and V.M. Machinder

Department of Veterinary Medicine, College of Veterinary & Animal Sciences, MAU, Parbhani

Canine ehrlichiosis is gaining importance as a clinical disease in India (Thilagar *et al.*, 1990 and Jain and Gupta, 1997). It is caused by *Ehrlichis canis*, an obligated parasite of the mononuclear cells and is distributed world wide. This rickettsial disease is transmitted by the brown dog tick *Rhipicephalus sanguineus* (Groves *et al.*, 1975). Fleas and mosquitoes have also been recorded as possible vectors. (Troy *et al.*, 1980).

### History and Clinical examination :

A six year old male German Shepherd was referred to the veterinary Polyclinic, Marathwada Agricultural University, Parbhani with the history of epistaxis and ataxia. It was treated with blood coagulants, Ampicillin, Vitamin B complex injections by the local veterinarian. In spite of the vigorous therapeutic efforts, no improvement was observed. Clinical signs with physical examination revealed rough coat, weight loss, anorexia, pyrexia, ataxia, seizures, dyspnea, shivering, pneumonitis, depression and congested mucous membrane. Bleeding was manifested as epistaxis and haematemsis. Ticks infestation were not found on the body of dog.

### Laboratory examination :

The radiograph of nasal area did not reveal anything abnormal. The blood examination revealed haemoglobin (Hb) 5.2 gm percent, packed cell volume (PCV) 21%, total erythrocyte count (TEC)  $5.07 \times 10^6$  /cmm and differential leucocyte count (DLC)

showed lymphocyte -12%, neutrophils-87%, monocyte-1%, eosinophils-nil and basophils-nil. Blood smears (ear tip) were stained with Giemsa's stain. Inclusion bodies of *E. canis* was found in monocytes.

### Discussion :

Ehrlichiosis is an important disease with the symptoms of pyrexia, anaemia, vomiting, weakness and lethargy (Kuehn and Gaunt, 1985) while acute nervous symptoms were recorded first time in this case. There was no evidence of tick infestation and possibly, fleas or mosquitoes could have been the possible source of vector for the transmission of the infection. Similar findings have been reported by Troy *et al.*, (1980). In this case morulae of *E. canis* were found in monocytes which co-relate the study of Ewing *et al.*, (1971). Leucocytosis has also been reported in canine ehrlichiosis by Thirunavukkarasu *et al.*, (1994). In severe cases, epistaxis occur because of thrombocytopenia (Huxsoll, 1976) or due to functional defects of platelets (Gaunt *et al.*, 1990).

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## Case Report : Congenital Abnormalities in Gaddi Lamb

A.A. Vamerzani and G.N. Sheikh

Department of Veterinary Medicine, Sher-e-Kashmir University of Agricultural Sciences & Technology, Srinagar

This report is intended to present here regarding the observations made on congenital abnormalities in Gaddi lamb. Gaddi lamb born during 1995-96 at the unorganized farm in Kashmir. An ewe gave birth at the age of four to a male lamb. His mother gave three normal births before this case. The abnormal lamb born alone typically from a normal ewe, but hereditary background of the ewe was unknown. The lamb was born with curved lumbo-sacral region, forelegs were bent backwards and hind legs were long and bent from the hock joints and not flexible (See Fig.). The lamb was not able to stand, but survived by hand fed mother milk. Arthrogryposis, which has been used to convey the description of joint fixation, strictly means fixation. In fixation the term congenital articular rigidly has been introduced (Swatland, 1974). The immobilization of the joint may be due to lack of extensibility of muscle, tendons, ligaments or other tissue around the joints or deformity of articular surfaces, or theoretically fusion between the bones of the articular surface.

Infection of ewe during early pregnancy with the Akabane virus results in the production of lambs with congenital arthrogryposts, but on investigation virus could not be isolated from affected lamb in the same enzootic area in which the lamb was born. If congenital arthrogryposis was due to Akabane virus, no treatment would be contemplated because affected lambs are not viable because the disease is transmitted by insect bites. Blood and Henderson (1974) and Dennis (1975) suggested that feeding on the plants such as

*Veratum Californum*, *Trachymene*, *chrocea* and *T.cyanankha* around the 14th day of gestation or due to trace elements deficient diet may produce this type of conditions in pregnant animals. The present case of such defect may not be the attributes of the above mentioned cause, as the animals in the farm were stall fed with mineral supplemented concentrate feed mixture. Hence the possibility of homozygosity of recessive genes as suggested by other scientists may hold good for the present observation. Similar skeletal defects such as arthrogryposis and cleft palate may also be due to hereditary factors as have been suggested earlier (Jubb and Kennedy, 1970).



**Fig. : Showing congenital abnormalities in Gaddi Lamb**

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## Case Report : Observations on a Clinical Case of Uraemia in Dog

**A. Bhaumik**

Veterinary Surgeon, Agartala

Uraemia may also occur due to post-renal causes like complete obstruction of the urinary tract or internal rupture of any of its parts (Blood and Radositits, 1989). The pathological alterations in such cases are also on record (Anon., 1986). The present communication deals with some of the haemato-biochemical changes in a clinical case of uraemia in dog.

A male spitz dog aged about seven years was brought for treatment with the complain of inappetence, dullness, weakness, irregular bowel evacuation and urination for about one week. Though scanty, the physical quality of urine was apparently normal. History revealed no previous incidence of urinary obstruction. Rough body coat, highly anaemic mucosae, rectal temperature of 102.4°F, and slight abdominal distension were noticed on clinical examination. Suspecting the case to be of anaemia accompanied by initial stage of ascites, treatment was advised comprising of oral ampicillin, frusemide and a liquid haematinic preparation containing protein hydrolysate; and intra-muscular vitamin B-complex with liver extract. On 5th day slight improvement in the clinical condition was noticed. As no anthelmintic was given during last one year, mebedazole tablets were also prescribed.

About 30 days later, the dog was brought with the signs of anorexia, severely distended abdomen, laboured breathing, pale and congested conjunctivae, mild oedematous, swelling of all the four legs, and oliguria. Rectal temperature was 101°F and pressure on tense abdominal wall evinced pain. Abdominal paracentesis could yield about

one litre of clear to straw (occasionally reddish) coloured fluid with frank smell of urine.

Examination of venous blood indicated the TLC-4.8 x 1000 $\mu$ l, Hb-4 gm/dl, PCV-8% and a neutrophilia on DC (neutrophils-85%, lymphocytes-15%). On blood biochemical analysis BUN-65 mg/dl, creatinine-1.6 mg/dl, and bilirubin-0.8 mg/dl were recorded. No protozoa / bacteria in the stained blood smear and no cells in the abdominal fluid sample could be found. Faecal examination also did not show presence of any helminth parasite.

Paracentesis and clinico-laboratory findings suggested uraemia resulting from uroperitoneum. Clinical symptoms and the significant increase in BUN in the present instance are in conformation with the reports of Sinha *et al.* (1986), and Tanwar and Saxena (1986) in cases of uraemia due to canine urolithiasis. Low Hb content and neutrophilia along with lymphopenia have also been recorded by Sinha *et al.* (1986). However, finding of a remarkably low PCV in the present case could not be interpreted because of paucity of relevant literature.

The dog died after two days of removal of fluid and the exact reason of uroperitoneum remained unascertained. But this might be attributed to rupture of the urinary tract as a result of obstruction due either to renal calculi, neoplasm, blood cells, or severe prostatic enlargement (Bush, 1990). And death in such cases may result from cardiopulmonary involvement as a consequence of uraemia as has been

described by Gangwar *et al.*, (1991) in experimentally induced post-renal uraemia in calves

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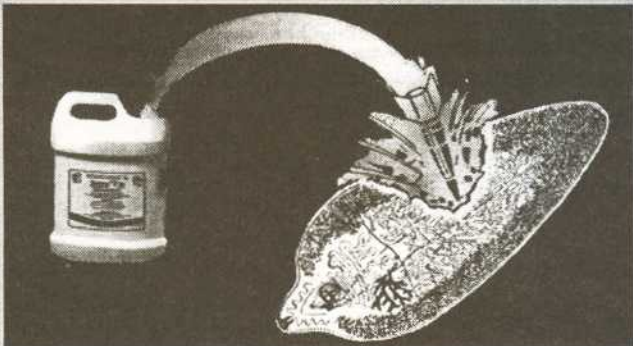
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## Case Report : *Dermatophilus* Infection in Cattle

K. Ananda Kumar and M. Krishnappa

Department of Animal Husbandry and Veterinary Services, Maddur Taluk, Mandya District, Karnataka

*Dermatophilus* infection is dermatitis occurring in all species of animals caused by organism belongs to genus *Dermatophilus* var *saccghan*. The name of the species which causes disease in cattle is *Dermatophilus congolensis*. Ten such cases of dermatitis in cattle were reported to Veterinary Dispensary, Nidaghatta and Honnalagere of Maddur Taluk, Mandya District.

Incidence was high during October and November. The lesions were restricted to distal parts of all four limbs, internal aspect of thigh and sometimes on udder. Lesions were small horny crust varying in colour, creamy to pink, measuring around 1.5cm to 2 cm. Beneath the lesion there was granulation tissue and pus like secretion. Lot of dandruff and thickening of skin in later stages (see Fig.) were also observed. Animals were reluctant to move due to severe pain. Except for this lesions animals were apparently healthy.



Fig.: Showing *Dermatophilus* Infection on the hind legs.

### Treatment and Discussions :

Different treatment regimes were in vague, like use of Tetracycline (5mg/kg b.w.), repeated weekly and other workers reported the use of Procaine Pencillin at the rate of 70,000 IU/kg b.w. and Streptomycin (70 mg/kg b.w.) suppose to be 10% effective (Ilenobade, 1984). However, in this case injection of Procaine Pencillin, Streptomycin and Tetracyclines did not show any improvement as it is expected. Some of the condition relapsed after few days. Animals were also given Diclofenac sodium (Zobid) 25ml I/M for lameness. Coupled with above treatment, simultaneous use of Gamagrine (Gama Benzene Hexachloride), Himax ointment as local application had given beneficial results. Outbreaks of this disease was recorded in sheep, goat and horse in Africa resulting in economic losses.

In sheep, this disease is called mycotic dermatitis and caused economic loss in terms of death and loss of skin (Oduye and Lloyd, 1971). The findings in this case report are in accordance with the report of Macadam (1964) that treatment regimes followed in particular place has little or no value elsewhere. Incidence of this disease, so far in this area in sheep and goat was not recorded.

In Africa, in case of sheep where large numbers are affected, 0.2 to 0.5% of Zinc Sulphide as dip or spray found to be very effective. Hard and Tyrzkiewichzh (1968) reported the beneficial effects of 1% Alum as a dip.

### Control Measure :

- 1) Isolation of affected animals
- 2) Avoid contact with infected material like grooming tools
- 3) Tick Control

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## Case Report : Concurrent *Anaplasma marginale* and *Theileria annulata* Infection in a Jersey Cow

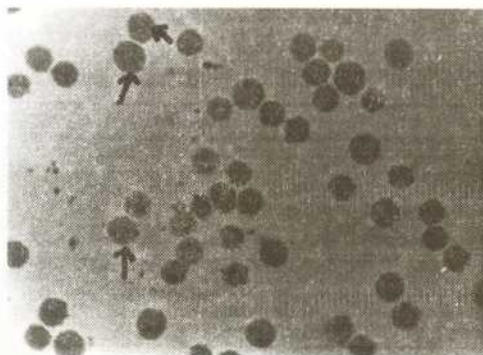
A.K. Sharma, V.K. Gupta, R.S. Kistwaria and B. Pal

Veterinary Clinics, College of Veterinary & Animal Sciences, HPKV, Palampur, H.P.

The present communication documents a rare report of concurrent infection of *Anaplasma marginale* and *Theileria annulata* in a jersey cow from Himachal Pradesh. A jersey cow about 8 years old referred to Himachal Pradesh Krishi Vidyalaya, Veterinary clinics (Regd. No. 1131) with the history of anorexia, nasal discharge, coughing with laboured breathing and reduced milk yield. Clinical examination revealed temperature (102°F), respiratory rate 28 per minute, ruminal motility 1/3 per minute. Conjunctiva were pale, pre-scapular and pre-femoral lymphnodes were highly enlarged. Jugular pulsation were observed on both sides. Blood was collected for haematological investigation. Smears were prepared, fixed in methanol and stained in Giemsa.

Haemogram revealed severe anaemia (haemoglobin-4.8 gm%, reduced packed cell volume-11%), total erythrocytic count (TEC) 2.17 million/cmm of blood. Differential leucocyte count (DLC) revealed neutrophilia (N-50%), lymphopenia (L-50%) and severe leucopenia, 1630 thousand /cmm of blood. Microscopic examination of blood smear (Giemsa stained) confirmed the presence of intra-erythrocytic bodies, indistinguishable from *A.marginale* and *T.annulata* (see Fig.). Animal succumbed before any treatment could be administered. Necropsy findings revealed icteric subcutaneous body tissue an mucous membranes, generalised engorgement of blood vessels and haemorrhages; pre-scapular and pre-femoral lymphnodes were enlarged three to four

times to their normal size. Spleen was also enlarged. Liver had patchy areas of necrosis.



**Fig.:** Blood smear showing *Theileria annulata* & *Anaplasma marginale* (piroplasma) in erythrocytes. (Giesma : X100)

Mixed infections of haemoprotozoans in crossbred animals in bovines are frequently encountered Gautam *et al.* (1984) and in the present case also the animal was Jersey cow. Clinical and post-mortem findings recorded in the present investigation were typical as described by other workers in haemoprotozoans diseases. (Jitenderen, 1977, Rodostitis *et al.*, 1994, and Gautam *et al.* 1984) The disease was in terminal stage as evident from haematological findings and animal died of severe anaemia leading to tissue anoxia but Galhotra *et al.* (1979) reported that intense erythrophagocyte cused by auto-immune phenomena could be the possible cause of death in such cases. Mixed infections of *T.annulata* and *Babesia*

*bigemina* has been reported from Himachal Pradesh (Jitenderen *et al.*, 1977) but perusal of literature did not reveal any report of mixed infection of *T.annulata* and *A.marginale* from this area hence the same is being placed on the record.

#### Acknowledgement :


Authors are grateful to the Dean, College of Veterinary & Animal Sciences, HPKV for the facilities provided and Dr. A.K. Mishra, Senior Scientist, Division of Parasitology, IVRI, Izatnagar (U.P.) for confirmation of the diagnosis.

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	Mange	10 ml.	5	1 lt. Taktic per 500 lt. of water added.	1.5 lt. Taktic per 500 lt. of water added.
Sheep Goat	Ticks	10 ml.	5	1 lt. Taktic per 500 lt. of water added.	1.5 lt. Taktic per 500 lt. of water added.
	Mange Lice/ Keds	20 ml.	5	2 lt. Taktic per 500 lt. of water added.	3 lt. Taktic per 500 lt. of water added.

- **Presentation** : Available in 50 ml. and 250 ml. tin packs with measuring Caps.
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## Case Report : *Stephenofilaria* Infection in Buffalo

Rajesh Kapoor and R.P. Gupta

Disease Investigation Laboratory, Animal Husbandry Department, Jammu

Disease Investigation Laboratory was approached to investigate a case of dermatitis in a 10 year old buffalo at village Sai (R.S. Pura area). The skin lesions started from ventral region near udder and consequently spread over almost 80% of body surface with pus and blood oozing, skin forming crusts and shedding off. The case was earlier treated by local veterinarian with antiseptics and Penicillin for five days without any response.

### Observation :

Blood smear revealed absence of haemoprotozoa. Skin scrapping was dissolved in 10% Potassium Hydroxide solution and examined under microscope, *Stephenofilaria* sp. was isolated.

### Treatment :

Animal was given 0.2% Butox (Deltamethrin, Hoechst Roussel Vet) solution by spraying method. Three sprays in a week's time cured the animal completely.

*Stephenofilaria* sp. was isolated from skin scrapping of a buffalo and treated successfully with 0.2% Butox solution. *Stephenofilaria* sp. (nematode), spread by flies *Musca conducens* and *Musca autumnalis*, in the skin of large animals is being reported for the first time from Jammu region in J&K.


### Acknowledgements :

Thanks are due to Dr. Iqbal Singh, Livestock Development Office, R. S. Pura and to Dr. R. L. Handoo, Director, Animal Husbandry, Jammu for providing necessary help and facilities.

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## Case Report : An Outbreak of Rumen Acidosis

**T. Umakanthan**

Veterinary Dispensary, Uthamapalayam (P.O.), Theni (District)

Pongal - the important Hindu festival is celebrated on January 15th to show the farmers gratitude to their livestock. As a custom, on the day, farmers provide a little quantity of cooked sweet rice to their livestock. On January 16th and 17th an outbreak of Rumen Acidosis in nine cattle recorded within a radius of three kilometers from this dispensary. During this outbreak a new group of antacid (Nilcid MPS - Abbotts Laboratories) was also tested for its potency in rumen acidosis.

History of all the cases revealed the excessive feeding of sweet rice on the festival day as a gesture of gratefulness. The following symptoms were noticed in the affected animals; anorexia, temperature between 37°C and 39.5°C, depression, staggering gait to lateral recumbancy, laminitis especially in hind limbs, reduced rumen motility, heart rate between 80-120/mt, *polypnoea*, *oliguria* and *anuria*, soft dung and recumbancy with intermittent kicking of limbs in some animals.

All the nine animals were withheld from water and feed for 24 hours, 50 Nilcid tablets (each Nilcid tablet contains Magaldrate 400mg and Simethicone 60mg) dissolved in one liter of water, given orally twice daily for two days and Pheniramine maleate 136.50 gms to 227.50 gms (Avil, Hoechst Roussel Vet) administered intramuscularly once daily for two days and severely affected animals were also given Ringers lactate (Kokad) 540ml x 2 to 4 bottles dissolved with or without Hivit 20 ml (Ranbaxy Ltd) intravenously for two days. Limited water and dry feed provided on second day.

All the animals were completely recovered in 24-60 hours of treatment.

### Acknowledgement :

The author thanks the Director, Department of Animal Husbandry, Tamilnadu for the facilities provided.

# butox

**A New Generation Ectoparasiticide**



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## Case Report : Deltamethrin (Butox) as treatment for Myiasis

Jenny E. Fernandes, B.P. Dandge and S.P. Mehesare

Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola

Myiasis or maggot infected wounds have been a source of problem to the veterinary surgeons to treat them effectively, since they not only eat the healthy flesh and decaying tissue of the wound, but they also delay the healing process of the wound. This delay in healing, increases the chances of secondary bacterial infection. The larvae of diptera flies accidentally or obligatory take on a parasitic existence after hatching from their eggs (Soulsby, 1975 and West, 1990).

Previous treatments for such myiasis cases at the Campus Veterinary Hospital of Dr. P.D.K.V., Akola included Turpentine oil and Eucalyptus oil. In the present study Butox (Deltamethrin), a synthetic pyrethroid, manufactured by Hoechst Roussel Vet, was used in various clinical cases involving myiasis. Butox was diluted in 1:10 parts with water, and introduced as cotton soaked swabs. Complete death of maggots was observed within 12 hours. An average of a single application of Butox was required on these cases for complete eradication of the maggots. No tissue damage or any local

inflammation was noticed. Immediate destruction of the maggots ensured a faster healing of all wounds and thus reduced chances of any secondary bacterial infection.

Deltamethrin being amongst the most potent ectoparasiticide has been indicated in all stages of ticks, mites, lice and other insects. (Rossi and Lanfranchi, 1992 and Sharma *et.al.*, 1992). This study confirms the use of Butox as an efficient maggotocide.

### References :

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Just as iron rusts from disuse,  
even so does inaction spoil the intellect.

- Leonardo da Vinci

## READERS' COLUMN

1. **Dr. D. N. Rajguru**

Professor of Medicine, MAU, Parbhani

The article entitled, "*In-vitro* Efficacy of Cefquinome (INN) and Other Anti-infective Drugs Against Bovine Bacterial Isolates from Belgium, France, Germany, The Netherlands and The United Kingdom" is unique and use of Cefquinome is the need of today. This unique booklet is a great boon for veterinarians and livestock owners / farmers including pet and wild life in particular. My good wishes to "*The Blue Cross Book*".

2. **Dr. Shirendra Nath Sabarwal**

Ex. Chief Veterinary Officer, Kanpur, U.P.

It contains a very useful article "Post-bite efficacy of Candur-R in dogs". It gives very practical research and useful information on veterinary practice. This is excellent book for dog practitioners like me.

3. **Dr. H. G. Apte**

'Vet Care Pet Clinic', Pune, Maharashtra

Articles on "Post-bite efficacy of Candur-R in dogs" is very useful in the field. Please try to publish few more articles on small practice, which will be of practical use to private vets.

4. **Dr. Nair Velayudhan**

Dy. Director, Directorate of A. H. & Veterinary Services, Panji, Goa

I like this issue because NEWS - "Starving Mosquitoes to Death" was interesting. Its a way to control Malaria in India.

5. **Dr. G. P. Patgiri**

Sr. Epidemiologist, Veterinary College, Khanaopara, Guwahati.

All the issues of "*The Blue Cross Book*" are of excellent quality, certainly helpful to vets in general and field vets in particular. Looking forward for more informative issues. Best wishes to Dr. A. K. Datta, Editor, "*The Blue Cross Book*".

6. **Dr. D. Swarup**

Senior Scientist, Div. of Experimental Medicine, IVRI, Izatnagar, U.P.

The "*The Blue Cross Book*" provides valuable information for veterinary clinicians and researchers. The paper entitled "Effects of

## READERS' COLUMN

Aflatoxin on Immune Response in Viral Diseases" is of immense interest.

7. **Dr. M. U. Siddiqui**

Head, ET & SS, Salon, Rae Bareli, U.P.

It provides good information on the latest products and their field trials. But it does not speak about field trials on animal reproduction performance. The objective of this publication should be broad based to accommodate more areas of veterinary profession. However, it is quite informative and useful in its present form.

8. **Dr. A. K. Sinha**

Professor & Chairman, Ranchi Veterinary College, Bihar

Like other previous issues, 10<sup>th</sup> issue is simply an excellent presentation. Some articles on pet practice, management, breeds etc. may be included from time to time.

9. **Dr. K. A. Doraisamy**

Professor & Head, Univ. Training & Research Centre, Salem

The research articles in this issue are practical oriented. Practically the research article entitled, "Flavomycin-40 as a Feed Supplement in Dairy Cattle for High Milk Yield, by Narsapur, Vaidya and Datta, clearly says the quantity of Flavomycin-40 to be added and its effect on milk yield and composition have been clearly studied. Such practical oriented findings will be useful to farmers.

10. **Dr. B. R. Boro**

Retired Professor of A.A.U., Khanapara, Guwahati

I like this issue because of a good article, "Effects of Aflatoxine on Immune Response in Viral Disease". Further, this is an excellent informative booklet for the practising veterinarians in the field.

11. **Dr. K. K. Vaish**

Varanasi, Kabir Marg, U.P.

This issue gives good details on Flavomycin-40 as feed supplement in dairy cattle for high milk yield for the field practice. I would like to have more information for treatment of canine animals and latest investigation in the line of different diseases.

## READERS' COLUMN

**12. Dr. V. S. Raghavan**

Veterinary Hospital, Tiruvannamali, Tamilnadu

The articles published are highly informative and keep abreast with latest development in the veterinary field. Article on the 4th generation antibiotics by A, Bottner *et al.*, provides latest information in regards to antibiotics and their usages.

**13. Dr. K. Dayanand**

Soondapet, Tirukkoitur

It gives useful case report eg. "Polioencephalacia in a Kid" which is very useful for field applications. Spelling mistakes are seen in this issue eg. Page No. 25 last paragraph, first line instead of harbours, the wrong spelling 'arbours' is noted.

**14. Dr. H. K. Palanisamy**

Clinician, Erode, Tamil Nadu

"Ruminal Acidosis in Cattle and Buffaloes" a problem is not only in Patna but it exists in India as a whole. You have commented the same in a correct form. "Post-bite Efficacy of Candur-R Vaccine in Dogs" gives me a clear picture of my doubt whether to use it or not.

**15. Dr. S. K. Basu**

Hooghly, West Bengal

This book is very helpful to practising and non-practicing veterinarians. The book will be very more helpful if articles on farm animals, canines and poultry are separately dealt with.

**16. Dr. U. V. Pednekar**

Veterinary Officer, Panji, Goa

In the article, "Enrofloxacin (Floxin Vet) in the treatment of sub-clinical mastitis in cows", (Ref. on page 33) it is mentioned as Pednekar U.V.T. and Swarup, D. (1991). This may please be corrected as Pednekar, U.V.T. and Swarup, D. (1991).

# *The Blue Cross Book*

for the Veterinary Profession

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M.Sc., Ph.D., D.M.L.T.(JU)  
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