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The Editor
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I am really glad to know that the 8th issue of 'The Blue Cross Book' is going to be published shortly. I take this opportunity to congratulate you and all the Editorial Board Members on this occasion.

The success story of The Blue Cross Book goes back to the veterinary department of HVG in Germany in the year 1958. The basic idea of the then 'BLUE BOOK' was to publish new research findings from all over the world in veterinary medicines and to make it available to field veterinarians. I understand that the same practice is being maintained in India.

Let me also take this opportunity to say here that the feedback we get from you that the reader has become a very positive one. Your response is the main motivator for the Editorial Board Members to make the publication in future even more interesting and beneficial to the field veterinarians in India.

I am convinced that the enthusaism and professionalism of the Editors will go a long way to establish close relation between Hoechst Roussel Vet and the veterinary friends in the field.

I wish you all success.

Wolf-Jochen Bader

Mr. Wolf-Jochen Bader

Mr. Wolf-Jochen Bader joined Hoechst GmbH in the year 1987 as Manager, Accounts Headquartered at Munich, Germany, after obtaining the basic University Education with the professional Diploma, Betriebswirt (F.H.) at Germany. He continued in the same position till December 1989 and subsequently from January 1990, he took over the charge of Manager, Controlling. During February 1993, he left Germany for South Africa to guide the Finance Dept. of Hoecht AG. Vet Pty. Ltd. as Financial Controller. From there, during March 1995, he moved to Hoechst U.K. to head the Finance & Logistics Department.

In the meantime, Hoechst merged its Animal Health activities with Roussel on a worldwide basis and formed a new stand alone company under the name "Hoechst Roussel Vet". One of the company's objectives is to strengthen its business activities in South East Asia. Hoechst Roussel Vet India has been identified as a key country in this region with focus on building up its own production facilities, further strengthening of Research and Development and expanding its export business activities. Mr. J. Bader is heading Hoechst Roussel Vet India as Managing Director with effect from November 1996.

Editor

Efficacy of Enrofloxacin in Bovine Mastitis

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Introduction

Mastitis is an important disease in bovines which inflicts heavy economic losses due to reduced milk production and adverse effects on manufacturing of the milk products from the infected milk (Rogers and Mitcell, 1986; Beck *et.al.*, 1992), It is a common finding that when organ damage to the udder due to mastitis is severe it is irreversible. Thus, the loss in milk production to the infected udder quarter is perpetual (Blood and Rodastits, 1989). It is, therefore, important to diagnose this disease in the subclinical stage and if needed the treatment should be carried out immediately or at the time of drying off (Morre and Heider, 1984).

A number of antibiotics have been recommended for use in mastitis and like other bacterial infections the incidence of the development of drug resistance is quite high. The rate of introduction of newer antibiotics for treating mastitis, therefore, is relatively high. Enrofloxacin is a newer generation antibiotic which is known to have a wide spectrum of antibacterial activity. It was, therefore, planned to take up studies to evaluate the efficacy of the drug in treating mastitis in bovines.

Materials and Methods

The trials were conducted at various dairy farms around Mumbai. The farms were first screened for subclinical mastitis for which modified california mastitis test (MCMT) (Schalm, 1962) and the newly developed Marathwada Agricultural University Mastitis (MAUM) test were done. The later test is rapid and is based on detection of threshold levels of antiproteases in milk (Samad and Awaz, under publication). Animals eliciting positive response to both the tests were diagnosed as mastitic and taken up for the studies. The response to treatment was based on reduction of antiprotease titre before and after treatment. The animal was declared as cured only when the antiprotease was not detectable in milk.

In all 138 cows belonging to different farms around Mumbai were screened to detect mastitis. We have recorded a quarter infection rate of more than 25% at individual farms. Out of these, 38 positive quarters were selected for the study. The cows diagnosed positive for mastitis were randomly allocated to three groups. Group I cows were administered Enrofloxacin intramammary, whereas to the Gorup II cows, in addition to intramammary, Enrofloxacin was also administered parenterally. Group III animals constituted control to whom no treatment was given during the span of the trials. Animals in the treatment group I were infused with Enrofloxacin 250mg (Hoechst Marion Roussel) per quarter per day intra-mammary per day whereas the group II animals were given 250mg Enrofloxacin intramammary and 600 mg Enrofloxacin subcutaneously every day until complete recovery or maximum five days. The milk samples from these cows were subjected to antiprotease titre daily.

The efficacy of the drug was evaluated in terms of number of infusions needed to reduce the milk antiprotease titre to non-detectable levels.

Results and Discussion

Mastitis is a disease of major economic importance for the livestock owners. High yielding animals are at greater risk of the

disease. To reduce new clinical cases in the farm the only plausible way is detection and treatment of the disease at the subclinical stage. We have used a recently developed spot test to detect mastitis since it is sensitive and specific as compared to other routinely used indirect tests. The new test is easy-to-perform and interpret. In the present study antiprotease titre assay was also used to evaluate the efficacy of treatment, since the antiprotease concentration in milk reflects the presence and intensity of inflammation due to infection (Awaz and Samad, 1994). We have consciously not undertaken bacterial culture and sensitivity since many veterinarians in the field have to take the therapeutic decision without an aid of milk culture. Moreover, the *in vitro* culture and sensitivity does not necessarily reflect the in vivo situation (Prescott and Baggot, 1985). It was, therefore, appropriate to evaluate the efficacy of Enrofloxacin intramammary and intramammary plus parenteral without doing a bacterial culture and sensitivity.

Administration of 250 mg Enrofloxacin intramammary appear to be well tolerated by the udder tissues since no untoward reaction to the drug was recorded. The details of the animals in treatment groups and the response to the treatment are given in tables 1 and 2.

The results indicate that in gorup I which was administered Enrofloxacin intramammary, 12 out of 15 infected udder recovered fully whereas in three quarters no improvement was recorded even after 5 days of treatment.Thus, the efficacy of Enrofloxacin given intramammary in the present trial was 80%. The cows included in the present study were not from one farm but belonged to different units around Mumbai. The higher efficacy, therefore, cannot be attributed to a single bacterial isolate from the same farm.

The group II animals were administered

Table 1 : Efficacy of Enrofloxacin in bovines when given intramammary

Sr. No.	Quarter affected	BEN units per ml.		Duration of treatment days
		TI titre before treatment	TI titre after treatment	
1	Right hind	400	not detectable	3
2	Left hind	1600	not detectable	4
3	Right front	800	not detectable	3
4	Right hind	1600	not detectable	4
5	Left hind 800	800	not detectable	3
6	Right hind	400	not detectable	4
7	Right front	800	800	5
8	Left hind	1600	not detectable	3
9	Right hind	3200	1600	5
10	Right front	800	not detectable	4
11	Right front	N.D.	not detectable	3
12	Left hind	800	1600	5
13	Left front	400	not detectable	3
14	Right hind	800	not detectable	3
15	Right hind	N.D.	not detectable	4

Table 2 : Efficacy of Enrofloxacin (intramammary + parenteral) in bovine mastitis

Sr. No.	Quarter affected	BEN units per ml.		Duration of treatment days
		TI titre before treatment	TI titre after treatment	
1	Left hind	1600	not detectable	3
2	Right hind	800	not detectable	4
3	Right hind	1600	not detectable	4
4	Right hind	800	not detectable	3
5	Left hind	1600	not detectable	4
6	Right hind	3200	not detectable	4
7	Right front	800	not detectable	3
8	Right front	400	not detectable	3
9	Right hind	1600	not detectable	3
10	Left front	400	not detectable	3
11	Right front	1600	not detectable	3

BEN units : Benzoyl-arginine-p-nitroanilide equivalent neutralizing units.

Enrofloxacin intramammary and parenteral. The results indicate that combined administration through the two routes is definitely superior over the single intramammary route. As is evident from the table 2 all the cows treated in the group responded within 3-4 days of treatment. This is in agreement to other workers who have reported better recovery rates with systemic antibiotic treatment (Ziv, 1980). The control group animals however remained infected in that the antiprotease titre remained similar to or more than the initial levels. These animals were later on treated with another antibiotic under our trials.

The reduction in the antiprotease titre after treatment to undetectable level provide an objective criterion for evaluation of mastitis treatment since this can be attributed to mitigation of the inflammation.

Based on the data of the present studies, it is recommended that Enrofloxacin can be used to treat mastitis in bovines and that the administration by intramammary and parenteral route simultaneously is superior over single intramammary route.

If the drug is to be recommended for intramammary use, it is imperative to study the drug residues in milk after intramammary and parenteral use. This will provide the data to recommend the mandatory milk withdrawal period.

Acknowledgement

We are indebted to M/s. Hoechst Marion Roussel for the financial assistance and supply of the drug. We also acknowledge the co-operation extended by Dr. A. K. Datta, Manager Technical, Hoechst Marion Roussel. Thanks are also due to various practising veterinary Officers for extending their cooperation.

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Concentration of Cholesterol in Follicular Fluid and Follicular Growth in Buffalo Ovaries (*Bubalus Bubalis*)

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Follicular fluid (FF) is composed of secretion from the follicle and exudates from plasma. Its composition reflects changes in the secretory process of the granulosa and theca intern layers (Edwards, 1974). The FF provides a suitable microenvironment for the growth and maturation of oocyte and is vital for the maintenance of fertility in the female (Kulkarni, 1988). The constituents of FF are considered as regulatory factors in follicular development and steroidogenesis (Brantmier, 1987). Among the constituents, cholesterol is one of the precursor for synthesis of various steroid hormones such as androstenedione, progesterone and oestrogen. On perusal of literature very few attempts have been made to assess the level of cholesterol in growing follicle in farm animals. (Swine : Chang, 1976; Cow : Savion, 1981; Brantmier, 1987; Buffaloe : Parmar, 1991, Goat : Sharma, 1996).

Hence this work was undertaken in buffalo to delineate the relationship between cholesterol level in follicular fluid and follicle growth.

Materials and Methods

Fifty pairs of ovaries were collected from nonpregnant buffaloes irrespective of stage of oestrus cycle immediately after slaughter from local slaughter house, Chennai. Ovaries were categorised as left and right and transported to the laboratory in sterile polythene bags containing ice. 110 follicles from left and 196 follicles from right ovaries were measured and classified into 3 groups based on their diameter (Kulkarni, 1988) viz. small (2-5mm), medium (5-10 mm) and large follicles (<10mm). In all 56 small follicles, 126 medium follicles 82 large follicles collected from left and right ovaries were analysed. FF from each follicle was aspirated using syringe fitted with 24 guage needle and cholesterol was estimated (IZZOC, 1981). The Data analysed by student 't' test (Snedecor and Cochron, 1967).

Results and Discussion

In the present study, the estimated total cholesterol (mg/dl) level in follicular fluid of different size follicle found to be increased as the follicle size increased. The mean value of cholesterol in three group of follicle was 53.85 ± 0.2726, 53.50 ± 0.5090 for small follicles, 75 ± 0.2869, 73.50 ± 0.4000 for medium follicles and 86.70 ± 0.6492, 83.46 ± 0.4342 for large follicles of left and right ovaries respectively. The statistical analysis revealed significant difference ($P<0.01$) in total cholesterol level in follicular fluid of different size follicles from same ovary (right or left) but no such significant difference ($P<0.01$) was observed between similar size follicles of left and right ovaries.

Similar observation was made by Brantmier (1989) in cows whereas no such relationship was observed by Chang (1976), Enk (1982), Parmar (1991) and Sharma (1996) in swine, human, buffalo and goat respectively.

Based on our results it can be concluded that the total cholesterol in follicular fluid increased as follicle grows. The increase in level may be contributed to the higher need of the avasculargranulosa cells for the synthesis of progesterone after LH surge and before ovulation. This observation was supported by Dielman (1984) who reported that the bovine granulosa cells produce large quantity of progesterone after LH surge and prior to

ovulation and vascularization.

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Efficacy of Enrofloxacin in Bovine Mastitis - Clinical Study

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When antibiotics were introduced to the therapeutic arsenal of veterinarians some 40 years ago, there seemed finally a hope of controlling mastitis. However mastitis is as common as it was before the antibiotic era (Sandholm *et. al.,* 1990.) In countries where antibiotics have been widely used *S. aureus* is gradually replacing Streptococci as a dominant isolate. Very few antibiotics achieve effective intracellular concentrations to eliminate *S. aureus* when the bacteria are sequestered within the phagocytes (Craven and Anderson 1984). Rifampicin, Paulomycin, Paldimycin and Fluroquinolones have been shown to reduce viable intracellular *S. aureus* bacteria in the macrophage system (Sanchez *et. al.* 1984). Enrofloxacin is the latest antimicrobial of fluroquinone group available to the veterinary clinicians (Gatne and Ranade, 1996). This study was designed to assess the efficacy of enrofloxacin in Staphylococcal mastitis.

Materials and Methods

Animals attending the large animal clinic outpatient unit of Madras Veterinary College hospital were taken for this study. Milk was collected from the mastitic animals and subjected to methylene blue reduction test (Hopps 1939) and culture and antibiotic sensitivity (Bauer and Kirby, 1966).

Ten animals (Jersy-6, Holstein, Friesian cross-1, Murrah graded-1, and mixed breed-2) tested positive for Staphylococcus mastitis were selected for treatment. Enrofloxacin was given at a dose rate of 4 mg/Kg B. wt intramuscularly by along with intravenous fluids and NSAID.

Results and Discussion

Out of the ten animals stuided complete recovery was recorded in nine animals. The observed recovery rate was ninetypercent. Since this trial was conducted at a teaching referral hospital which receives was conducted at a teaching referral hospital which receives many referred cases not responding to usual treatment locally and the delay in reporting may also have contributed to the failure in one case. Cullor (1993) stated that *S. aureus* may invade the adjacent tissues, the milkducts or glandular cavities. It then becomes established, grows and produces minute to large abscesses. In such cases antibacterial substances administered parenterally or intravenously cannot reach the organisms in therapeutically adequate concentrations because the connective tissue wall does not allow penetration of the agent to the infection site. In these cases no current treatment method is successful.

Methylene blue reduction test (MBRT) in the animals studied ranged from 5-15 mts. Gram positive cocci group were the powerful reducers of methylene blue (Wilson 1939). Vijayalakshmi (1995) reported that gram positive pathogenes reduced dye faster than coliforms and added that it will be useful in identifying the etiological agent on the first day of antibiotic therapy at field level which will be immensely useful in selecting the antibiotic. Culture test revealed *S. aureus* in all the ten cases and the isolates were sensitive to enrofloxacin, ciprofloxacin and other antibiotics (Fig 1). Sandholm (1990) stated that the methods of *invitro* sensitivity testing does not closely simulate *in vivo* conditions and

added that rifampicin, erythromycin and fluroquinolones were stated to be effective against *S. aureus*. Rifampicin is widely used in the treatment of tuberculosis in human beings and this limits its use in dairy animals. Erythromycin, even though stated as effective against *S. aureus*, parenteral perparations of this drug are not available to the practitioners.

Craven and Anderson (1984) stated that very few antibiotics achieve effective intracellular concentrations to eliminate *S. aureus*. Apparently this organism can be present in the mammary gland as naked "L" forms which means the betalactam group interfering with cell wall synthesis are ineffective (Ownis 1988), so *S. aureus* readily develops resistance to various antibiotics.

Fluroquininolones have been shown to reduce viable intracellular *S. aureus* bacteria in the macrophage system (Sanchez *et. al.* 1988). Among the fluroquilonones enrofloxacin was found to be cost effective (Fig.2) and an ideal choice for *S. aureus* mastitis therapy.

Further Mengozzi *et. al.*, (1996) reported that enrofloxacin when administered intramuscularly is characterised by high bio-availability and high volume of distribution implying high tissue concentrations exceeding MIC values for most pathogens. Hence enrofloxacin Intramuscular preparation was used in this study.

Complete recovery was recorded in all the cases studied in 4-5 days and the enrofloxacin therapy was continued for 5 days. Funhe (1982) suggested that the therapy of intramammary infections due to *S. aureus* by antibiotics would require long treatment periods. In lactatinal therapy there is not much difference between results from single treatments and those extended for 3 days, however extension beyond three days increases the cure rate.

Figure 1

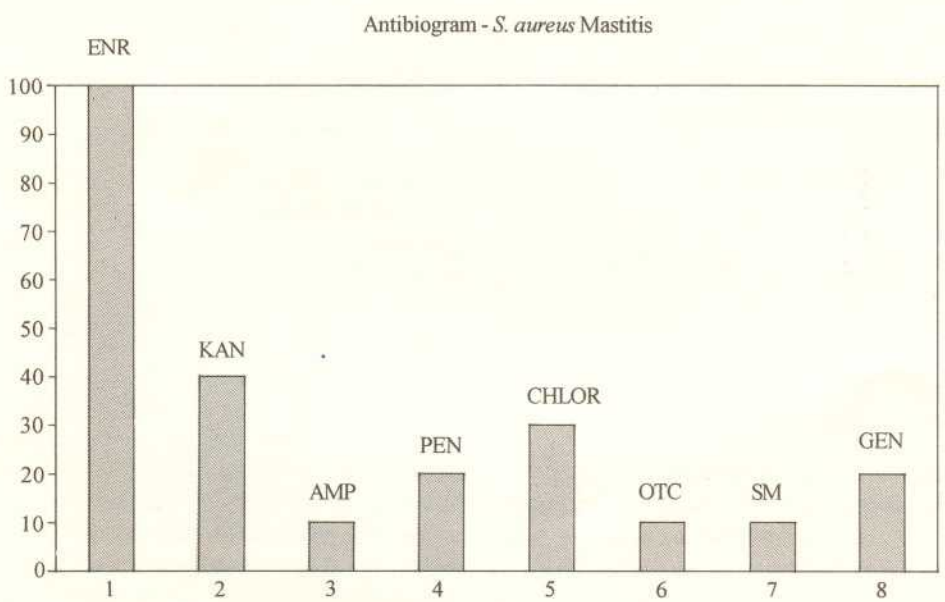
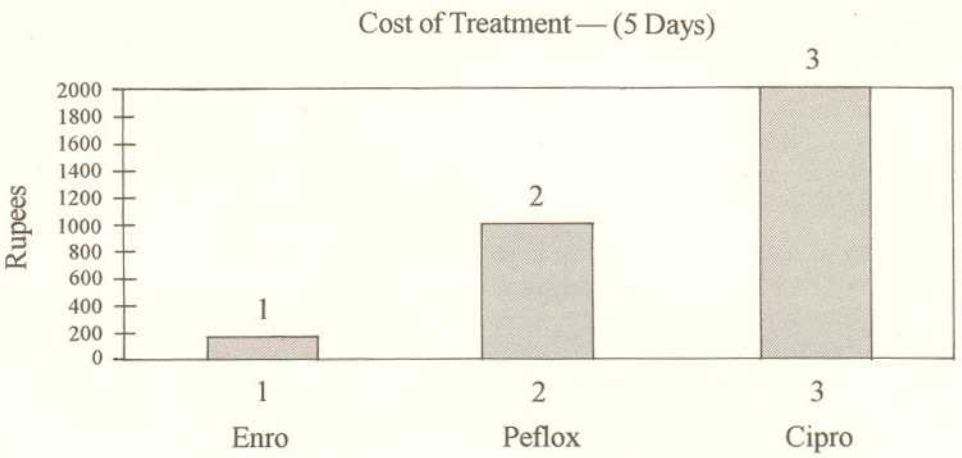


Figure 2



Summary

Enrofloxacin was given at a dose rate of 4mg/Kg B.wt. intramuscularly by to ten animals with *S. aureus* mastitis. The recovery rate was found to be ninety percent.

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Self Medicating Anthelmintic Delivery Devices for Ruminants

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Introduction

The problem of helminth infection in large ruminants is vast by anyone's reckoning as these parasites represent the single most important gorup of infections. The most commonly found helminth parasites are round worms i.e. members of the class Nematoda. Many different species of nematodes are found in the gastrointestinal tracts of cattle and buffalo. Broadly they are classified into **Haematophagous** nematodes of the genera *Haemonchus, Mecistocirrus* and *Bunostomum* and **Non-haematophagous** nematodes consisting of the rest (Soulsby, 1982). The economic importance of nematodosis in dairy animals has long been recognized and it is well known that the treatment of dairy animals results in their increased milk production, reduced intercalving period, thus providing a rationale for regular treatment (Sanyal *et. al.,* 1992a; Sanyal *et. al.,* 1993).

Control of gastrointestinal nematodosis is primarily executed through the use of antiparasite drugs, called anthelmintics, Several effective anthelminitics are available in the market for use in domestic livestock. But if one looks into their chemical structures, their number seems to be limited. Their number is further reduced when the anthelmintic resistance in nematodes is considered. Prichard *et. al.,* (1980) grouped the major anthelminitics into :

Group 1 : Phenothiazine

Group 2 : Salicylanilids and substituted nitrophenols

Group 3 : Organophosphates

Group 4 : Benzimidazole and Prodrugs

Group 5 : Imidothiazoles and Tetrapyrimidines

Group 6 : Avermectin

Out of these, benzimidazoles are very commonly used broad spectrum anthelmintics. With the discovery of thiabendazole in 1961, the benzimidazoles as a class of low dose broad spectrum anthelmintics with a high therapeutic index were established (Lacey, 1990). The subsequent cascade of patents during the next 30 years led to the development of 15 additional benzimidazoles or its prodrugs (Townsend and Wise 1990).

The use of anthelmintics in India is purely tactical, the animals are treated when found positive on faecal examination. This has resulted in indiscriminate use of broad spectrum anthelmintics in ruminants, at times with marginal or ineffective dose applications, leading to the survival of alleles which were drug tolerant. Progressive selection of tolerant individuals resulted into development of resistant strains. Further, dose rates set in naive animals are translated in animals having anatomical similarity, without understanding the host's pathophysiology after infection. The phenomenon of drug resistance in nematodes has both intra-group and inter-group expressions. Thus, worms resistant to thiabendazole and fenbendazole etc. Cross-resistance between phenothiazine and benzimidazoles as reported by Kelly *et. al.,* (1977) may be cited as inter-group cross-resistance.

While there is wide-spread anthelmintic resistance reported in the nematodes of small ruminants, the problem has started emerging in the nematodes of large ruminants (Prichard,

1990). Although, a drug resistant nematode strain is yet to be reported in cattle and buffaloes in India, there is evidence of multiple drug resistance in nematodes of small ruminants (Yadav *et. al.,* 1995). The choice of drugs available for use against benzimidazole resistant strains is limited, and most of them have a narrow spectrum of activity. Research for developing new drugs is capital intensive and time consuming. Therefore, it would be of considerable practical significance, if the performance of the already available benzimidazole anthelmintics could be improved.

While undertaking studies on pharmacokinetics of benzimidazole anthelmintics in ruminants in the Biotechnology Laboratory, National Dairy Development Board (NDDB), Anand, we have made some interesting observations which could partly explain the mechanism for emergence of drug resistant strains (Sanyal and Singh, 1993a). Studies on the pharmacokinetic behaviour of benzimidazole anthelmintics have indicated that their effectiveness and spectrum of activity can be improved by procedures which maintain plasma concentration of the anthelmintic beyond the periods that are normally recorded following a single drenching (Anderson *et. al.,* 1980; Zimmerman and Hoberg, 1988). Single intraruminal dose of fenbendazole or parbendazole or mebendazole is significantly more effective against benzimidazole resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep compared to their direct administration into the abosmasum (Kelly *et. al.,* 1977). Prichard *et. al.,* (1978a) showed that radiolabelled fenbendazole was slowly absorbed from the rumen and released over 24-48 hours into the abomasum where a more rapid rate of absorption occurred. Thus, an elevated plasma level of fenbendazole for an extended period were recorded. It was also demonstrated that administration of benzimidazoles in divided doses was more efficacious than single dose administration (Prichard *et. al.,* 1978b).

These observations encouraged us to work on drug delivery devices through which the anthelmintic could be delivered to the ruminant animals on a daily basis to maintain sustained plasma level of benzimidazole anthelmintic. This will not only reduce involvement of labours required to restrain large animals for drenching and eliminate spillage of medicine while drenching the animals forcefully but also delay emergence of drug-resistant parasite strains due to prolonged exposure of toxic concentration of the drug to the parasite.

Self medication

Self medication is a method of prolonged low level administration of anthelmintics in which the animal ingests the drug on a daily basis so that a sustained plasma level is maintained which is independent of the influence of parasite and host related factors. There is also evidence that the incoming larvae are more susceptible to the anthelmintics than the established larvae and adults. Thus, it may be possible to break the parasite's life cycle by daily dosing through self medication.

At the Biotechnology Laboratory, works have been carried out for developing methods for self medication of ruminants, selection of vehicle for the drug delivery and targeting dose rate so that the animals receive the desired dose of the drug on daily basis. The rationale for using the self medication is to prevent the establishment of the parasites during the favourable months of their development and survival, i.e. strategic treatment.

Anti-nematode block lick

The anthelmintic carrier

These urea molasses nutrient supplement blocks (UMB) for improving were used as the carrier of anthelmintics. The anthelmintic incorporated blocks not only ensure slow and constant release of the drug but also supplement the nutrition in the form of urea, molasses and mineral mixture. Thus, in a country like ours where roughage is the major part of animal feed, medicated blocks not only control

parasites, but also improve nutrition resulting in improved health and production.

Use of blocks as carrier of anthelmintic saves labour of drug administration, avoids spillage of drug and eliminates the risk of underdosing., Further in this method of anthelmintic delivery device, change of diet and oesophageal groove reflex do not affect the anthelmintic uptake.

Selection of anthelmintic

The criteria for selecting anthelmintic to be incorporated into the urea molasses licks are :

1. should have broad spectrum of activity worms
2. should be stable in blocks
3. should not be excreted in milk beyond permissible limit
4. should have a high safety index
5. should preferably be insoluble in water
6. should be cost effective.

Fenbendazole is the drug which fulfils all the criteria. It is stable in blocks, has no milk withholding period, insoluble in water, and its safety index 67 in cattle and 1000 in small ruminants.

Targeting dose rate

Studies were conducted to understand the pharmacokinetics of fenbendazole in buffalo and cattle using the recommended single intraruminal doses at 7.5 mg/kg body weight (Sanyal, 1993a; 1994; Sanyal and Singh 1992). After a series of experiments on continuous intraruminal dosing, it was found that effective concentration of oxfendazole in plasma could also be reached by daily intraruminal administration of fenbendazole at the rate of 0.5 mg/kg body weight. The effective level is reached between day 4-6 and a plateau was maintained thereafter (Sanyal, 1993a). The curve shows typical zero order absorption-elimination from day 4-6. In other words, by daily low level dosing the parasites would be exposed to the toxic concentration of the drug for much longer periods without additional use of the drug.

Medicated urea molasses block

Different levels of fenbendazole were then incorporated in UMBs and the availability of the drug in the block was estimated by solid phase extraction followed by its run in high performance liquid chromatography (HPLC; Ali *et. al.*, 1990). The medicated blocks were offered to the animals and the plasma samples collected at different intervals were analysed for determining plasma concentrations of the drug and its metabolites through reverse phase HPLC. This enabled us to select a dose to be incorporated in blocks which would ensure the desired plasma concentrations of the anthelmintic, with zero order absorption-elimination in cattle and buffalo (Sanyal and Singh, 1993b; Sanyal 1993b).

Efficacy of medicated blocks

Trials were conducted in calves experimentally infected with Haemonchus placei for evaluating the efficacy of fenbendazole incorporated medicated block (Sanyal and Singh, 1993c). In one of the experiments, the blocks were offered for 15 days after patency of infection. The ouput of eggs in the faeces became zero within 4 days of medicated block feeding and after 15 days of observation period, no worms were detected in the abomasum of treated animals. In another experiment, the animals were dosed with infective larvae of *Haemonchus placei* daily for 10 days with simultaneous licking of medicated blocks. It was observed that at no stage worm eggs were present in the faeces of treated animals and worms were not detected in their abomasum. The results suggest that the medicated blocks could effectively remove established adult parasites and also prevent establishment of infection by killing larvae (Sanyal and Singh, 1993c). Thus, it is concluded that medicated urea molasses blocks are highly effective for the treatment as well as prevention of parasitism in cattle and buffaloes.

Productivity trial

Trials were conducted on replacement heifers and lactating cross-bred cattle and buffaloes in

farms around Anand.

Forty heifers of 20-33 months of age, weighing 165-240 kg were randomly divided into 2 groups, one was allowed to lick medicated blocks and the other non-medicated blocks. The trial was continued for a period of 5 months. Faecal egg counts indicated moderate worm burden in the animals licking non-medicated blocks and consistent zero counts in the animals licking medicated blocks. Medicated blocks treated animals gained more weight with a net gain of 60 gm per day (Sanyal and Singh, 1995a).

Forty lacting buffaloes selected for the study were divided into 2 groups and offered medicated and non-medicated blocks, respectively. A four month trial indicated consistent zero egg counts in medicated block treated group with a net gain of 0.8 litre milk per buffalo per day (Sanyal and Singh 1995b).

Twenty eight cross-bred lactating cows were divided into 2 groups and offered medicated and non-medicated blocks, respectively. The trial was continued for 4 months. The cows receiving medicated blocks passed no eggs in the faeces with a net gain of 0.58 litre of milk per cow per day (Sanyal *et. al.,* 1995).

Anti-fluke block lick

As liver fluke infection is rampant in our country, an attempt was made to develop a flukicide delivery device using the principle of prolonged low-level administration. Triclabendazole (Fasinex, Ciba-Geigy, Switzerland) which is the only effective drug against immature fasciolosis in our country, was selected for incorporation in block licks.

While targeting dose rates in blocks, single dose pharmacokinetic studies were organized using the recommended therapeutic doses . To our utter surprise, we observed very poor anthelmintic uptake in buffalo compared to cattle (Sanyal, 1995). Critical efficacy studies against experimental bovine and bubaline fasciolosis using different dose schedule of triclabendazole indicated that the drug is very effective against both immature and mature fasciolosis in cattle at the recommended dose rates of 12.0 mg/kg body weight (Sanyal, in press), while buffaloes require 24.0 mg/kg body weight or above to control immature and mature fasciolosis (Sanyal and Gupta,1996)

Daily low-level administration of triclabendazole against experimental bovine and bubaline fasciolosis indicated that 10 daily dosing are required to eliminate mature liver flukes in cattle and buffalo at the rate of 0.5 and 1.5 mg/kg body weight, respectively (Sanyal and Gupta, in press). Therefore, different dose rates were targeted in urea molasses blocks for cattle and buffalo, so as to deliver a minimum daily dose of 0.5 and 1.5 mg/kg body weight for cattle and buffalo, respectively.

Dewormer concentrate feed pellets

Another self medicating device in the form of "medicated feed pellets" was developed to control parasitic gastroenteritis in small and large ruminants by incorporating albendazole and fenbendazole anthelminitcs. Initial trials indicated that consecutive 2 and 4 days of medication are required to control nematode parasites for large and small ruminants, respectively. Detail studies are in progress.

Strategic application of self medicating devices

Gastrointestinal parasites cause significant economic losses in ruminants, primarily because of their high morbidity rates. Apart from poor productivity and poor health of infected animals, occasional deaths are also reported, especially in calves. Tactical treatment has been the only way to minimize losses due to parasitism in clinically sick animals. Effective control strategy could not be formualted in India due to paucity of information on parasite epidemiology in different agroclimatic zones of India. For formulating strategies for worm control in Gujarat, studies were organized to understand the epidemiology of parasitic nematodes in dairy animals (Sanyal *et. al.,* 1992b). Later, similar studies were organized in 7 other agroclimate zones of India. The information on the nation-wide surveys is now available

(Sanyal and Singh, 1995c). It is logical to base the timing of treatment of daity animals on the knowledge gained from these studies on parasite epidemiology.

Conclusion

Control of parasitic disease, at least in the near future will depend on the use of chemotherapeutic agents. Although attempts are being made for developing vaccines against helminths and developing parasite resistant hosts, chemotherapy will continue to occupy an important place in the integrated parasite management programme. As described previously, for achieving maximum efficacy of benzimidazole anthelminitics the parasites should be exposed to the toxic concentration of the drug for as long a duration as possible. A square metabolite concentration-time profile, i.e., zero order absorption and elimination would be ideal. This can be achieved using medicated urea molasses blocks. Constant circulating concentrations of fenbendazole and its active metabolite oxfendazole are particularly efficacious against the adult worms and the incoming larvae. Such a low level prolonged administrations is found efficacious against larvae of benzimidazole resistant strains of parasites (Barger *et. al.,* 1993).

Prolonged low level and divided dose administration of anthelminitics through urea molasses blocks and feed pellets have the following benefits :

1. The involvement of labour and spillage of medicines that occurred during conventional oral drenching can be avoided.
2. The low but prolonged administration of the drug not only increases its efficacy against existing worms but also prevents reinfection.
3. The urea, molasses and minerals incorporated in the blocks, greatly improve nutritional status of the animals which makes them more tolerant to parasites. This, in turn, increases the productivity of the animals.
4. The emergence of anthelminitic resistant parasites is likey to be delayed and the larvae of already resistant parasite strains are likely to be killed.
5. Strategic application of these anthelminitic delivery devices, depending on the epidemiology of parasitic worms, will help in reduction of worm egg output in the faeces of the animals resulting into reduced level of pasture contamination and finally reduced level of parasitic challenge to young calves which are highly susceptible to worm infections. Epidemiological studies on parasite prevalence in dairy animals being conducted in various parts of India indicate that the most appropriate periods for medication of animals are rainy and post-rainy seasons which are mose favourable for development and survival of the pre-infective stages of the parasites.

Thus, medicated blocks and feed pellets are effective tools for integrated parasite management as it is useful in treatment of existing infection, prevents reinfection and reduces pasture larval contamination, thus improving performance of the animal.

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Simultaneous Uses of Floxidin Injectible and Oral Solution to Prevent Early Chick Mortality in Broilers

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Introduction

Mortality during first three weeks of life in broilers is often due to bacterial infections causing Oomphilitis, inflammation of yolksac and body cavity and pasted vents. Several species of Bacteria viz. *E. coli.*, *Salmonella* spp., *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Arizona* spp. have been isolated from such cases. Even with strict hygienic precautions at breeder farms and hatchery levels, certain degree of mortality is common in chicks.

In order to prevent this mortality, now-a-days it has become a common practice to give antibiotics through parenteral route to the day old chicks followed by oral route of antibiotic treatment for 3 to 4 days at farm level.

Gentamycin injection has been used at post-hatch stage followed by oral administration of different antibiotics at farm level. Although this strategy works well initially, it is not advisable in the long run to expose chicks to different antibiotics in a short span. Gentamycin has the disadvantage since it cannot be administered orally to maintain MIC level in the blood. It was therefore decided to try Floxidin injections at post hatch level followed by Floxidin oral solution for four days to prevent early mortality due to bacterial infections in chicks.

Methods

The trial was conducted on a commercial broiler farm near Mumbai which has its own hatching unit. The trial was based on 1,02,372 chicks from 13 batches of hatch. The trial design was as given in Table I.

Table I : Design of the Trial

Trial	Number of Batches	Av Number of Chicks per Batch	Injection Treatment	Oral Treatment
Trial I	5	7874	Gentamycin (On day of hatch)	Furasol + Tiamutin (1-4 days)
Trial II	5	7876	Floxidin (On day of hatch)	Floxidin (1-4 days)
Control	3	7857	—	Furasol + Tiamutin (1-4 days)

Table II : Mortality pattern during first three weeks Broiler chicks in Trial & Control batches

Trial I

Treatment	Batch	No. of Chicks	Mortality				
			IW	IIW	IIIW	Total	Percentage
Gentamycin	1	7960	35	31	42	108	1.356%
Injection	2	7836	52	119	26	197	2.154%
+	3	7868	56	180	30	266	3.38%
Oral	4	7866	43	61	51	155	1.97%
Furasol & Tiamutin	5	7838	40	159	24	233	2.845%
Total		39368	226 (0.574%)	550 (1.397%)	173 (0.439%)	959	2.41%

Trial II

Treatment	Batch	No. of Chicks	Mortality				
			IW	IIW	IIIW	Total	Percentage
Floxidin	1	7940	28	22	22	72	0.907%
Injection	2	7833	27	54	40	121	1.545%
+	3	7873	33	56	26	115	1.46%
Floxidin	4	7925	40	75	33	148	1.867%
Oral	5	7861	39	70	44	153	1.946%
Total		39432	167 (0.42%)	277 (0.702%)	165 (0.418%)	609	1.544%

Control

Treatment	Batch	No. of Chicks	Mortality				
			IW	IIW	IIIW	Total	Percentage
Oral	1	7682	130	110	53	293	3.84%
Furasol	2	7950	71	226	47	344	4.32%
+	3	7940	66	143	88	297	3.74%
Tiamutin							
Total		23572	267 (1.13%)	479 (2.03%)	188 (0.797%)	934	3.962%

Figure 1 : (Floxidin Trial) Mortality Pattern in 3 weeks

Doses of drugs used were as follows :

Floxidin Injection	—	5 mg/Kg
Floxidin oral solution	—	5 mg /Kg
Gentamycin Injection	—	5 mg/Kg

Furasol — 1 gram/Litre of drinking water.

Tiamutin — 5 gram/1000 chicks per day.

The flocks were observed upto three weeks for symptoms, development and mortality and the post mortem examination was carried out every week.

Observations (Refer Table II and Figure 1)

1) In all the three groups, mortality was lower in the first week. But the mortality rose to high levels in the second week and declined in the third week.
2) Total mortality at the end of third week was highest (3.96%) in controls which did not receive any antibiotic injection. Mortality was lowest (1.544%) in Trial II which received Floxidin injection + oral treatment and was intermediate (2.41%) in Trial which received Gentamycin + oral Furasol & Tiamutin.
3) The total mortality at the end of third week was less by 61% in Floxidin treated flocks and by 39.14% less in Gentamycin + Furasol + Tiamutin treated flocks as compared to mortality in control batches. In all the three weeks the same trend was noticed.
4) Vent pasting and lesions of Omphilitis, yolksac infection were common in controls, mild in Gentamycin treated batches and were rare in Floxidin treated batches.
5) Floxidin injection did not cause any adverse reaction and the drug could be safely administered with Marek's vaccine.

Conclusion

Floxidin injection at the dose rate of 5 mg per 1 kg body weight on day of hatch followed by

oral administration of Floxidin at the same daily dose from 1st to 4th day was found more effective than other antibiotics in the prevention of early chick mortality in broilers.

Chronic Udder Odema in a Primigravid Jersey Crossbred Cow

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Slight mammary odema at parturation in cows is a physiological response and does not warrant veterinary attention (Radostits *et. al.*, 1994). But severe udder odema persisting for weeks after parturition, is of great concern to the owners. The present report describes a case of chronic udder odema in a primigravid Jersey crossbred cow and its successful management.

A Jersey crossbred primigravid cow aged about three years, was referred to Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar with a complaint of excessive swelling on udder since last 46 days. The history revealed that the cow had calved a month before and was being fed high grain ration (6.0 Kg grains) with 80 gm. of common salt daily. Despite treatment with antibiotics, anti-mastitis tubes, anti-inflammatory drugs, diuretics like frusemide and other drugs, the condition had remained refractory.

Clinical examination showed odematous swelling right from perineum, udder (Fig. 1), belly to navel area, normal temperature (101 F),crackling of the skin of left hind teat and in between teats. On a subjective score of 10 points (Tucker *et. al.*, 1992), the odema in the present case was graded as +10

Based on history, clinical examination and examination of milk, the diagnosis of chronic udder odema was arrived at. Therapy with acetazolamide (Diamox 250 mg. tablets) 1.0 g orally twice daily for 6 days, pheniramine maleate (Avil 22.75 mg/ml) 10 ml intramuscularly daily for 5 days, manitol 20 percent solution 200ml intravenously once and cessation of common salt and grains in diet yielded remarkable results.

Discusion

Clinical findings in the present case were resembling with those described for chronic udder odema (Radostits *et. al.*, 1994). Occurrence of chronic udder Odema during pregnancy and persisting weeks after parturation in primigravid heifers agrees with Schmidt, 1971). Continued feeding of high grain ration (Johnson and Otterby, 1981) and common salt (Randall *et al.*, 1974) pre and post partum was possibly responsible for prolonged persistance of udder odema in the present case. Therapeutic management was adopted with acetazolamide diuretic which showed improvement on 3rd day and complete regression of swelling by 6th day. Nevertheless, cracking of skin between teats and udder, persisting before start of therapy, required intensive wound management . Effectiveness of acetazolamide in the management of udder odema has also been reported earlier by Gouge *et. al.*, 1959 and Wagner, 1962. The refractiveness of diuretics like frusemide in this case before coming to this Polyclinic seems to be due to continuance of high grain ration and sodium chloride and a casual treament approach.

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Results and Discussions	:	
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Bioavailability Studies of Floxidin (Enrofloxacin 10%) in Buffaloes

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Introduction

Enrofloxacin is the latest antimicrobial from quinolone group developed exclusively for veterinary use. It was identified in 1983 by M/s. Bayer, Germany through extensive research efforts. A series of laboratory and clinical investigations proved its efficacy as regards spectrum of activity, stability, coverage for different clinical indications in different species of animals. The serum enrofloxacin level data are available in literature for most of the species of animals but buffaloes. In India, major production of milk is from buffaloes. It was thought necessary to generate data on serum enrofloxacin profile in buffaloes.

Recently Hoechst (India) has launched Floxidin (Enrofloxacin 10%) injection for veterinary use. It was used for the present study with a view to generate useful data and deciding the dosage schedule of enrofloxacin in buffaloes.

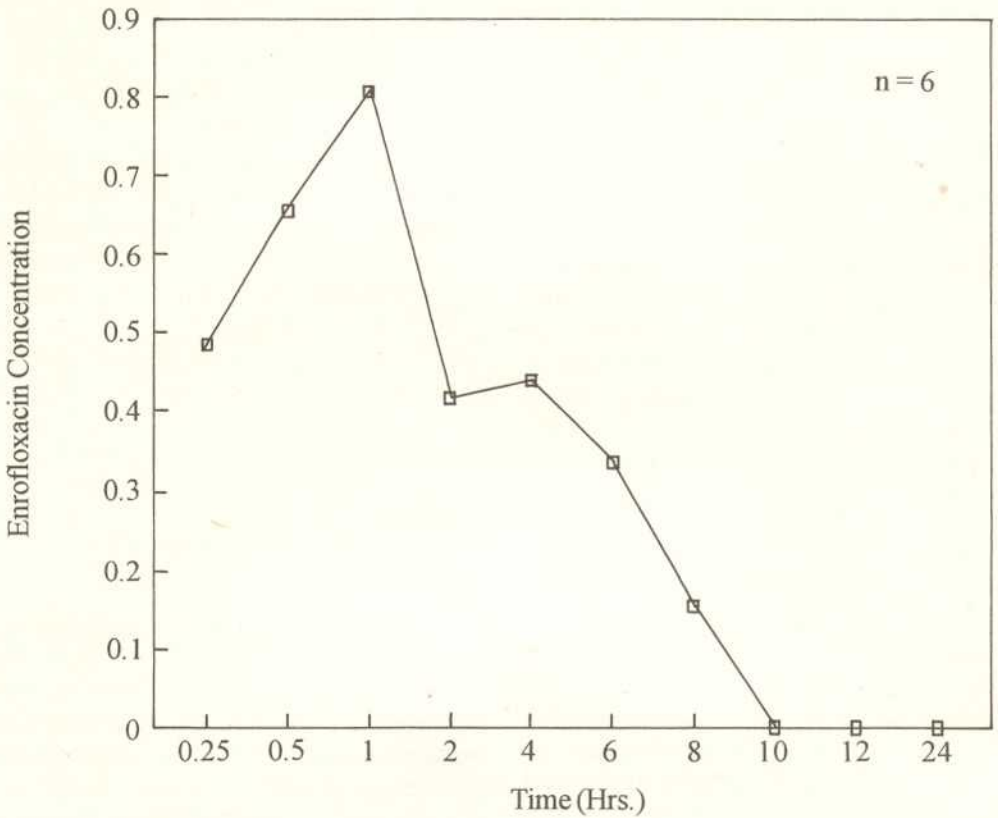
Table 1. Serum Enrofloxacin concentration (Mcg/ml) after administration (2.5 mg/kg I.M.) of Floxidin in buffaloes

Hrs. After Drug Administration	Buffalo No. I	II	III	IV	V	VI	Mean conc. (Mcg/ml) ± SE
0.25	0.55	0.49	*	*	0.30	0.60	0.485 ± 0.065
0.50	0.41	0.58	0.27	1.45	0.68	0.56	0.658 ± 0.168
1	0.30	1.20	0.9	0.85	0.5	1.1	0.808 ± 0.139
2	0.35	0.6	0.3	0.56	0.30	0.4	0.418 ± 0.053
4	0.34	0.52	0.46	0.26	0.5	0.56	0.44 ± 0.049
6	0.45	0.30	0.46	ND	ND	0.82	0.338 ± 0.127
8	ND	0.30	ND	ND	ND	0.64	0.156 ± 0.110
10	ND	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND
24	ND	ND	ND	ND	ND	ND	ND

* Sample was not available

ND = Not Detectable

Figure 1
Mean Serum Enrofloxacin Concentration (Mcg/ml) in Buffaloes After Administration of 2.5 mg/kg Enrofloxacin (Floxidin) Intramuscular



Materials & Methods

The studies were undertaken at Bombay Veterinary College, Parel, Mumbai. Buffaloes (5 Females & 1 Male) weighing 300 -350 kg were administered enrofloxacin 2.5 mg/kg by intramuscular route. Blood samples were collected at 0.25, 0.50 ,1,2,4,6,8,10,12 & 24 hours. Serum was separated and was processed for microbiological assay by the method of Benett *et. al.,* (1966) using *Bacillus subtilis* as the test organism. The sensitivity of the assay method was 0.1 mcg/ml.

Results

The results of the serum enrofloxacin levels in buffaloes are presented in Table 1 and the data are presented graphically in fig. 1. The mean peak serum enrofloxacin level was attained at one hour and was 0.808 mcg/ml. However, if individual animal is considered , level as high as 1.45 mcg/ml could be obtained.

In general enrofloxacin could be detected upto eight hours in serum though there was variation in persistence of enrofloxacin in serum in individual animals from 6 to 8 hours. The MIC of enrofloxacin for majority of pathogenic organisms is reported as 0.06 to 0.1 mcg/ml. It will be advisable to administer Floxidin injection with 12 hours interval in routine practice. However in acute cases classically it should be administered thrice in a day.

Canine Neosporosis - a Potential Cause of Posterior Paralysis in Indian Dogs

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History

Until recently, all cases of neuromuscular disease due to *Toxoplasma gondii* like organisms were classified as toxoplasmosis (Dubey, 1985). Recent research shows that such cases with posterior paralysis followed by an ascending paralysis may be produced by another cyst-forming protozoan named as *Neospora caninum* (Dubey *et. al.,* 1988). Examination of 23 dogs with toxoplasmosis like illness indicated that 10 of them were not of Toxoplasmosis. Since the organism did not fit in any known genera, a new name was coined for it : *Neospora caninum* (Dubey *et. al.,* 1985). Retrospective studies show that the disease existed at least since 1957 in the United States (Smith, 1993). In 1990, three cases, as old as 1971 (2 cases) and 1977 (1 case), previously reported for encephalomyelitis were examined immunohistochemically. These samples were taken from the paraffin-fixed slides of cerebrum and spinal cord. They revealed tachyzoites of *N. caninum* (Munday, 1990).

The random amplified polymorphich DNA (RAPD) polymerase chain reaction (PCR) technique revealed the high genetic divergence of *N. caninum* from *T. gondii* and *Sarcocystis* spp. It has been confirmed that *N. caninum* is an independent protozoan species (Guo *et. al.,* 1995).

The organism

N. caninum is an apicomplexan parasite in family Sarcocystidae with tachyzoites and bradyzoites very similar to that of *T.gondii.* It multiplies by endodyogeny. There are three major structures associated with *N. caninum.* They are - tachyzoites, bradyzoites and the tissue cyst wall. The tachyzoites are located within the host cell cytoplasm within a parasitophorous vacuole. The bradyzoites are 6-8X 1-1.8 µm in size and slender. Organelles are same as tachyzoites but has more periodic acid schiffs (PAS) - positive granules and fewer rhopteries. The tissue cyst wall measures upto 107 µm in length and may be round to oval in shape. It is usually 1-2 µm in thickness. It may become as thick as 4 µm if the infection persists for a longer duration (Dubey *et. al.,* 1996). The tissue cyst wall harbours the bradyzoites in it and is found only in neural tissues i.e. brain, spinal cord and retina (Dubey *et. al.,* 1988).

The *N. caninum* organism of both canine and bovine origin are same, as done by nucleotide sequencing. The *N. caninum* differs from its counterpart organism *T. gondii* in four nucleotides. Hence the two are closely related (Marsh *et. al.,* 1995). Besides the strain discovered in USA i.e. NC-1 (Dubey, Carpenter, Speer, Topper, Uggla), NC-Liverpool has been recognized in UK (Barber *et. al.* 1995).

Life cycle

Other than the canines, animals affected include cattle, sheep, goat, cats foxes pigs, rabbits, primates, coyotes, mice, horses, rats and gerbils (Cuddon *et. al.,* 1992).

The true life-cycle of *N. caninum* is unknown. The hypothetical cycle starts from a suspected carnivore definitive host. It is so suspected because the bradyzoites are resistant to HCl pepsin solution. Cat is not the definitive host (Cuddon *et. al.,* 1992). Various carnivorous bird species have been tested and are not the definitive host of *N. caninum* (Baker *et. al.,*

1995).

This unknown host, likely, discharges Neospora oocysts in its faeces. This stage has not yet been found. Abbit (*et. al.*, 1993) have presented a circumstantial evidence of *Hammondia pardalis* oocysts as a Neospora life-cycle stage. However, this has been questioned by Dubey (1993) and Mc Allister (1993) (Dubey *et. al.*, 1996).

Herbivores such as cattle, sheep and goats consume the feed and water contaminated with the Neospora oocysts.

Dogs and other carnivores eat their flesh which possibly contains tachyzoites and tissue cysts. These tachyzoites in the carnivores invade the CNS and the muscles. Bradyzoites are found only in the brain, spinal cord and retina with tissue cysts.

The last connecting step of the life cycle may be achieved by four different routes :

i) Injected by subcutaneous (SC), intramuscular (IM), intra peritoneal (IP) or intravenous (IV) routes.

ii) **Transplacental Mode.**

iii) **Transmammary Route** (Cole *et. al.*, 1995).

iv) **Intestinal phase** : It is a probable phase which leads to shedding of oocysts in the faeces (Smith, 1993).

No circumstantial evidence is available.

Signs and symptoms

The N. caninum infection is clinically manifested mainly by posterior paralysis or weakness of hind limbs.

The hind limbs paralysis may occur due to involvement of both or either of the hind limbs (Cochrane *et. al.*, 1993). This may be of varying intensity depending upon the site which has been prasitized (Jackson *et. al.*, 1995). Intially, there might be no pain in the spinal region or hindlegs. However, as the disease progresses, there may be superficial as well as deep pain response in both the hindlegs. Myelopathy is the possible cause of this as has been confirmed by electromyographic examination (Gasser *et. al.*, 1993). Ascending paralysis is a characteristic sign (Knowler *et. al.* 1995). The upper motor neuron paralysis, combined with myositis. may cause rapid progressive fibrous contracture of the muscles. This may fix the joints, hence, leading to hyper-extended limb (Dubey *et. al.*, 1996) The lower motor neuron deficits of the pelvic limb, bladder and rectum have been recorded in young dogs (Knowler *et. al.*, 1995).

Newly born pups from infected mothers, may show prioception deficits, increase in muscle tonicity, and spastic hind limbs. Abortions may be there (Cole *et. al.*, 1995).

Severe multiple cutaneous abscesses may appear especially over the hind limbs (Dubey *et. al.*, 1991a). Skin biopsy may reveal tachyzoites of *N. caninum*. Ulcerative and fistulous dermatitis is one of the major lesions in the dog (Dubey *et. al.*, 1988). Cutaneous neosporosis characterised by pyogranulomatous dermatitis with multiple draining nodules has been reported (Dubey *et. al.*, 1995).

A crouched stance posture may accompany with paresis (Hoskins *et. al.*, 1991)

An isolated case of involvement of the cranial nerve has been reported. However *N. caninum*, was not confirmed (Poncelet *et. al.*, 1990).

A dog met with a sudden death. Necropsy revealed severe myocarditis and subsequently *N. caninum* tachyzoites were isolated (Odin *et. al.*, 1993). Hence, changes in vital cardiac values may be a good indicator in a suspected case.

Involvement of lungs has also been documented from time to time. Pneumonia has often been found in positive cases. Besides this urinary incontenance may also be seen (Uggla *et. al.*, 1989).

Diagnostic Methods

Diagnosis of Neospora organism is often

difficult. This is because it is found in the tissues which are often not examined. Also, the number of the organisms so less that they may go unnoticed. The organism is a very close associate of *T. gondii*. The only distinguishing factor between the two lies with the immunoserological tests.

The organism can be detected by various immunological and molecular biological tools such as indirect fluorescent antibody technique (IFAT), Enzyme linked immunosorbent assay (ELISA), Polymerase chain reaction (PCR), Immunohistchemical diagnosis with Avidin-Biotin Peroxidase complex, Western blotting, Immunoelectron microscopy and Bradyzoite specific antigen-5 (BAG-5) test. Various tachyzoite antigens can be exploited for the specific diagnosis of *N. caninum*.

In blood, serum creatinine kinase activities are found elevated due to necrotizing myositis. Simultaneously, blood alanine and aspartate aminotransferase may increase. Blood eosinophils may also increase.

Differential Diagnosis

1) *Toxoplasmosis* : IFAT with *N. caninum* antibodies does not cross-react with *T. gondii* organism. Also, an immunoperoxidase test can distinguish the same. Clinical examination and histopathological scanning is of no use to differentiate the two organisms.
2) *Sarcocystis* spp : In immunohistochemical test, *Sarcocystis cruzi* antiserum is highly specific for *Sarcocystis canis* sp. nov and does not react with *N. caninum* (Dubey *et. al.*, 1991).
3) All neurological disorders must be differentiated from *Neospora* by various serological tests (Bjorkman *et. al.*, 1994)
4) *Pyogranulomatous dermatitis* : The bioassay of counts of lesions may confirm Neosporosis.
5) *Myositis* : Myositis, due to *N. caninum* should be differentiated from myositis due to other parasites (Craig, 1989).

Post-Mortem lesions

In *N. caninum* most of the post-mortem lesions are found in the skeletal muscle, brain, spinal cord, liver, skin and adrenal glands.

Necrotizing fibrosuppurative dermatitis is seen on the skin. There is focal retinitis, choroiditis, mild non-specific iridocyclitis and myositis of extra ocular muscles (Dubey *et. al.*, 1990). Skeletal muscles are much pathognomic for *N.caninum*. This is an important specimen for diagnosis. Pale and streaked muscles with polymyosites, discriminating necrotizing myosites and myonecrosis may be seen.Gross atrophy of hind limb muscles is usually seen (Uggla *et. al.*, 1989).Brain and spinal cord show generalized encephalomyelitis. In the brain, severe or chronic non-suppurative meningoencephalitis, with multi focal lesions, are seen. In the spinal cord, protozoal cysts, (Ruchlmann *el. al.*,1995) and polyradiculomyelitis (Dubey *et. al.*,1988) may be encountered.Myeloradiculitis, at the lumbosacral level,with foci of inflammatory cells,where macrophage are dominant, may also seen (Poncelet *et. al.*,1990).Severe pneumonia and pulmonary oedema is often present in neosporosis.The liver may show congestion or hepatic necrosis (Dubey *et. al.*,1990).Placenta may show multi focal necrosis and haemorrhage with intralesional tachyzoites. Blood vessels may exhibit perivascular cuffing.Spleen and adrenal glands have been found to be affected (Hoskins *et. al.*, 1991).

Treatment

The disease is incurable and complete recovery has not yet been achieved. However,early detected cases may have an effective treatment to reverse the paralysis development. The following therapeutic regimens have been proposed with variable results:

1. Trimethoprim and sulphadiazine at a combined dose of 15 mg/kg body wt. a day, with pyrimethamine at 1 mg/kg b.wt.once a

day, for four weeks,is quite effective.This can even reverse the paralysis due to *N. caninum* infection in early stages (Mayhew *et. al.*,1991).

2. Clindamycin hydrochloride has been successfully used, at the rate of 7.5 mg/kg b. wt, for 45 days, to treat pyogranulomatous dermatitis in dogs (Dubey *et. al.*, 1995).

3. Spiramycin has been effectively used to cure toxoplasmosis. Hence, it can be used for *N. caninum*. However, its property of not crossing the blood brain barrier or reaching cerebrospinal fluid, limits its use. Spiramycin can prevent verticle transmission of toxoplasmosis. It is not very effective in established fetal infections (CIMS, 1995).

4. Ionophorous antibiotics, macrolides, lincosamides and tetracyclines have been found to be effective against the tachyzoites. However, these need a thorough test before they are put to clinical use.

Prevention and control

The disease cannot be prevented as the life-cycle is not fully known. No vaccine has been developed till date.

Neosporosis in India

Neosporosis is spreading all over the world. May be, spreading is a misnomer. Rather, it is gradually being detected in different parts of the world. Although, no case has been reported from India till date (Dubey *et. al.*, 1996), synonymous cases are being attended in various clinical establishements of the country which are unreported. These cases of canines have posterior and/or ascending paralysis with or without incontenance of urine and faeces (due to low motor neuron deficit of bladder and rectum, respectively). Various neurostimulants, nutritional supplements and dewormerns have failed to cure the syndrome. Some dogs die or have to be euthanised.

It cannot be concluded that these cases are of Neosporosis. However, the possibility can neither be culled out. No doubt, the diagnosis requires sophisticated laboratories and reagents but eyes cannot be swiveled away from this direction.

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Efficacy of Butox (Deltamethrin)in Oil & Aquabase Against *Demodex Canis* Infection in Dog.

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A large number of acaricides are in use for the treatment of demodicosis in canines topically, orally and parenterally with varying degree of success. Due to problems of appearance of drug resistance, newer agents are being needed to be screened continuously. In this study the efficacy of Butox (Deltamethrin) was evaluated against recurrent demodicosis in dogs. There seems to be no report on the efficacy of Butox in the oil base other than its traditional use in the aquous base. The present plan could help to assess the actual concentration of butox to be used in the oil base to control demodicosis in dogs.

Materials &. Methods

Butox commercially available acaricides in aquous base at 0.6, 0.4 and 0.1% in oil base in chalmogra oil was used at weekly intervals for three weeks. Naturally infected 30 dogs of either sex, different age group and breeds, in and around Kalyani Calcutta belt were included in the present study. These dogs were divided into 3 groups randomly for each trial of each concentration. To determine the residual effect, the treated animals were observed till they became infested with either or any of these parasites.

Results and Discussion

The drug deltemethrin at 0.6 in aquous base and 0.1% in oil base were found to be most effective (100%) against demodectic mange infection. At 0.1% the drug showed absolute efficacy (100%) but comparatively better efficacy was observed at 0.1% after first and second application. The drug at 0.1% concentration did not require third application in 50% casses if applied thoroughly after proper dressing of hairs. Similar findings of superior efficacy of pyrethroid compound was reported by various workers in aquous base (Kumar and Rahaman, 1988; Khan and Srivastava, 1992) but no comparative report with oil base is found. The drug in aquous base showed lower efficacy and both the concentrations require 3 applications for satisfactory recovery but if not further applied there was relapse of the disease after two months.

The effectiveness of the treatment against demodectic mange was noticed by subsidence of itching, stoppage of scab formation and keratinisation, smoothing of skin and normal hair growth. The period of protection from reinfection was observed to be highest in Deltamethrin in oil base followed.by 0.6% and 0.4% aquous base. Out of these three preparations Butox 0.1% in oil base is recommended for use against demodectic mange of dog for complete cure.

Summary

A synthetic pyrethroid Butox (Deltamethrin) was used at 0.6% and 0.4% in aquous base and 0.1% in oil base for topical application against demodectic mange infection in dog. The drug 0.1% in oil base found most effective followed by 0.6% and 0.4% in aquous base.

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Clinical Incidence of Reproductive Disorders in Caprines

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Reports on the incidence of reproductive disorders in caprines in India seem to be meagre. Hence an attempt was made to place on record the incidence of certain reproductive disorders in caprines.

Data was collected from the clinical cases that were brought to the Obstetrics ward of the Large Animal Clinic of Madras Veterinary College for a period of 5 years (1991-1995). Out of the total 12,627 cases attended in the Obstetrics ward, 1458 were caprines (Table I). The percentage of caprines attended ranged from 10.67 to 13.27 with an average of 11.55%.

In the 1458 cases attended, the incidence of the clinical disorders and their percentage is shown in the Table II. The incidence of percentage of Retained foetal membrane (RFM) (24.48), abortion (13.37), dystokia (12.69) were higher when compared to prolapse (1.85), metritis (8.02), mummified foetus (2.67) and vaginitis (1.03). Animals that had normal parturition and no pathological condition but were brought for postpartum check up formed 35.87% of the cases attended.

The incidence of retained foetal membrane is highest (24.48%) when compared to other conditions. Srivastava *et. al.,* (1985) reported under farm condition the incidence of abortions to be more in local breed of goat (3.61%) when compared to other conditions like metritis (0.34%), Vulvitis (0.33%0 and dystokia (0.03%). This significant high incidence of retained foetal membrane in the present study may be due to the difference in the source of study material.

Winter - Dec. Jan, Feb,

Summer - March, April, May,

South west monsoon -June, July, Aug,

North east monsoon - Sept, Oct, Nov.

Though all conditions were prevalent throughout the year retained foetal membrane and metritis were observed to be more during North east monsoon and Winter seasons. Similarly Prolapse was more common during North east monsoon and winter and recorded a low incidence in Southwest monsoon. A similar observation was made by Krishnamurthi *et. al.*

Table I : The number and percentage of Clinical cases attended in the Obsterical ward.

Year	Total cases attended	Total caprine cases attended
1991	2,554	300 (11.75)
1992	2,605	278 (10.67)
1993	2,667	354 (13.27)
1994	2,508	273 (10.89)
1995	2,293	253 (11.03)
Total	12,627	1,458 (11.55)

Table II : The incidence of the various disorders and their number and percentage

Condition	Number and percentage of the clinical disorders attended
Retained foetal membrane	357 (24.48)
Prolapse	27 (1.85)
Metritis	117 (8.02)
Dystokia	185 (12.69)
abortion	195 (13.37)
Mummified foetus	39 (2.67)
Vaginitis	15 (1.03)
Postpartum check up	523 (35.87)
TOTAL	1458

Table III : The seasonal incidence

Condition	Winter	Summer	South west monsoon	North east monsoon	Total
RFM	31.09	18.49	20.17	30.25	357
Prolapse	29.63	22.22	3.70	44.44	27
Metritis	33.33	16.24	17.09	33.33	117
Dystokia	27.57	26.48	20.00	25.95	185
Abortion	31.79	17.95	23.59	26.66	195
Mummified foetus	35.89	20.51	15.38	28.21	39
Vaginitis	26.66	20.00	26.66	26.66	15
Postpartum Checkup	25.05	21.03	24.66	29.25	523
Total	420 (28.81)	296 (20.30)	315 (21.60)	427 (29.29)	1458

(1983) in bovines. Abortion and mummified foetus were more common during the winter season. These differences can be attributed to the seasonal breeding in caprines.

Summary

Clinical incidence of reproductive disorders in caprines were analysed and discussed. The incidence was on an average 11.55%. Retained foetal membrane was the most common disorder in caprines (24.48%). Variation in the seasonal incidence of different disorders is noticed in caprines.

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A Field Trial of Butox (Vet) in Fish Culture Ponds

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Introduction

The Indian Major Carps (Fishes) *Catla Catla* (Ham) and *Labeo rohitha* are cultured in 60,000 Hectares of fresh water fish culture ponds in Andhra Pradesh. Carp lice or Argulosis is a common ectoparasite disease of culture carp fishes, effects fish around the year causing heavy losses to the Fish Farmers. Although, the carp lice does not cause mortality of fish but adversely effects the fish production by making wounds, injuries at the place of attack, resulting invade of secondary disease causing organisms like fungus and bacteria.

The carp lice Argulus Sps is a parasite copepod, oval in shape with flattened body and small bilobed abdomen, attaches itself to the fish by means of a pair of disc like suckers on the ventral side of its body.

Materials and Methods

Earthen dug out fish culture ponds 3 numbers each 20 cents in size, 4 feet in water depth were chosen for conducting field trials of Butox (vet) Deltamethrin 1.4 e.c. an Argulosis effected fish. These ponds are known as nursery fish ponds, owned by a private fish farmer. The ponds are located in Ganapavaram Mandal of West Godavary District, A.P. After thoroughly examining the fish (75-150 Gms. in size, stocked at density of 25,000 numbers/Ha.) suffering from Argulosis as diagnosed by the naked eye observations were taken into consideration for conducting the experiments.

All these nursery pond waters were treated by broadscattering the Butox (Vet) at the dose rate of 250 ml/Ha. The aquatic insects (water fleas), copepods died within ten minutes of application of Deltamethrin. Generally fish suffering from heavy infestation of Argulus Sps jumps out of the water, try to rub its body to the available plantations, bamboo poles are fixed for tying the feed bags, keep the tail fin upward and beats the water making snapping sounds. After treating the pond waters with Butox (Vet) there was pindrop silence, showing the signs of control of Argulosis. After an hour of time carp fishes were netted out by using cast net, for making eye observations on the control of Argulosis. The dead Argulus Sps were found on the scales, finroots and body of the fishes.

Similar type of trials were conducted in another larger fish culture pond. Grow out ponds are 4 to 10 Hectares in size, with 6 to 9 feet in depth, a higher dose rate 500 - 750 ml./Ha. (200/300 ml./ acre) of Butox was broad scattered in such lager and deeper fish culture ponds.

Results and Discussion:

Argulus infestation was effectively controlled administering Butox (Vet) at minimum dosages. Argulosis is a very common ectoparasite disease reported from many fresh water fish culture ponds from all over the world. Organophosphates like Malathion and Dichlorovos were used against Argulosis. As the higher dose rate of Organophosphates are required (500/1000 ml./acre or 1250 - 2500 ml./ Ha) for control, high investments are made. Usage of Butox is economical, results in quick relief when compared to other treatments. The Butox treatment to the pond water did not produce any adverse effects. It is because of which Butox can be used for effective control of Argulosis in fish culture ponds.

Evidence of Passive Transfer of K99 *Escherichia coli* Antibodies in Calves

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Lactogenic passive immunity from dam of the calf through colostrum is known to play its role in reducing the incidence of colibacillosis among farm animals. Prevalence of K99 fimbriae bearing *E. coli* and their role in bovine diarrhoea has been well established. In addition to lactogenic antibodies, *E. coli* K99 antibodies are often detected in colostrum due to its high antigenicity. Detection of K99 specific antibodies in colostrum, therefore can be a good indicator to know the prevalence of K99 *E. coli* in the herd. In the present study K99 specific antibodies were detected in the colostrum samples collected from dams of organised farms where neonatal diarrhoeic cases due to K99 fimbriae bearing *E. coli* were recorded 6-8 months before this study (Taku *et. al.*, 1991).

Colostrum samples were collected from the dam during period ranging from 0-20 days after parturition and stored at -20 C. The K99 specific antibodies were detected employing ELISA test. Test samples were prepared by adding 1% Rennet and then centrifuged at low speed and whey was collected for the test. ELISA plates (Linbro) were sensitised with 200 µl of heat extracted K99 fimbrial antigen at 4 C over night. The wells of the sensitised plates were then washed with PBS-T (0.1 PBS, pH 7.2 containing 0.5% tween 20) and unbound surface blocked with bovine serum albumin (3% w/v) for 1 hour at 37 C. After washing with PBS-T for three times. 0.1 ml of colostrum whey was added to all the wells and incubated at 37 C for two hours. This was followed by three more washing with PBS-T. A 0.1 ml of antirabbit IgG alkaline phosphate conjugate (Sigma) was then added and plates were incubated for further two hours period at 37 C followed by three more washings with PBS-T. The washed wells were now loaded with .0.2 ml of P-nitrophenyle phosphate (Sigma 1.0 mg/ml in tris buffer pH 7.6) and incubated at 37 C for 30 min. in dark. A total number of 18 colostrum samples representing different herds as sample survey were tested. Out of 18 samples, 11 (61.1 1%) were found positive for the presence K99 specific antibodies. It was also observed that two samples which were found negative originated from 20 days after parturition. In this study undiluted samples were tested in view of lack of information whether K99 antibody secretes in colostrum or not.

It was observed that in case of rotavirus infection passive lactogenic immunity within the gut lumen plays an important role in protection (Saif and Smith, 1983). Further, it was also observed that most of the seropositive cows for rotavirus antibodies secrete high level of antibodies in colostrum but low levels in milk (Acres and Babiuk, 1978, Flewett and Woode, 1978, Saif I *et. al.*, 1983). However, quantitative level of secretion of K99 antibody in colostrum is not yet known and its effective role in protection needs evaluation. Present study only evidenced that there were presence of K99 antibodies in colostrum samples tested and it needs further study on degree of transfer of antibodies in calves particularly in the area where evidence of prevalence of K99 *E. coli* are well known. In view of present evidence, *E. coli* K99 fimbrial preparations can be considered as a candidate for vaccine preparation to extend its future scope for passive immunisation of dam to circumvent the problem of neonatal diarrhoea of farm animals.

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Clinical Studies on Coccidiosis in Angora Rabbits in Himachal Pradesh

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Abstract

Coccidiosis caused by *Eimeria* species, is a major parasitic disease of rabbits and is responsible for a high incidence of morbidity and mortality in commercial rabbitries. The disease occurs in two forms, hepatic and / or intestinal, the latter being more common than the former. Presently, the control of rabbit coccidiosis relies almost entirely on chemical coccidiostats besides improved management practices. The present communication is an insight into the problem, the causative agent and preventive measures of the rabbit coccidiosis in Sub-Himalayan region of our country.

Introduction

Rabbit production has already attained commercial status in many parts of the world including India and has the potential to become one of the world's major livestock species. This industry is picking up for wool production in hilly areas of Himachal Pradesh, Uttar Pradesh and Jammu & Kashmir and as a broiler industry in areas of temperate and subtropical climate in West Bengal, Assam, Manipur, Andhra Pradesh, Tamil Nadu, Kerala and Karnataka. Coccidiosis caused by different species of the protozoan parasite, *Eimeria* is one of the important diseases of rabbits and is a major cause of morbidity and mortality (Lebas *et. al.*, 1986). In commercially reared rabbits coccidiosis occurs in subclinical form leading to growth retardation and altered feed conversion (Peeters, 1981). In India, coccidiosis is a major impediment to rabbit production and the most common parasitic disease affecting all age groups of Angora rabbits (Rai *et al.*, 1985). Prevalence of different coccidial species of domestic rabbit has been reported earlier (Sanyal and Srivastava, 1986; Jain, 1988; Meitei *et. al.*, 1988; Chandra and Ghosh, 1990). However, information on coccidial species of Angora rabbits is scanty. The present investigation was conducted to study the prevalence of different coccidial species during outbreaks of clinical coccidiosis in different farms in the Kangra valley of Himachal Pradesh.

Materials and Methods

German Angora rabbits of either sex belonging to different age groups viz., weaners (< 6 weeks), growers (6-24 weeks) and adults (> 24 weeks) died in different rabbitries located in and around Palampur in Kangra valley, Himachal Pradesh. The carcasses were subjected to detailed post-mortem examination. Smears from the intestinal content and /or mucosal surface were examined for the coccidial oocysts. The liver and the bile was examined for the presence of hepatic coccidial species. Both the biliary and intestinal contents were processed using the simple saturated salt floatation method. The concentrated oocysts were placed in a thin layer in petri-dishes with 2.5% potassium dichromate solution and allowed to sporulate at 25-28°C. The species identification was made by exogenous study done based on sporulation time, morphology and morphometry of sporulated oocysts purified after centrifugal floatation (Catchpole and Norton, 1979; MAFF, 1984). The data on prevalence of various species of coccidia was recorded.

Results and Discussion

The main clinical symptoms reported in these animals were diarthoea, anorexia progressive

loss of condition and emaciation before death. The clinical coccidiosis observed in organised rabbit farms in various age group is shown in Fig. 1. The overall infection/mortality due to coccidiosis was found to be higher in weaners (46) than in the growers (24) and adults (6). Of the 202 animals died in different rabbitries, 57 (28.2%)deaths were found to be due to intestinal coccidiosis, 14 (6.9%) due to hepatic coccidiosis and 5 (2.5%) due to both the types (Fig. 2). Microscopic examination of the scrapings from white patches in the liver revealed *E. stiedai* oocysts in plenty (Fig. 3). In advanced cases the liver showed a number of white to yellowish spots or streaks and later nodules. Hepatomegaly and dilated bile ducts were commonly associated with hepatic coccidiosis. However, older rabbits developed a strong immunity to *E. stiedai*. Various degrees of haemorrhagic enteritis were common in intestinal coccidiosis. Examination of mucosal smears revealed active endogenous developmental stages and oocysts. The OPG in the present study varied from $16.5X10^3$ - 666.2 $X\ 10^3$. Although there is no correlation between OPG and the severity of the disease in rabbit (Coudert, 1989), this parameter provides an indication of the degree of contamination in the farm in relation to managemental practices.

Prophylactic medication in drinking water and feed was found to drastically reduce oocyst production and almost total inhibition of hepatic and intestinal lesions in rabbits. Many drugs have been tried against coccidiosis in rabbits such as Sulpha drugs, Clopidol, Nitrofurans, Amprolium, Monensin etc. However, the most common drugs are Nitrofurans, Amprolium and Sulpha drugs. The following drugs have been found to be effective against clinical coccidiosis in rabbits during the course of the present study in various farms.

BIFURAN (Nitrofurazone + Furazolidone), SKF-1 tablet / litre of drinking water for 7 days. PEQUIN (Sulfaquinoxaline), RANBAXY - 0.05% in drinking water for 7 days.

CODRINAL (P-toluensulphonyl-beta methoxyethyleurethane + Tetracycline), HOECHST- 1gm / litre of drinking water for 7 days.

AMPROLSOL (Amprolium 20%), GLAXO - 0.06% in drinking water for 7 days.

Figure 1

Mortality in Angora Rabbits due to coccidiossis

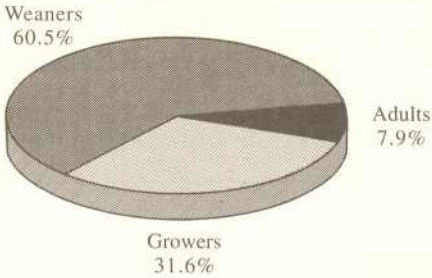


Figure 2

Clinical coccidiosis in Angora Rabbits on necropsy examination (n=202)

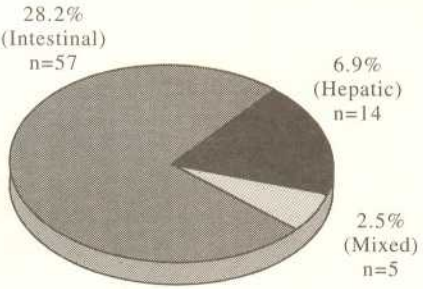
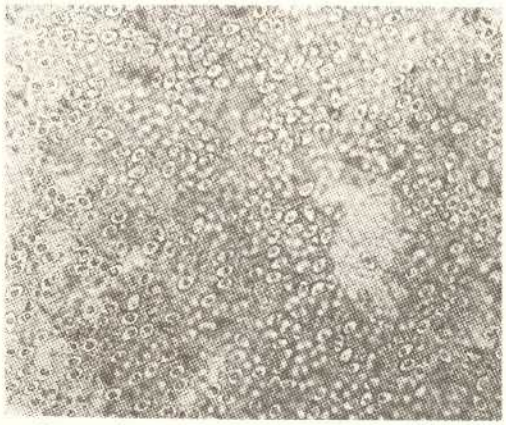


Figure 3



Impression smear of *E. stiedai* infected liver.

SULMET 16% solution (Sulphadimethylpyridin), CYNAMIDE - 7.5 ml / litre of drinking water for 7 days.

The treatment should take into account the possibility of re-infection especially due to coprophagy in rabbits. Thus a minimum of 2 periods of treatments are recommended preferably 2 periods of 7 days each with a pause of 7 days in between. Apart from medication, good preventive hygiene is the key to successful rabbit production. Further studies are in hand in our laboratory to study the efficacy of other compounds such as Monensin, Halofuginone, Salinomycin and other indigenous coccidiostats in broiler as well as wool rabbits.

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News

Control of Aquatic Insect Pests

Aquatic insects (Family Notonoctidae) are often found to infest nursery ponds of carp fish and kill nearly 70% of Spawns and hatchlings causing losses. Experiments conducted by G. Gnaneswar (Ganapavaram, Andhra Pradesh), revealed that application of Deltamethrin (Butox) to water in nursery ponds killed the insect pests in 10 minutes and ensured 100% survival of hatchlings.

Hindu(5.3.92)

Dr. B. Panda Honoured

Dr. B. Panda Founder Director (Retd.) of Central Avian Research Institute is elected to the prestigious 'International Poultry Hall of Fame" in 1996 He is the first Indian to get this honour.

World Poultry Congress, New Delhi 1996

A heart from back Muscle

Dr. Norbert Guldner, Chief Surgeon at Luebeck University Hospital Germany has transformed *Latissimus dorsalis* (back muscle) of a billy goat into a supplementary heart which could be stimulated to beat continuously and pump blood.

Times of India 19.8.96

New approach to fight Autoimmune disorders

In American College of Rheumatology in Orlando Florida, Scientists have found three new lines of treatment for rheumatoid arthritis in Man viz i) Temporary blocking of T cells with specific antibodies ii & iii) Blocking by genetically engineered proteins the destructive proteins (Tumor necrosisfactor interlukin produced by macrophages responsible for causing infammation of cartilages.

The treatment although "literally had patients dancing in the hall" and without side effects, symptoms recurred in few weeks after discontinuing the treatment. Nevertheless this is hailed as a new approach for treatment of auto-immune disorders in general.

TIME Oct-28, 1996 148 (18) : 70

News

Nobel prize 1996

Dr. Peter Doherty, Veterinarian from Australia and Dr. Rolf Zinkernagel specialist in tropical diseases Switzerland received Nobel prize in Physiology/ Medicine this year for their theory on immune rejection response explained in 1970s. They showed that WBCs of immune system look for changes in a Key marker called self protein which identifies cells as belonging to ones, own body. Any alterations in this protein tag the cell for destruction. This is similar to body's virus defence system

TIME Oct-21, 1996 148 (17) : 42

Rabbit calcivirus Disease (RCD)

Rabbits are the worst vertebrae pests in Autralia causing severe losses to Agriculture and damage to environment.

The rabbit calicivirus, which after escape from laboratory had reduced rabbit population by more than 95% in an area was extensively tested for its safety to other fauna in field trials and has been declared as an agent organism for control of rabbits under the biological control Act.

(RCD view has been released this year in nature in some parts of Australia)

Newsletter of CSIRO Animal Health, May'96
and News Paper Reports Aug - Dec'96 Australia.

Global Fever

Paul Epstein, Epidermologist with Havard School of Public Health has stated that climate changes and upheaval that can be expected from global warning, favour the opportunistic pests viz rodents, insects, bacteria, viruses and protozoas. The real threat may not be a single disease, but armies of emergent microbes raising havoc among host of creatures- Man, animals and plants. The animal & plant diseases can rip through the economies and prove more disastrous than those affecting man.

TIME July, 8 1996 **148** (2) : 40-41

M/s. Anuradha J. Desai

M/s. Anuradha J. Desai Chairperson of Venkateshwara Hatcheries group is elected as President of World Poultry Science Association with effect from September'96. She is the first Indian to get this highest honour and first lady to adorn the presidency of this world association. She is the daughter of the late Dr. B.V. Rao, father of poultry industry in India.

Readers' Column

Dr. T. N. Ganesh, Asso. Prof., Madras Vet College, **Chennai** 7

I like this issue because of interesting clinical case reports like "An unusual case of Rhinitis in Buffalo." Contents page (front innerside), it is very difficult to read under dark blue background. Please make white background for the whole page.

Dr. V. Velan, Indian Herbs, **Bangalore**, Karnataka

The technical review and articles are of excellent quality. I have nothing to dislike in this issue. Many congratulations for the good job.

Dr. Subhash Chander, Gurdaspur, Punjab

The 7th edition contains the information regarding "Diagnosis & Management of common chemical poisoning in animals". I like this article. But I don't like the 7th issue because it does not contain sufficient number of case reports for practising veterinarians under field conditions. I suggest to include articles of advancement in veterinary surgery, gynaecology & medicine.

Dr. Girish Kalra, Gurgaon, Haryana

It is providing technical update to me in the field and I am being benefited by the latest knowhow at my doorstep.

Dr. Md. Sami Ahmed, Vet Hospital, **Mysore** Dist. Karnataka

It is very much useful in the field practices and also very important in daily usage. Kindly arrange to give information regarding etiological factors of anoestrous animals & its treatment and also for repeat breeders.

Dr. V. Marivel, **Dharapuram**, Periyar Dt., Tamilnadu

I had a chance of reading your 6th & 7th issues of the BCB. They are very much useful and informative especially the latest trials & their results of some preparations of your company and some of the other companies as well. I am keen to get the BCB for my personal reference to follow thereby.

Dr. Jayachandran, **Kottayam**, Kerala

The articles, viz. "Diagnosis & Management of Common Chemical Poisoning in Animals" and "Management of Early Embryonic Death in Cows" were very refreshing & informative. The BCB is actually a very good publication for refreshing the professional knowledge and for imparting current information about the profession as well as new products.

Dr. R. C. Behera, Vet Asst. Surgeon, **Kalahandi**, Orissa

I like this issue because of review article on "Bovine Spongyform Encephalopathy (BSE)." Own experience in treatment (in a particular disease) of veterinarians should be published.

Dr. A. Bhanu Murthy, **Dhenkanal**, Orissa

I like this issue because of publication of clinical articles authored by field veterinarians which will encourage the doctors working in remote areas in regard to their professional activities. Let such booklets be circulated to more number of veterinarians.

Animal Models for AIDS

Dr. Ranjana A. Deshmukh
Haffkine Institute, Mumbai

The definition of animal models for HIV infections of humans will be crucial for furthering our understanding of the pathogenesis of AIDS. Animal models will also be important in the development of efficacious drugs and for testing vaccines against HIV infection. While HIV isolates have been shown to infect a variety of non human species under experimental conditions, the virus host interactions in those infections appear to be significantly different from those seen in HIV infected humans.

Thus HIV infected laboratory animals have proved inadequate as models for studies of pathogenesis of AIDS. However HIV is a lentivirus and is therefore related to a variety of other pathogenic viruses that infect ungulates, cats and non human primates. Emerging data are now indicating that the study of these lentiviruses and the diseases they induce will be of enormous value in elucidating the pathogenesis of AIDS and assessing strategies for treatment and vaccine protection against HIV infections.

HIV infected non human species

PRIMATE MODELS : The ideal animal model for AIDS would be one in which HIV 1 and 2 infects and induces an AIDS like disease in an inexpensive, readily available laboratory animal (Table1).

Table 1 : HIV infections in different animal species.

Species	Isolates	Clinical manifestations	Limitations as AIDS model
Chimpanzees Gibbons	HIV 1	None	— scarcity of animals. — expensive — absence of disease.
Old world monkeys	HIV 2	None	— sporadic infectability — absence of disease.
Rabbit	HIV 1	Abnormal immune responsiveness	— low level infection — absence of disease.
Humanised SCID mouse	HIV 1	Rapid T-lymphocyte ablation	— abnormal human lymphocyte repopulation in mouse host.

Great apes have been shown to be susceptible to experimental HIV 1 infection . Extensive work has been done with HIV 1 in the chimpanzee (*pan troglodytes*,) and a limited number of gibbons (*hylobates* spp.) , have also been infected with the virus . Although virus can often be isolated from peripheral blood lymphocytes (PBLS) of inoculated animals,and although these animals develop high titre virus specific antibody responses after infection,

neither the HIV 1 infected chimpanzees nor gibbons have developed any signs of the disease. These findings, the endangered species status of the animal , their lack of availability and their cost make plans for the extensive use of great apes in AIDS research unrealistic .

A number of isolates of HIV 2-the AIDS inducing virus, endemic in humans in West Africa have also been studied in non human primate species Under experimental conditions HIV 2 can infect a number of African and Asian monkeys , however no disease has developed in these infected animals.Although HIV 2 infected non human primates are not useful models for studying the pathogenesis of AIDS they will be important in testing potential vaccines for protection against infection with AIDS virus.

NON PRIMATE MODELS: Interest has recently focussed on non primate models for HIV infections.The rabbit has been shown to be susceptible to a low level persistent infection with HIV 1.

Although AIDS like disease has not been demonstrated in these animals , after infection recent studies indicate that HIV 1 infected rabbits cannot generate normal antigen specific immunological responses.The eventual role that the HIV - rabbit model may play in AIDS research is still unclear.

Mice with severe combined immunodeficiency (SCID) 'reconstituted with either human PBLs or human foetal thymus and liver or lymph nodes have been shown to be capable of harbouring HIV infection . This observation generated a tremendous amount of optimism when it was first reported raising the possibility of using the HIV infected "humanised" SCID mice as an inexpensive easily accessible small animal model for AIDS .

More recent work has however indicated that functional CD8+ human lymphocytes do not persist in SCID mice reconstituted with human PBLS. More over HIV-1 in humanised SCID mice appears to spread rapidly and ablate T cells rather than replicate a chronic low grade fashion Thus like the HIV 1 infected rabbits the value of this murine model in AIDS research remains to be demonstrated. LENTIVIRUSES OF UNGULATES: The human AIDS viruses are members of the retrovirus sub family Lentiviridae and are therefore related in their morphology and nucleotide sequence to a variety of pathogenic Lentiviruses of non primate species The best studied of these viruses are those that infect and induce disease in ungulate species : Maedi - visna virus (MVV) of sheep, caprine -arthritis-encephalitis virus (CAEV),equine infectious anaemia virus, (EIAV), and newly recognised lentivirus of cattle,bovine immunodeficiency virus (BIV). Like the human AIDS virus, these ungulate lentiviruses appear in the electron microscope studies as enveloped particles with dense cylindrical cores. Like HIV, the ungulate viruses contain gag, pol and env genes, as well as small open reading frames between pol & env and at the 3' terminus that codes for regulatory proteins. The chronic disease induced in the ungulates by those lentiviruses are analogous in many ways to HIV induced disease in humans. The common features of the disease and the virus host interaction include prolonged periods of clinical latency,the development of only a weak neutralizing antibody response with resulting persistent viremia, a tendency for the virus to undergo extensive genetic mutation and antigenic drift, significant neuropathology, and lytic viral infection of selected bone marrow derived cells.

FELINE IMMUNODEFICIENCY VIRUS : The best available small animal model described to date for AIDS may well be the feline immunodeficiency virus (FIV) infected cats. FIV a T lymphotropic lentivirus was first isolated from cats with an immunodeficiency syndrome While FIV inoculated pathogen free cats have developed only a transient fever and persistent lymphadenopathy, cats coinfected with the distinct retrovirus , feline leukemia virus ,

develop more severe disease and opportunistic infections . FIV infected cats also develop encephalomyelitis and a vacuolar myelopathy.

SIMIAN IMMUNODEFICIENCY VIRUS :Soon after the initial isolation of HIV 1 a number of HIV related viruses of non human primates were identified . These simian immunodeficiency viruses (SIV) include SIVmac, first isolated from a rhesus monkey with a lymphoma SIVagm first isolated from healthy African green monkey, SIVsm first isolated a healthy sooty mangabey,SIVmnd first isolated a healthy mandrill, and SIVcp2, isolated from healthy chimpanzees in Gabon .

It can be presumed that further SIV isolates from other non human primate species will be described . These isolates all share striking nucleotide homology with HIV 1 and HIV 2 as well similar tropism for CD4 bearing lymphocytes and monocyte-macrophages.

The nucleotide sequences of SIVmac and SIVsm are so similar that it is assumed that the macaque isolates arose in the recent past from the mangabey virus . These two SIV isolate also show striking sequence similarities to the West African human AIDS virus isolates of HIV-2. These similarities in nucleotide sequence provide the most compelling evidence to date that simian and human immunodeficiency viruses have arisen in recent evolutionary history from a common ancestor.

The mangabey, African Green monkey , mandrill and chimpanzee isolates all share certain epidemiologic features. They have all been isolated from African non human primate species . Furthermore none has yet been shown to induce disease in their presumed natural host . However SIVmac and the closely related SIVsm after experimental infection of a variety Asian macaque species induce a disease with remarkable similarities to human AIDS.

SIV Induced Disease in Macaques

One to three weeks after inoculation with these SIV isolates macaques frequently develop a transient rash on the face , trunk and groin that is similar to clinical and histological appearances described in humans after HIV infection. Six weeks to one year after the experimental inoculation of SIV, some monkeys develop transient axillary and inguinal lymphadenopathy. When this has been noted clinically , CD4+, PBL counts have been substantially decreased , usually to less than thousand mm-3.Biopsy specimens of lymph nodes show same histopathological appearances as that in HIV infected humans with AIDS related complex .

The B cell containing follicles are active and expanded. The T cell rich paracortex of these nodes is reduced and the usual 2:1 ratio of CD4 :CD8 cells is altered with the CD8 subsets of the lymphocytes present in greater than normal numbers equalling or exceeding the number of CD4 lymphocytes.

Macaque monkeys infected with SIV develop immune abnormalities similar to those of HIV infected humans.

The absolute number of CD4 cells in their blood falls to one half that of normal monkeys as early as two weeks after experimental infection. Whereas the number of circulating CD8+ cells remain unchanged . Thus the ratio of CD4 to CD8 cells decreases in experimentally infected monkeys The blastogenic response of their PBLs to the plant lectin concanavalin A and in mixed lymphocyte reactions does not decrease significantly. However, the T cell dependent profileration of their B cells after stimulation with pokeweed mitogen is dramatically and persistently diminished after infection.

After experimental infection with SIV many of the macaque monkeys have died with weight loss, opportunistic infections and primary SIV encephalitis. Some have developed profound wasting losing upto 60% of their body weight Disseminated adenovirus and cytomegalovirus infections disseminated Mycobacterium avium intracellulare infections and Pneumocystis carinii pneumonias and intestinal

cryptosporidium have been seen.Some SIV infected monkeys have developed a lymphoma like lymphoproliferative syndrome Some have also died with a lentivirus encephalitis in which perivascular infiltrate or SIV containing macrophages have been present throughout their brains.

Although most of the reported disease in these animals occur within months to a year after infection, a fatal disease has been induced with idiosyncratic Pbjl4 isolate of SIVsm This isolate induces rapidly fatal syndrome characterised by bloody diarrhoea and abdominal lymphadenopathy.

Conclusion

The SIV macaque model has already proved valuable in assessing novel approaches to AIDS treatment , vaccine trials and learning more about pathological responses in animals.In fact many of the central questions in AIDS research can only be addressed in lentivirus infected animals. The precipitating events leading to CD4 depletion , the pathogenesis of CNS complication in AIDS, can be clarified with animal models. Much can be learnt about the protective immune responses for AIDS in animal models.

Abstracts

(1) Anthelmintic resistance in Australian Sheep nematode populations

On 88% sheep farms evaluated, 85% farms had sheep infected with nematodes resistant to benzimidazoles 65% to levamisole and 34% to combination of the two drugs. Resistance to Ivermectin was not detected. The prevalence of resistance was not corelated to stocking rate but was more in areas with average rainfall greater than 500 mm.

The parasites involved were, *Telodorsagia, Circumcinata, Trichostrongylus* sp., *Chabertia, Ovina and Haemonchus contortis.*

Overend D.J., Phillips M; Poulton A.L. and Foster CED (1994)
Aust Vet. J. **71** : 117-121

(2) Sequential Studies of endochrondral ossification and Serum 1,25-dihydroxy cholecalciferol in broiler chickens between one and 21 days of age.

In the two distinct strains (AαB) of commercial broiler flocks, incidence of tibial dyschondroplasia (TD) was different (10 to 20 % in A and 10 to 70 % in B). The serum concentrations of 1,25-dihydroxy cholecalciferol which was equal in AαB on day one was 40 to 50% lower in B than in A at 7, 14 α 21 day of age. At 14 day age the birds of 'B' showed bone lesions of calcium deficiency rickets. It is concluded that TD is related to inherent predisposition and also to lower serum concentration of 1,25 $(OH_2)D_3$.

Parkinson, G; Thorp B.H.; Azuolas J and Vaiano S. (1996)
Research in Veterinary Science (Australia)
60 : 173-178

3) Serum total calcium, phosphorous, 1,25-Dihydroxychole calciferol and Endochloderal ossification defects (EOD) in commercial broiler chickens.

Sequential studies of six Australian broiler flocks representing three major genetic lines revealed that flocks with high incidence of EOD (50% at day 14) had significantly lower bone ash and 1-25 $(OH)_2$ D_3 compared to mildly affect flocks (12 to 16% incidence at day 14) It seems that higher systemic concentrations of 1,25 $(OH)_2$ D_3 between 7 to 14 days age will enhance the ability of broiler chicks to effectively mineralize the cartilegenous growth plates in the appendicular skeleton during early bone maturation

Vaiano S.A.; Azuolas J.K.; Parkinson G.B. (1994)
Poultry Science **73** : 1296-1305.

4) Vaccination against hydatidosis using a defined recombinant antigen

A vaccine based on a cloned recombinant antigen from oncosphere of *Echinococcus granulosus* has been developed by the authors. Sheep vaccinated with antigen designated EG. 95 are protected (Mean 96-98%) against hydatidosis developing from an experimental challenge infection with *E. granulosus* eggs. The vaccine provides valuable tool to control hydatidosis in man and animals. This research also holds promise for development of vaccine against other zoonotic parasites of *Taemia* sp.

Lightower M.W; Lawrence S.B.; Gauci C.G. Young J.; Ralston M.J.; Mass D & Heath D.D. (1996)
Parasite Immunology **18** : 457-462

5) Dose titration of Moxidectin oral gel against migrating strongylus Vulgaris and parascaris equorum larvae in pony foals

In the experimental infection of ponies with *Strongylus vulgaris* (600 larvae on 0 day of exp.) and *Parascaris equorum* (3000 embryonated eggs on 45th day) three doses of Moxidectin as oral gel were tested viz 300 µg, 400 µg, and 500 µg,/kg, on the basis of aecropsy findings & larval recovery on 56th day of experiment. The drug was 99.6 to 100% effective on L4 & L5 of S vulgaris and 100% effective on P. equorum at all dose levels. There was strong indication that prior infection with S vulgaris affects adversely the establishment of P. equorum infection.

Monahan C.M.; Chapman M.R.; Taylor H.W. French D.D. and Klei T.R. (1995)
Vet parasitology **60** (1-2) : 103-110

(6) Comprehensive study of anti pruritic action of Terfenadine in Canine Dermatoses

A study of 48 cases dermatosis accompanied by pruritis in dogs. Terfenadine (Trexyl 60 mg tablets) was given at dose rate of 5mg/kg twice a day. The drug was highly effective in 23 (47.9%), Moderately in 13 (27.0%) poorly effective in 8 (16%) and ineffective in 4 (8.3%). No side-effects were seen. Mean duration of recovery was 5.2 days. Overall efficacy was 91.67%

Ashok Kumar & Joshi B.P. (1996)
Indina Vet. J. **73** : 1264-1266

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