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A Tribute to a Scientist

Dr. C. R. Sane
G.B.V.C., FRVCS (Sweden) D.Sc.

Professor C.R. Sane is a pioneer in the field of Animal Reproduction who ushered in an era of Scientific breeding by Artificial Insemination in India fifty years ago. Following an advanced training in Sweden and U.K., he established the cattle Sterility Scheme in the erstwhile Bombay state, recording the existence of various types of infertility and sterility in cattle.

Subsequently a chair of Professor of Obstetrics and Gynaecology was created at the Bombay Veterinary College and Dr. Sane was the first to be honoured to grace the same. In this capacity he started postgraduate courses in Animal Reproduction and created a band of dedicated workers in this field. Later he was elevated as the Principal, Bombay Veterinary College. He was also elected as the Dean of the Faculty of Technology of the University of Bombay.

He is also the founder President of the Indian Society for the Study of Animal Reproduction (ISSAR) a scientific forum devoted to the dissemination of knowledge and advancement of education and research in animal reproduction. This organisation has now blossomed into a body of international recognition.

A high point of his efforts is the publication of "Textbook of Reproduction in Farm Animals (Theriogenology)" under his chief editorship.

Prof. Sane is the recipient of many honours which include Doctorate of Science from Konkan Agricultural University, Fellowship of ISSAR and those from All India Veterinary Association. He had been the Vice President of Association of Fertility and Sterility Indian Society of Endocrinology and established a closed liaison with scientific fora dealing with problems in Human Reproduction.

After his retirement he devoted more than two decades as a Scientific advisor to a renowned cattle breeding farm and energetically collaborated with different organisations viz., Feed Factories, Pharmaceuticals & Agricultural universities for conducting clinical trials to improve the performance of animals in respect of reproduction.

Various institutions and scientific bodies pioneered by Prof. Sane bear an eloquent testimony to his singleminded devotion to the advancement of animal science.

(Courtesy B.R. Deshpande, Mysore)

Diagnosis and Management of Common Chemical Poisonings in Animals

D. Swarup and **S.R. Sharma**

Division of Medicine, Indian Veterinary Research Institute, Izatnagar

A poison is defined as a substance which by chemical action can kill or injure living organisms including man and animals. The major types and sources of naturally occurring poisonous substances for animals include bio-toxins such as toxic plants, phytotoxins, mycotoxins, bacterial toxins and zootoxins (snakes, toads, insects, etc.), toxic minerals in geothermal waters, feed and fodders and naturally occurring toxic inorganic and organic compounds. Major man-made sources of poison for animals are toxic substances in domestic and industrial wastes and effluents, automobile exhausts, agrochemicals (i.e. pesticides, rodenticides, herbicides, fertilizers etc.) toxic drugs, synthetic chemicals, mining and metallurgy wastes etc. Many of these chemicals are readily available to animals whereas some may be a rare or accidental cause of poisoning. Although cases of poisoning in animals remain undiagnosed many a times, there are ample evidence of an emerging trend of chemical toxicosis in animals, especially due to expansion of chemical industries and widespread use of agro-chemicals in the modern farming system.

Diagnostic approaches

Treatment and prevention of animal toxicoses require sufficient knowledge of the hazardous properties of and potentials for exposure to toxic agents. This can only be possible by correct diagnosis by the history, clinical and post-mortem examination, sero-biochemical and chemical test results, response to therapy and most importantly the professional judgement and experience of Veterinarian with toxicological problems. There are four main approaches applied for diagnosis of poisoning (1) Clinico-epidemiological observations (2) Environmental monitoring and circumstantial evidences (3) Pathological examinations and (4) Laboratory analyses comprising serological chemical and microbiological tests. Informations pertaining to clinicoepidemiological and circumstantial evidence are readily available to the field veterinarian enabling him to diagnose tentatively the cases of poisoning and make presumption of possible cause. The presumptive diagnosis can be confirmed by laboratory examinations.

1. Suspicion and tentative diagnosis of poisoning

Sudden illness in a number of previously healthy animals without any immediately apparent cause usually arouses suspicion of poisoning. The veterinarian can further his suspicion and make tentative diagnosis by obtaining a detailed history, observing and examining sick animals and noting lesions at autopsy. In most instances poisoning is accidental and a large number of animals may be affected exhibiting some signs at a given time. The necropsy lesions in dead animals are mostly identical. Although some infectious, deficiency and metabolic conditions also present similar outbreak profile, a differentiation is possible by thorough review of history and if possible by locating possible source of poisoning. At this point veterinarian's professional knowledge and skill play a key role. The appearance of ration/pasture or medication or spraying and painting, etc. is a common history with most chemical poisoning cases which can help in making a tentative diagnosis. For example, animal showing

nervous signs, frothy salivation and colic in a newly painted house or in an area near lead smelter should be considered tentatively for lead poisoning. Many a times poisoning in animals is deliberate or criminal. This type of poisoning is suspected when selective population of animals is affected without change in diet, pasture or the environment.

2. Presumptive diagnosis

The diagnosis becomes presumptive when a source of toxicants appears to be accessible to the animals and there is an evidence of actual exposure. Geographical distribution of toxic plants, industrial locality and application of farm chemicals are some of the potential sources of poisoning to livestock. The response to a specific antidotal therapy also assists in making presumptive diagnosis. However, lack of response does not necessarily preclude a suspected poisoning because no antidote is 100% effective under all conditions or the treatment may have been too late to evoke a proper response.

3. Confirmatory diagnosis

Diagnosis of poisoning may be confirmed by laboratory tests and chemical analysis of suspected materials. Measurement of functional profile of liver, kidney, gastro-intestinal tract and haemopoeitic systems assists in differential diagnosis. It also helps in understanding pathogenesis of poisons so as to provide a suitable supportive therapy. For example, on the basis of clinical signs, an acute lead poisoning can be mistaken for polioencephalomalacia, infectious thrombolic meningeoencephalitis, nervous form of coccidiosis, rabies, listeriosis or insecticide poisoning. However, measurement of urinary delta-aminolevulinic acid (ALA) level, which increases in lead poisoning (300-400 µg/dl in sheep and upto 500 µg/dl in cattle) may distinguish lead poisoning from the other conditions.

The final diagnosis and specification of causative agent are usually established by chemical analysis of rightly selected, preserved and transported samples. The field veterinarians share this responsibility of proper selection, preservation and transport of samples to the laboratory. It is, therefore, imperative that they have a knowledge of how to submit right sample in a proper manner to the laboratory for toxicological analysis. Some general points need to be considered in this regard are provided herein.

Specimen for chemical analysis should invariably include material suspected as sourceof toxicity (i.e. feed, water, pasture grass, plant, etc.), specimen of alimentary tract together with ingesta from rumen and intestinal segments, and a sample of liver in any suspected cases of poisoning. Skin or lung specimen should be sent, when exposure via skin or respiratory tract is suspected. Additional specimen required by the laboratory vary according to the type of poisoning suspected. The following list can be used as ready reference for submitting additional specimens for some chemical poisons :

Lead	—	Kidney, whole blood and bones.
Fluoride	—	Urine, bone, teeth and kidney.
Arsenic	—	Hair, skin, kidney
Mercury	—	Kidney
Selenium	—	Hair, urine and milk
Copper and Molybdenum	—	Blood and kidney
Phosphorus	—	Kidney and muscles
Cadmium	—	Kidney
Zinc	—	Blood, pancreas and kidney
Hydrocyanic acid	—	Ingesta i and air tight container, blood and muscle
Nitrate and nitrite	—	Ingesta in chloroform or formalin in an air tight container, blood
Halogenated hydrocarbons (DDT, BHC, etc.)	—	Omental and perirenal fat
Strychnine	—	Blood kidney and urine

Following precautions should be observed while sending material for chemical analysis.

i) Do not add any preservative (except in case of suspected nitrate poisoning) to tissue specimen. If a preservative has to be used for long distance transportation, use ice packs or ethyl alcohol (1mg/g tissue). In this case, sample of preservative must be sent alongwith the specimen with details about the quantity of the preservative used.

ii) Carefully pack the specimen to avoid loss of some toxicants by escape as gas or microbial fermentation.

iii) Preferably use heparin as anticoagulant for whole blood samples.

iv) Plasticware is preferred for sending material in cases of suspected fluoride poisoning.

v) Submit sufficient quantity of specimens e.g. : Feed and fodder 100 g; water-200 ml; liver 100 g; kidney 100 g; rumen content 100 g; rumen fluid 100 ml; whole blood/plasma/serum 10-25 ml, urine 50-100 ml, milk 50-100 ml, faeces 50 g.

vi) In case of suspected malacious/criminal poisoning samples should preferably be submitted in duplicate.

vii) The material should accomplish with a report indicating full record of history, clinical signs, postmortem findings, results of environmental investigations (for the search of accessible source of pollution) details of treatment and poison or group of poison suspected.

Treatment of poisoning

The treatment of poisoning is aimed mainly at : (i) Removal of residual poison to reduce its absorption from sites of absorption i.e. gastro-intestinal tract or skin and (ii) detoxification and elimination of absorbed toxicants by specific or non-specific antidotes to reduce its toxicological effects. Ancillary treatment may be necessary to combat the side effects such as diarrhoea, nervous manifestation fever, shock, dehydration and other metabolic alterations.

1. Removal of residues :

Residues of poison in contact of skin (such as organophosphorus compound after dip or spray) can easily be removed by thorough washing with soap, soda or detergent mixed water. In farm animals removal of residual poison from the alimentary tract depends largely on use of adsorbant and purgative because gastric lavage and emetics are of title or no practical value.

Activated charcoal is used to absorb common chemical poisons such as chlorinated hydrocarbons, organophosphorus compounds, mycotoxins, plant alkaloids, feed additives and antibiotics. The oral dose of charcoal is 1-3 g/kg body weight which can be repeated if necessary. Charcoal does not adsorb heavy metals, nitrite, chlorate, sodium chloride etc. These can be removed by oily or saline purgative. It is also necessary to remove adsorbent and poison combination for which purgative should be administered simultaneously with adsorbent. Some other agents such as tannic acid preparations, milk, egg, sulphate of sodium, magnesium or aluminium, etc. are used to neutralize toxic residues in the alimentary tract.

2. Detoxification and elimination of absorbed poison

Toxicants which have already been absorbed can in some instances be neutralized or eliminated by specific or non-specific antidotes. For example, calcium EDTA is an effective antidote for lead; sodium-thiosulphate and sodium nitrate can neutralize effects of hydrocyanic acid, methylene blue is an effective anitdote for nitrate and nitrite poisoning and injection of a calcium salt is physiological anidote in cases of overdosing with magnesium salts. Specific and non-specific antidotal treatments which can be used in cases of some common chemical poisons are listed in table 1.

3. Ancillary treatment

The objective of ancillary or supportive therapy

is to combat systemic effects of poisons. This includes provision of fluids in cases of diarrhoea, corticosteroids and fluids in cases of shock, corticosteroids and tranquilizer in excitement, stimulants in case of CNS depression, demulcents in gastroenteritis, antibiotic along with corticosteroids to reduce stress and bacterial invasion, and multiple vitamin therapy to tone-up liver for better detoxification.

The above principles for treatment can be applied to all cases of poisoning. However, the veterinarian attending the case is the best judge of the situation and can therefore alter his approach according to the need. Further, when treating a lactating or meat producing animal, it is important to take an account of possible unsuitability of the milk or meat for human consumption due to presence of the poison or antidote.

Table 1 Treatment approaches for some common chemical poisons

Poison	Treatment approach	
	Specific antidote dose and route	**Non specific/Ancillary treatment**
Lead	Calcium versanate (Calcium-EDTA) : 12.5% soln. i.v. @ 1ml/kg b.wt. 6 hrly or 2 ml/kg 12 hrly (For s.c. 1-2% solution should be used in 5% dextrose) (BAL, D-Penicillamine, DMSA are also used)	Thiamine-HCl : 5-10 mg/kg b.wt.s.c., Dextrose saline and liver tonic
Arsenic	Sodium thiosulphate : 8-10 g as 10-20% soln. i.v. + 20-30 g in 300 ml water orally 6 hrly (higher doses of 30-40 mg/kg i.v. + 60-80 mg/kg orally 2-3 times daily are also recommended)	Mineral oil purgative, Adequate supply of drinking water Dextrose saline
	BAL (Dimercaprol) for large animals; 3 mg/kg as 5% soln. in a 10% soln. of benzyl benzoate in oil, i.m. 4 hrly for the first 2 days, 6 hrly 3rd day and twice daily for next 10 days	Mineral oil purgative, Adequate supply of drinking water Dextrose saline
Mercury	BAL : 6.5 mg/kg i.m. 4 hrly	Oral astringents Parenteral fluid
Copper	Ammonium thiomolybdate : 168 mg/100 kg b.w. in saline i.v. at 2-3 days interval (BAL, Ca-EDTA, Penicillamine are also effective)	0.5% Ascorbic acid in feed Gastrointestinal sedative + Fluid therapy in acute cases.
Hydrocyanic acid	Sodium thiosulphate (15g) + sodium nitrite (5g) in 200 ml D.W. i.v. for cattle, (3g + 1g in 50 ml water for sheep and goats) (Sodium thiosulphate and sodium nitrite may be used alone also. Cobalt chloride @ 10.6 mg/kg b.wt. can be used in place of Sod. nitrite	Cooling, diluting and acidification of rumen content by 3.5-4 litre Vinegar in 12-20 litre cold water Mineral oil

Poison	Treatment approach	
	Specific antidote dose and route	**Non specific/Ancillary treatment**
Nitrate and nitrite	Methylene blue upto 20mg/kg i.v. as 1% soln. 6-8 hrly for ruminants.	—
Chlorinated hydrocarbons (DDT, BHC etc.)	—	Activated charcoal (9g) orally + chloralhydrate or pentobarbital i.v. Scrub animal with soap and water. Calcium borogluconate i.v. may be given
Organo-phosphorus Compounds	Atropine 0.25mg/kg (cattle), 1 mg/kg (shep) i.v./s.c.i.m. (depending on severity) 3-6 hrly. 2 PAM : 50-100 mg/kg i.v. or TMB (Trimedoxime bromide) 10-20 mg/kg b.wt as a 10% saline solution	Activated charcoal orally Scrubbing with water + detergent Mineral oil purgative

Animal Food for 21st Century : Scope for Veterinary Drugs

S.A. Mir

Department of Pharmacology & Toxicology,
Faculty of Veterinary Science, SKUAST, Shuhama, Srinagar, Kashmir.

Human food includes foodgrains such as cereals and pulses, and animal origin food such as eggs, milk, fisheries and meat from ruminants, poultry and swine. Animal origin food is considered superior mainly as a source of high quality protein with higher biological efficiency (97%) compared to that of pulses (85%) and cereals (78%). The preponderent number of vegetarians in the world, nearly 67% (Wilson *et. al.*, 1971), lean heavily on pulses for derivation of basic proteins. This is particularly relevant in India (Mahajan 1983). Food production has to keep pace with growing human population. A serious concern has been felt that the pace is not satisfactory from global or Indian context (Booth, 1988; Mahajan 1983; Sahay 1996). The gap between agricultural production and human demand is anticipated to widen during the 21st century. The world has to feed additional 2 billion mouths, and India has to feed over a billion mouths by 2000 A.D. The importance of improving food-producing animals to ensure ample and wholesome food-supply to human beings now and for the 21st Century has been stressed (Booth, 1988). All attempts are needed to accomplish the objective satisfactorily.

India needs to boost its animal origin food production for obvious reasons; to meet growing needs, to earn foreign exchange and to generate employment for needy population. Fortunately, animal food production enterprise in India has shown considerable signs of progress over the preceding decades. The

Table 1 : Food Production Performance in India Over Preceding Decades

Food	Growth rate (% p.a.)	Reference
Foodgrains	2.3-3.0	Mahajan, 1983; Survey of Indian Agriculture 1995.
Cereals	2.97	Sharma, 1995
Pulses	1.41	
Meat, milk, fish, eggs	10-15	
Milk	5.19	Rajan, 1995
Eggs	6.60	Panda, 1993
Broiler	18.00	
Fisheries	6.00	Sinha, 1995
Marine	5.50	
Inland	6.80	
Honey	12.70	Mishra, 1995

production performance has been more impressive than that concerning foodgrain production both in terms of annual growth rate (Table1) as well as in enhancing net per capita availability (Mahajan, 1983:Sharma, 1995). Since early sixties *per capita* availability of milk has increased nearly 2-fold (Kurien, 1987; Paroda, 1995), eggs 5-fold (Panda, 1989; Rao, 1995), broiler meat 3-fold (Panda, 1989; Rao, 1995) and fish nearly 3-fold (Mitra, 1982; Santhanam *et. al.*, 1987; Sinha, 1995). This impressive growth in animal food production has been largely responsible to compensate the decline in *per capita* availability of pulse over the decades (Sharma, 1995). In fact pulse production in India has never been satisfactory (Mahajan, 1983; Sharma, 1995). Cereal production, torch bearer of Indian green revolution myth (Mahajan, 1983; Sahay, 1996), has not been able to enhance *per capita* availability of cereals in India. Thus, animal origin food production enterprise constitutes an important food area to be nurtured and boosted now and for 21st century as an important human food sector. Dairy, poultry and fisheries have particularly shown considerable potential for growth and development. Meat production potential of India, mainly from ruminants, is considered to be very high (FAO assessment) but needs proper care and planning. The role of drugs and chemicals in enhancing animal food production for human consumption is anticipated to be more important for the 21st century (Booth, 1988).

The scope of veterinary drug extends to all food-producing animals including meat-or milk-producing animals, poultry, fish and bees, whether used for therapeutic, prophyletic or diagnostic purposes or for modification of physiological functions or behaviour, as per the definition extended by US Codex Committee (Barragry, 1994).

The major objective of animal food production is to enhance magnitude of production, improve the quality of produce and reduce the cost of animal food for the consumer. The key to the success of preserving and improving the productive potential of food-producing animals, even in genetically improved stocks, lies in the accomplishment of the following objectives i) Reduction of mortality, morbidity and stress, ii) Efficient nutrient utilization, iii) Manipulation of fertility and augmentation of reproductive efficiency, iv) Manipulation of animal growth and production, and v) Improvement in the quality of animal food. The veterinary drugs, including biologicals, constitute indispensable tools in fulfilment of these objectives. Efficient feed/nutrient utilization has more than one objective viz., i) reduce feed cost, feed cost being 60-70% of the cost required for rearing livestock, ii) achieve rapid gains in production, and thus save managemental costs, iii) reduce load on agricultural produce, and iv) reduce the amount of animal waste to alleviate problems of environmental pollution (Booth, 1988).

The drug in relation to animal production may be broadly categorized as those;

1. Preserving production,
2. Augmenting reproductive efficiency, and
3. Improving growth, production and quality.

Drugs Preserving Animal Production

Drugs for health

A vast number of prophylactic and therapeutic agents are used to preserve health of food-producing animals. The basic aim is to reduce in production owing to animal mortality, and more often owing to animal morbidity. the field, conventional objective of advent of veterinary drugs, is well established, and discussion beyond the scope of this article. The impact of anticoccidials alone in poultry industry has been immense; a 5-fold increase in broiler industry in USA (1940-1980) has been largely attributed to advent of these drugs. Timely vaccination and appropriate use of anticoccidials and antibacteruials are pre-requisite for the survival of poultry industry. In dairy industry, apart from usual prophylactic

and therapeutic measures, greater emphasis is attached to control mastitis, and production-related disorders especially in highyielding stocks. In fisheries, the scope of veterinary drugs is limited to fast-growing aquaculture fish production (Santhanam *et.al.*, 1987 Schubert, 1987; Sedgwick, 1985). Control of fish diseases and parasites is of paramount significance in enhancing fish production especially carps (Chaudhury, 1982). A major blow to bee industry in India in nineties, a major setback in honey production, has been due to lack of availability of proper medication against Thai Sac brood virus disease (Mishra, 1995)

Anti-stress drugs

The drugs are valuable in minimizing losses in animal production consequent to environmental stresses especially that concerning animal transportation. Stress raises circulating corticosteroids that are potential pro-catabolic and anti-anabolic. Tranquillizers have reduced shrink in cattle when given prior to transportaiton (Singh and Moore, 1982). Chlorpromazine or related tranquillizers are effective aids in transport of dairy cattle (Nair and Razdan, 1982). Pentobarbitone, and to some extent diazepam have been shown to reverse corticosteroid-elevating effects of transportation in goats (Sanhouri *et. al.*, 1991 a, b). Benzodiazephines such as elfazepam have additional effect of improving appetite in ruminants. Stress losses are presumably higher in chicken owing to their increased tendency to excitement. Metoserpate is the recommended CNS depressant for mass treatment in chickens for safe-handling (Booth, 1988). Vitamin C supplementation in chickens have improved egg production and egg-quality under environmental stresses (Balnave *et. al.*, 1991; Bhat *et. al.*, 1996). Zinc methionine also preserves egg-quality under saline stress (Moreng *et. al.*, 1992). Transportation losses are considerably great in fisheries; mortality during and after transport of spawn, fry and fingerlings is very high (Chaudhury, 1982) The recommended drugs include sodium amytal, tertiary amyl alcohol and chloral hydrate. Tricaine methanesulfonate is the only FDA approved anaesthetic for safe-handling of food-fish including rainbow trout and lake trout (Booth, 1988). The use of drugs in safe transportation of fish especially for inbreeding and culture purposes in India need appropriate stadardization.

Drugs Augmenting Reproductive Efficiency

Hormonal agents have proven valuable in augmenting reproductive efficienty of major food-producing animals viz., ruminants, fisheries and swine, paving scope for increasing the population of improved genetic stocks. The drugs are used not only to correct reproductive disorders in farm animals but also to regulate reproduction for better and cost-effective management (estrus synchronization, parturition induction etc.) and to augment reproductive efficiency in an effort to overcome the limitations on productivity set by normal breeding methods and by natural production (Munro and Marriner, 1983). Multiple ovulation and embryo transfer (MOET) has emerged as a powerful tool to revolutionize animal food production particularly in India. The major emphasis in India is to upgrade the Indigenous germplasm as well as to expand the same at faster rate. Hormonal agents are required to induce superovulation, to improve conception rates (e.g. LH-RH or its analogue Buserelin), and to maintain pregnancies in incubator stocks (Menon , 1989; Munro and Marriner, 1983; Taneja *et. al.*, 1989). The drugs used in these endeavours include mainly gonadotrophins (FSH, LF, HCG, PMS), estrogens, progestins and prostaglandins, natural ($PGF_{2\alpha}$) or its analogue cloprostenol.

Use of gonadotrophins or related drugs has revolutionized fish-culture worldwide. Hypophysation technique, making at present use of crude pituitary fish extracts, has considerably improved prospects of increased fish production in India and abroad. It has enhanced production of fish-seed and implementation of selective breeding and hybridization particularly in difficult-to-spawn

major carps. A major breakthrough has been already achieved by evolving catla-rohu hybrid with high growth rate (catla germplasm) and small head (rohugermplasm) (Chaudhury, 1982; Chaudhury and Singh, 1984). The need of the time is to develop and standardize an effective potent and cheap alternative to currently used crude fish pituitary gonadotrophin (Chondar, 1984).

Drugs Improving Growth, Production & Quality

Nutritional Agents :

Mineral and vitamin supply in livestock ration in appropriate quantities is considered a desired approach for achieving optimal food production even in improved stocks. Ruminants do not require exogenous vitamin B-complex supplementation. A large number of trace elements are required to improve ruminal fermentative processes. Of special importance is cobalt. required for both vitamin B_{12} biosynthesis as well as for efficient utilization of propionic acid, a major rumen metabolite, into glucose in liver. ZInc in addition to fulfilling metabolic functions is also known to enhance taste acuity, and thereby feed-intake. Iodine is required by all growing animals to favour growth and production, lactating animals need higher amounts to compensate loss of iodine vai milk. Manganese supplementation in poultry and iron supplementation in piglets is of special significance to favour adequate growth. Vitamins particularly B-complex are required to improve feed utilization and assimilation by the animal body. Vitamin C supplementation in nursery piglets (first 2 weeks of post-weaning) has improved live weight gain (LWG) and feed conversion efficiency (FCE) by about 10-11% (Crenshaw, 1995). Intraduodenal infusion of proline in dairy cattle has improved milk yield by about 11% improved milk quality (fat-corrected milk yield) and efficiency of dietary energy utilization (Brukental *et. al.*, 1991). Micronutrients, yeast, ruminant stomach extract and vitamin B-complex have been reported to enhance the growth and survivability of nursery fisheries- cobalt chloride has proven particularly beneficial in improving survivability in fish (Chaudhury, 1982).

Anabolic agents :

These include drugs that are intended to selectively improve growth and production in food-producing animals. These are also called growth promotants or growth-ptomoting agents. The agents are used worldwide particularly in USA and UK to boost animal food production. Anabolic agents, in general, improve both LWG as well as FCE - improvement in quality of the produce is also recorded particularly with repartitioning agents. The agents may be grouped into three major categories : i) Hormonal agents - Sex-hormone related drugs and growth hormone or GH-related drugs, ii) Feed additives - mainly antibacterials, probiotics and of late herbal feed additives, and iii) Repartitioning agents including beta-adrenceptor agonists.

Sex-hormone related drugs :

The agents include naturally occurring steroids e.g. 17-*B* -estradiol, testosterone and progesterone and xenobiotic hormonal agents e.g. zeranol (estrogenic), trenbolone acetate (TBA, androgenic) and melengesterol acetate (MGA, progestenic). They alone or in combination have, on an average, increased LWG by 10-20% and FCE by 5-10%, estradiol inceased lean meat content by 1-3% (Barragry, 1994; Fraser, 1991; Heitzman, 1983). Estrogen -androgen combination is considered most efficient in maximising meat production in ruminants or in enhancing lean meat content in swine (Heitzman, 1983). The use in fish production is most likely to increase in near future since androgens have increased growth rate in young trout (Heitzman, 1983). The use of gonadal agents has not yielded significant response in poultry

Growth hormone :

Natural or recombinant GH has yielded impressive performance in enhancing meat and milk production particularly in ruminants and swine. The utility in fisheries and poultry has not been demonstrated. The hormone has increased LWG in cattle by 10-50%, besides improving meat quality (Fraser, 1991). Growth rate in swine has increased by 12-20% and FCE by about 15% besides improving meat quality (Crenshaw, 1995; Fraser, 1991).

GH has increased milk production in dairy cattle by about 20% (range 10-50%), and improved FCE by 5-15%; the quality of milk and udder health is not adversely affected (Barragry, 1994; Burton *et. al.*, 1994, Fraser, 1991; Sud, 1991; Varga and Putnam, 1995) The hormone is likely to bost production of dairy industry in India (Menon, 1989). The prediction that immunization of livestock with somatoshtatin release inhibiting factor (SR IF), to keep endogenous GH release continuous and unblocked, would enhance meat production in ruminants has not yielded desired results so for (Dawson *et. al.*, 1991; Zainur *et. al.*, 1991).

Feed additives :

Gut-active feed additives mainly antimicrobials in sub-therapeutic concentrations constitute most widely employed drugs in favouring increased animal food production. The drugs include ionophore and non-inonophore antibiotics, as well as synthetic antimicrobials with choice (s) varying with animal species.

(Armstrong, 1986; Barragry, 1994; Hudd, 1983; Fraser 1991). Most commonly favoured feed additives include avoparcin, zinc bacitracin, virginiamycin, flavophospholipol, nitrovin in ruminants poultry and swine. There is an increased tendency to use carbadox in swine, monensin or related ionophores in ruminants, and in India, penicillins and tetracyclines in poultry (Singh and Moore, 1982). On an average feed additives have improved LWG and FCE by 10% or more in cattle, 4-5% in swine and veal calves, and 3-4% in broiler chicken (Fraser, 1991; Hudd, 1983). Singh and Moore (1982) documented increase in growth rate in chickens by 10-15%, improved FCE by 5% and feed saving about 6%. Of various anticoccidial feed additives tried in poultry, narasin is considered to be best (Parsons, 1995). The potential of feed additives in swine production (Stahly, 1995) and fish production (Chaudhury, 1982) has been encouraging.

Use of probiotics, non-pathogenic micro-organisms that aid efficent dietary nutrient utilization, in enhancing animal food production is also gaining popularity (Barragry, 1994 Crenshaw, 1995; Parsons, 1995). There is growing attention for evaluating and employing organisms that improve ligno-cellulose digestion, reduce ruminal energy losses and enhance efficiency of metabolic assimilation in ruminant species (Menon, 1989; Singh and Mehra, 1989). Of late, use of herbal feed additive in poultry production has been reported to enhance LWG as well as FCE appreciably (Majdanski, 1994).

Repartitioning agents

These agents include beta--adrenergic drugs that favour protein deposition and reduce fat content (increased lipolysis) in skeletal muscles; notable drugs are clenbuterol, cimeterol and ractopamine. The agents have improved LWG and FCE in swine and poultry by 1-3%, and decreased fat content in carcass by about 20%. Ractopamine is gaining wider acceptance in swine production with better performance (Crenshaw, 1995). In cattle and lambs, clenbuterol and cimeterol, have increased LWG by 20-30%, FCE by 15-30% and lean meat content by about 17% (Barragry, 1994; Fraser, 1991).

Concluding Remarks

The increasing demand for animal protein for 21st century is anticipated to enhance the scope for use of drugs and chemicals for animal food production more than that in the past. The potential applications particularly in India are quite great. The need of the time is not only to make extensive evaluation and use in all sectors

of animal food production, but also to rigorously enforce regulations concerning drug residue tolerances in edibles to ensure public health is not jeopardized.

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Mechanisms of Antimicrobial Resistance

G. P. Patgiri

Department of Microbiology, College of Veterinary Science, Khanapara, Guwahati.

The occurrence of antimicrobial resistant pathogenic bacteria in clinical materials as well as in normal flora of humans, in livestock and in foods is an emerging problem commonly encountered in treating various infections due to such bacteria. Human health, livestock production and agricultural developments rely on the use of antimicrobial substances not only for treatment, but also for the promotion of growth and prevention of diseases. Presently, numerous antimicrobial substances are commercially available. The most commonly used antibiotics are Aminoglycosides - (Gentamicin, Kanamycin, Streptomycin; Cephalosporins); Penicillins- (Amipicillin, Benzylpenicillin, Methicillin); other B-Lactams; Sulphonamides - (Sulphadiazine, Sulphamethazine;) Tetracyclines - (Chlortetracycline, Doxycycline, Methacycline, Oxytetracycline;) others are Amphotericin B, Bacitracin, Chloramphenicol, Erythromycin, Lincomycin, Nalidixic acid, Novobiocin, Nystatin, Polymyxin - B, Rifampicin, Trimethoprim, Vancomycin etc.

Antimicrobial substances are biochemically active substances which interact with molecules within the bacterial cell and cause its death.

There are three major types of mechanisms which produce antimicrobial resistant bacteria.

The first type of resistance is "natural resistance." Here the bacteria lack the "target site" with which the anti-microbial substance would have interacted. Such a resistance is inherited vertically among the offsprings.

Second type of resistance is "chromosomal resistance." At least one (or more) mutation occurs in bacterial chromosome, thereby altering the "target site" or preventing the penetration of the antimicrobial substance into the cell. Such a resistance is also vertically inherited.

Third type of bacterial resistance is due to the possession of bacterial plasmids located within the cell but outside the chromosome. Plasmids carrying resistance genes are called R-factors and can either be passed on the progeny of the cell or be exchanged among members of a bacterial population or to other bacterial species. The process of plasmid transfer is called conjugation and the plasmids involved are called "transferable R-factors." Sometimes resistance genes are spread by the process of transformation and transduction involving free DNA and bacteriophages as carrier of DNA.

There are several strategies by which resistance genes can prevent the action of antibiotics. The first is the biochemical conversion of the antibiotic into an inactive compound. Secondly the level of the antibiotic within the cell is kept low. The third involves in the alteration of the target site.

Individual Resistance Mechanisms :

Penicillins and cephalosporins :

These antimicrobial substances interact with binding proteins in the cells and interfere with the cell wall synthesis. For the B-lactam antimicrobial substances several resistance mechanisms have been demonstrated. Chromosomal resistance occurs in *Neisseria, Streptococcus, Staphylococcus* and *Pseudomonas*, where resistance due to plasmid encoded B- lactamase predominates. Their mode of action is the hydrolysis of the B-lactam ring. Numerous enzymes have been involved. Recently mutant plasmid mediated lactamases

have been described which inactivate third generation cephalosporins.

Tetracyclines

Tetracyclines inhibit protein synthesis by interacting with the 30S subunit of ribosomes. The B-lactam antimicrobial substances along with the tetracyclines have been used for a long time not only for therapy but also for growth promotion in animals and hence tetracycline resistance is wide spread. Ten different resistance determinates - 5 for Gram negative and 5 for Gram positive bacteria have been described. In the first mechanism of resistance, the influx of the drug is reduced by increasing the efflux of the drug. In the second mechanism of resistance, the ribosomes are protected from antibiotic action.

Aminoglycosides :

These exert multiple effects on the ribosomes and interfere with translation. Although chromosomal resistance can occur, most clinically significant resistance is due to enzymes specified by R-factors. These enzymes are situated in the periplasmic space and modify the antimicrobial substances so that they no longer bind to the ribosomes.

Chloramphenicol

Chloramphenicol acts on bacterial protein synthesis by interacting with the ribosome and blocking translation. The resistance is mainly due to the production of chloramphenicol acetyltransferase (CAT).

Sulphonamide and Trimethoprim

Sulphonamides and Trimethoprim exhibit their antimicrobial action by competitively binding to important bacterial enzymes viz., dehydropteroate synthetase blocked by dihydrofolate reductase blocked by trimethoprim. In both cases, resistance is due to plasmid-encoded alternative enzymes which are not inhibited by either antimicrobial substance.

Marcolide, lincosamides and streptogramins (MLS)

They inhibit the chain elongation step during protein synthesis and resistance is the result of the specified resistant gene modifying the 23S ribsome RNA. This leads to formation of ribosomes which are not susceptible to anti-microbial action. At least seven γRNA methylases have been described.

Quinolones

These act on susceptible cells by interacting with a subunit of DNA gyrase which breaks and rejoins strands of DNA. The resistance to oldest known agent nalidixic acid, is due to chromosomal mutations in the gyra A gene. Resistance to the newer quinolones seems to be due to rare chromosomal mutations.

Monitoring individual resistance genes

DNA-DNA hybridization is a valuable tool for monitoring the epidemiology of individual resistance gene. DNA-probes for the B-lactam, aminoglycoside, MLS, tetracycline, chloramphenicol and trimethoprim resistance genes are available.

Reference :

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Current Trends in Diagnosis and Treatment of Bovine Infertility

Inderjeet, Umed Singh and **S.K. Khar**

Department of Gynaecology & Obstetrics, College of Veterinary Sciences, Hissar.

The aim of economic dairy farming is to obtain 'a calf a year' for optimum productive & reproductive efficiency. Infertility in our cattle and buffaloes leads to huge economic losses - unrecognised by the Indian farmers, who consider dairying as an auxiliary chore rather than an industry, with little attention to profits. The farmer's own labour and fodder are unaccounted for. With the increased pace of economic development in the country, the farmers, particularly those who take dairying as the sole means of livelihood, must realise the extent of losses inflicted by different aspects of farm animals' poor efficiency, especially the reproductive efficiency.

Indigenous breeds, of both cattle and buffalo, achieve puberty at a much later age than the exotic and cross-bred cattle. In addition, the intercalving interval in indigenous livestock is very long - owing primarily to a period of post-partum ovarian inactivity (anoestrus). Cross-bred cattle usually end-up to be chronic repeat breeders due to poor quality semen available for artificial insemination and other factors. Both in the rural areas and at the organised buffalo & indigenous/exotic/cross-bred cattle farms, we have encountered the two most important reproductive dysfunctions to be :

(i) Anoestrus

(ii) Conception failure/repeat breeding

The incidence of anoestrus amongst the infertile animals presented for treatment in rural areas and at organised farms was around 55%. At the organised farms, 53% of these animals were true anoestrus, while 47% were having cyclic structures in the ovaries or were even pregnant - though misdiagnosed by the management and presented as anoestrus. The incidence of repeat breeding in field and farm conditions was 21 and 44% of total infertile animals examined, respectively. So basically , these two types of disorders, account for 80-95% of all infertility problems encountered in our dairy animals. With great economic significance of livestock infertility, it is pertinent that this is quickly diagnosed and proper therapy is instituted at the earliest.

Diagnosis of Infertility

Diagnosis of infertility requires farm records/ history; evaluation of structures present in the ovaries and determination of uterine contents.

Examination of the Ovaries and Uterus.

Hormone assays

In established laboratories, routine radioimmunoassays for circulating amounts of reproductive hormones can be undertaken to determine the reproductive status of the animals. The steroid hormones (estradiol and progesterone) can also be measured in the milk of lactating animals and ready-to-use cow -side kits have been developed which can immediately reflect the ovarian functional status by dip-stick / colour-change methods. Combined with history and/or ovarian palpation, these tests are confirmatory for the reproductive status of the animals.

Apart from these hormones, which are basically suggestive of ovarian activity, in the recent past, assays have also been developed for hormones which reflect pregnancy status of the animal. One of these is a protein (Pregnancy Specific Protein-B : PSPB) and the other is a steroid metabolite (estrone sulphate).

PSPB can be detected in peripheral plasma from as early as day 15 post-mating and continues until after parturition, due to its very long half-life. Estrone sulphate is detected later in the first trimester of pregnancy. Both these hormones also tend to reflect the number of fetuses *in utero*, though not yet very reliable for this purpose.

Unfortunately, determination of these hormones in blood plasma or milk requires well-equipped radioimmunoassay laboratory. However, the use of enzyme-immunoassay technique, which is equally sensitive and reliable, is more practical for field situations, though again, cost of the equipment remains a constraint for field use. Development of cow-side kits demonstrates field applicability of these tests.

Ultrasound scanning

Ultrasonography is the latest technology available to veterinary practitioners with the advantages of being visual, non-invasive, negligible operating costs and reliable repeatability without adverse effects on biological functions.

Ultrasonography works on the principle of differentialreflection of ultra high frequency sound waves by tissues of different density, which are then converted into a dynamic image on the monitor screen. The organs and structures can be identified by their image : shape, size and location, and any abnormality in the same can be detected. The technique is unmatched in its visualization of the structures in the ovaries & uterine contents. Fluid filled cavities like follicle,fetal sacs and blood vessels appear black on an ultrasound image (no impedence to ultrasound waves - thus no reflection i.e. non-ecogenic) while muscles and bones or hard tissues appear varyingly ecogenic(reflecting waves as per their density and size) It is practically used to find the number of fetuses in ewes and equines for proper feeding or prevention of twinning, respectively. The ovaries can be examined ultrasonographically for the presence, size and number of follicles; presence and location of the corpus luteum; cystic ovarian degeneration; type of cyst (luteal/follicular/cystic corpus luteum), tumors etc. Uterine contents can be accurately described as pregnant with singleton or twins; muco-/hydro- / pyo-metra; location of twins etc. When combined with history, useful information can be derived with ultrasonography. Attempts have also been made for fetal sexing in farm animals with the help of ultrasound.

Laparoscopy

This technique is not routinely employed as the method is invasive and requires incisions in the animals's body under good restraint, thereby limiting its repeatability, especially in bovines. Laparcoscopy can best visualize the ovarian structures, but for the uterus, only its surface can be visualized - not the contents. However, flexible endoscopes are now available for equines which are transcervially passed into the uterine cavity to visualise the cavity and its contents. These endoscopes are extensively used for research in early embryonic life, uterine morphology and some physiological/ pathological reactions of the uterus.

Therapeutic Measures

Exogenous progestogens

Exogenous natural progesterone and synthetic progestogens are being employed to alter the estrus cycle in bovines. Their use is based on mimicking the role of corups luteum for a certain period of time and upon withdrawal of this exogenous source, the sharp decline in circulating progestogen levels leads to pituitary release of gonadotrophic hormones (FSH &LH) which cause follicular growth and ovultion. When animals are treated in groups, the treatment results into synchronization of the estrus and facilitates fixed time insemination, saving on labour costs and ensuring good fertility. However, under conditions of poor nutrition and climatic stressors, insemination at detected estrus is desirable for better success/ conception rates. The treatment can be given

orally in feed (e.g. melengestrol acetate MGA), through intravaginal drug releasing devices (PRID), by subcutaneous ear implants (norgestomet) or even by injections of progesterone preparations in depot forms. In deep anoestrus cows/buffaloes, the regimen needs supplementation with 400-600 I.U. eCG (PMSG) at progestogen withdrawal.

Prostaglandin $F_{2\alpha}$

Prostaglandins have been in veterinary practice since over two decades and are used for various managemental and therapeutic purpose in cattle farming. Their foremost use remains in the treatment of sub-estrus or silent estrus animals to bring them to estrus at predetermined times, when estrus detection can be intensified or fixed-time insemination is practised to achieve pregnancy. An intramuscular dose of 25mg of natural or 500µg of synthetic salt is luteolytic, while the dose is reduced to one-fifth of this for intrauterine or intra-vulvo sub-mucosal (IVSM) administration. For estrus synchronization in cattle, without the need of ascertaining the presence of luteal tissue, a double-dose schedule of $PGF_{2\alpha}$ is more effective. Apart from use in silent estrus animals or those with unobserved estrus, the treatment, with fixed time insemination, tackles the inadequacy of estrus detection regimen at the farm.

Treatment with $PGF_{2\alpha}$ is also effective in evacuating the uterus of infected contents or in hastening the pace of uterine involution when given in early post-partum period. The single treatment is quite effective in the treatment of pyometra, metritis and endometritis, by removing the source of progesterone and thus opening the closed cervix and inducing uterine contractions for evacuation of the pathological uterine contents. The concurrent increase in estradiol also cleanses the uterus due to its function in stimulating endogenous defence mechanism. The $PGF_{2\alpha}$ therapy is quite effective in treating luteal cysts in the ovaries, and also for the treatment of follicular cysts when combined with hCG/GnRH treatment for luteinization of the same before $PGF_{2\alpha}$ is administered.

Gonadotrophin releasing hormone

GnRH and its synthetic analogues are employed in infertility conditions associated with ovarian dysfunction such as anoestrus, anovulation, delayed ovulation and cystic ovarian degeneration, besides improving conception rates in normal and repeat breeding animals by stimulating luteal function. Results with GnRH treatment of anoestrus cattle and buffaloes are variable and may require repeated of pulsatile administration. In anovulation or delayed ovulation, GnRH is recommended at the onset of estrus. Similarly for the treatment of cystic ovarian degeneration, particularly follicular cysts, GnRH or hCG can be effectively employed to luteinize the structure, which can then be destroyed with $PGF_{2\alpha}$ injection given 15 days later. This also circumvents the need to differentiate the cyst, whether luteal or follicular. In repeat-breeder cattle, GnRH is administered either on the day of estrus or 11-13 days later, aimed at ensuring ovulation or revitalising the luteal tissue for increased progesterone production to continue gestation by tiding the period of maternal recognition of pregnancy (for prevention of luteolytic mechanism) when majority of embryonic losses in domestic animal are recognised to be occurring.

It is, therefore most important that the reproductive dysfunctions and diseases are diagnosed and treated at the earliest opportunity for maintaining the economic viability of the dairying enterprise.

In Vitro Antibacterial Activity of Enrofloxacin

S.B. Majee, D.V. Undegaonkar, M.D. Patil and **A.A. Sherikar**
Department of Microbiology, Bombay Veterinary College, Mumbai.

Efficacy of Enrofloxacin as an antibiotic is gaining popularity in treatment of a wide spectrum of infections in animals and poultry. An attempt was made to study the *in vitro* efficacy of various concentrations of Enrofloxacin against bacteria commonly encountered in raw milk and milk products. A few isolates from raw milk and milk shake namely, *Staphylococcus aureus, Escherichia coli, Bacillus cereus and Salmonella* spp. were selected on the basis of their pathogenicity in both man and animals. Standard isolates obtained from Haffkine Institute, Parel namely, *Staphylococcus aureus* ATCC 3750, *Escherichia coli* ATCC 10148, *Bacillus cereus* and *Salmonella* spp. were used in parallel for comparing the respective inhibitory zone diameters of the pathogenic isolates. Enrofloxacin discs in concentrations of 10, 20 and 30 µg were provided by Hoechst Marion Roussel Ltd. Other commercially available discs of antibiotics were obtained from Hi-Media Ltd.

The *in vitro* drug sensitivity of the isolates was carried out by employing disc diffusion technique. Table-1 and Table 2 show the results of the *in vitro* sensitivity or resistance of the isolates to the various antibiotics.

All the isolates tested in the present study were sensitive to Chloramphenicol, followed by 83.3% of the isolates being sensitive to Ciprofloxacin, 68.3% to Gentamycin, 66.6% to Ampicillin, 58.3% to Amoxycillin and Amikacin, 41.6% to Doxycycline and 33.3% to Streptomycin and Co-Trimoxazole. Only 16.6% of the isolates were sensitive to Cloxacillin and 8.3% to Bacitracin and all the isolates were resistant to Furazolidane.

With respect to Enrofloxacin discs, (Floxidin vet, Hoechst), the inhibitory zone diameters of *S. aureus* ATCC strain and *E. coli* ATCC strain were much higher (30mm) and (24-26mm) respectively using 10, 20 and 30µg discs whereas the pathogenic isolates of *S. aureus* and *E. coli* showed a range of 20-24mm and 20-22mm respectively. The same was also observed in case of *Salmonella* spp, isolated from tanker milk which showed a zone size of 20mm with 10µg of enrofloxacin as compared to 22mm shown by *S. paratyphi A. Bacillus cereus* showed a steady zone profile of 20-24mm with standard isolate and 20-22mm with pathogenic isolates (Table1). The Minimum Inhibitory Concentration values of Enrofloxacin for *E. coli, Staphylococcus* and *Bacillus* spp. are already well established (Gatne and Ranade 1996). Therefore, considering the above range of diameters for both gram positive and gram negative organisms the inhibitory zone diameter range for Enrofloxacin may be fixed between 20-30mm and 10µg of Enrofloxacin would be most suitable for routine antibiotic sensitivity purpose.

With reference to the other antibiotics (Table2) used in this study the pattern of sensitivity or resistance to an antibiotic was more or less similar in the pathogenic isolates of a species as compared to their respective standards. *E. coli* isolated from tanker milk and organised dairy was found resistant to all antibiotics except Ampicillin, Amoxycillin, Amikacin and Chloramphenicol as also reported by Sharma *et al. (1992)* of *E.coli* isolated from chicken and pig salami and sausages. *E. coli* was resistant to Ciprofloxacin which is widely used in treatment of urinary tract infections in man and animals. *S. aureus* isolated from milk shake 1 and milk shake2 showed a wide variation in their antibiogram. *S. aureus* from milk shake 2 was sensitive only to Ciprofloxacin and Chloramphenicol as compared to *S. aureus* from milk shake-1 which was also sensitive to Ampicillin, Amoxycillin, Cloxacillin and Bacitracin. Such

Table 1 : Results of Enrofloxacin disc assay with pathogenic isolates from raw milk and milk shake and standard isolates strains.

Antibiotic	*S. aureus* ATCC 3750	*S. aureus* Milk shake (1)	*S. aureus* Milk shake (2)	*B. cereus* (Std.)	*B. cereus* raw milk tanker	*B. cereus* raw milk organised Dairy	*E. coli* ATCC 10148	*E. coli* raw milk organised dairy	*E. coli* raw tanker	*Salmonella Paratythi* A-(Std.)	Std. *Salmonella* raw milk organised dairy	*Salmonella* raw milk tanker
Enrofloxacin 10µg	*30	20	20	20	20	20	24	20	20	22	22	20
Enrofloxacin 20µg	30	22	20	[illegible]	20	22	24	22	20	24	22	22
Enrofloxacin 30µg	30	24	20	24	[illegible]	22	26	22	20	24	24	24

** Numbers indicate inhibitory zone diameter in mm.*

Table 2 : Results of antibiotic disc assay with pathogenic isolates from raw milk and milk shake and standard isolates strains.

Antibiotic	*S. aureus* ATCC 3750	*S. aureus* Milk shake (1)	*S. aureus* Milk shake (2)	*B. cereus* (Std.)	*B. cereus* raw milk tanker	*B. cereus* raw milk organised Dairy	*E. coli* ATCC 10148	*E. coli* raw milk organised dairy	*E. coli* raw tanker	*Salmonella Paratythi* A-(Std.)	Std. *Salmonella* raw milk organised dairy	*Salmonella* raw milk tanker
Enrofloxacin	S	S	S	S	S	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S	R	R	S	S	S
Gentamycin	S	R	R	S	S	S	R	R	R	S	S	S
Streptomycin	R	R	R	R	S	S	S	R	R	R	S	R
Doxycycline	S	R	R	S	S	S	R	R	R	S	R	R
Ampicillin	S	S	R	R	R	R	S	S	S	S	S	S
Amoxycillin	S	S	R	R	R	R	S	S	R	S	S	S
Cloxacillin	S	S	R	R	R	R	R	R	R	R	R	R
Amikacin	S	R	R	S	S	S	S	S	R	S	R	R
Bacitracin	R	S	R	R	R	R	R	R	R	R	R	R
Furazolidane	R	R	R	R	R	R	R	R	R	R	R	R
Co-Trimoxazole	S	R	R	R	R	R	R	R	R	S	S	S
Chloramphenicol	S	S	S	S	S	S	S	S	S	S	S	S

R=Resistant, S=Sensitive.

variation between *S. aureus* strains is widely known as is also reported in cases of mastitis (Tuteja *et. al.* 1992). *B. cereus* has been isolated from cases of pyometra in bovines (Rampal and Dwivedi, 1992). They have reported sensitivity to most of the commonly used antibiotics whereas the isolates in the present study were resistant to antibiotics like Ampicillin, Co-Trimoxazole and Furazolidane. *Salmonella* spp. isolated from raw milk showed sensitivity to Gentamycin, Ampicillin, Streptomycin and Chloramphenicol as compared to the findings of Rahman (1993) who found *Salmonella* spp. from foods of animal origin to be resistant to the above antibiotics. These results indicate the wide variation in the antibiotic susceptibility of various isolates.

The presence of R-factor in *E. coli* and other bacteria is well documented and it is possible that multiple antibiotic resistant strains of bacteria are consumed through raw milk and milk products. Consequently, a large section of the population may demonstrate resistance to several antibacterial agents and hence respond poorly to the commonly available antibiotics. Hence, utilisation of newer antibiotics like Enrofloxacin showing a broad spectrum of antibacterial activity would help in reducing the problem of drug resistance in animals.

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Efficacy GnRH Treatment on Pregnancy Rate in Suckled and Non Suckled Cows

A. Azizi Vamerzani*, B. Munilal Dubey** and **D.V. Arneja ***

Abstract

In the present study 88 cross bred cows comprising of 22 suckled and 66 non suckled cows were administered randomly with either 2.5 ml normal saline (Placebo therapy) or 2.5 ml. Receptal (Hoechst, Buserelin) (GnRH therapy) intra-muscularly one hour post artificial insemination. Pregnancy was confirmed pre-rectally between days 45 to 60 post A.I.

The conception rate was significantly higher in GnRH than Placebo therapy in non suckledcows (P0.01). Within GnRH treated groups the pregnancy rate was highest in suckled cows. Possible role of GnRH in reproduction in suckled and non suckled cows is discussed.

Introduction

GnRH treatment increased conception rate in normal cycling cows (Schels, and Mostafawi., 1978 and Rao and Rao., 1984) and in repeat breeder cows (Lee *et. al.*, 1983 and Stevenson, *et. al.*, 1990). In normal cycling cows (Ryan *et.al.*, 1991) found no additive effect on pregnancy rate from multiple GnRH treatment over that of single treatment. Howeer, reports of occurrence of peak luteinizing hormone (LH) concentrations and subsequent ovulation (Roche 1977) still varies among individuals.

The primary object of the present study was to determine the effect of GnRH administration on conception rate by administering it one hour post artificial insemination in crossbred cows and the effect of suckling and non suckling on efficacy of GnRH.

Materials and Methods

Eightyeight crossbred cows in moderate body condition were randomly selected. These animals were divided into two experimental groups the experiment I including normal suckling control group (Placebo therapy) and the experiment II including normal suckling treatment group (GnRH therapy) respectively.

Animals were under veterinary supervision for sexual health and disease control. In experiment I and II animals presented for A.I. for the first or second, third or more than three services after normal parturation were included. All the selected animals were disease free without gross anatomical abnormality or inflammation of genital organs.

To check the effect of suckling and non-suckling on efficacy of GnRH, all the results of experiment I and II were grouped into two groups namely Placebo and GnRH therapy group. The variation in pregnancy between and within two groups was statistically analysed by adopting test for proportion as described by (Snedecor and Cochran, 1967).

Results and Discussion

The administration on GnRH at the time of insemination creates certain beneficial physiological process resulting in higher conception. There is release of FSH and LH after administration of GnRH hormone in bovines.

Schels and Mostafawi (1978) concluded that GnRH administration during estrus synchronized the process of maturation of the follicle, ovulation and development of corpus luteum.

* Dept. of Gynaecology & Obstetrics, College of Vety. Sc., Hissar.
** Dept. of Gynaecology & Obstetrics, Vety. College, Bangalore.

Table I : Pregnancy rate in suckled and non suckled cows and GnRH efficacy.

Parameters	Placebo therapy		GnRH therapy	
	Total No. of animals	Pregnancy percentage	Total No. of animals	Pregnancy percentage
Suckled cows	17	64.70	5	100.00
Non suckled Cows	23	26.08	43	72.09

Significant between	t. value	inference
Placebo and GnRH treatment in suckled cows gorup	1.067	N.S.
Placebo and GnRH treatment in non-suckled cows gorup	2.876	* *
Suckled and non-suckled cows in placebo group	1.882	N.S.
Suckled and non-suckled cows in GnRH group	0.997	N.S.

N.S. = Non Significant

* * = Significant at 0.01 percent

In the present study the conception rate in placebo and GnRH treated groups were 64.70 and 100 per cent cows suckled by calves and 26.08 and 72.09 percent in cows non suckled by calves respectively. The percentage of pregnancy inceased significantly (p/_0.01) between Placebo and GnRH treated animals in non suckling group, whereas the variation in suckling group was not significant (Table 1). Further there was no significant differences in conception rate between suckling and non suckling groups both in Placebo or GnRH therapy (Table 1). However during post partum period, Carter *et. al.*, (1980) recorded significantly higher (P/_0.05) concentration of LH and significantly larger size of follicles in ovaries (P/_0.01) with GnRH therapy in non-suckled than suckled cows. Further during post partum period removal of calf 72 hours prior to experiment resulted in an increased pituitary response and more release of LH in GnRH treated than in control (Dunn *et. al.,* 1985).

The suckling stimulus inhibits hypothalamic release of GnRH *in-vivo* studies (Khobil and Neil *et. al.*, 1988) and in *in-vitro* studies when cow pituitary explants was exposed to GnRH, the LH secretion is doubled in the weaned than suckled cows (Walters *et. al.,* 1982). Nett (1987) stated that suckling and environment factors induces release of endogenous opioid peptides which inhibit GnRH release from the hypothalamus. It could be infered from the above studies that suckling has suppressive effect on pituitary gonadotropins release. The significantly higher per cent (P/_0.01) of conception rate in non-suckling cows in the present study (Table 1) could probably be due to weaning practices, which removed the suppressive effect on pituitary glands. It could be concluded from the present study that GnRH therapy is more effective in increasing the conception rate in cows that were not suckled by calves.

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Bovine Spongyform Encephalopathy-A Review

A.T. Sherikar,V. S. Naraspur and **S. Jagdish**
Bombay Veterinary College, Mumbai

Bovine Spongyform Encephalopathy (BSE) also known as "Prion disease" or "Mad Cow Disease" belongs to a group of diseases termed "Transmissible Spongyform Encephalopathies" which include the following:-

1 BSE - Cattle
2 Scrapie - Sheep
3 TME - Transmissible Mink Encephalopathy
4 CWD - Chronic Wasting Disease-Mule deer
5 CJD - Creutzfeldt Jacob Disease-Man
6 GSS - Gerstmann-Staussler Scheinker Syndrome-Man
7 Kuru- Man

The nature of infective agents may be a Prion (a minute infectious pathogen which contains a form of protein resistant to procedures that hydrolyse nucleic acids) or Virino (a small micro organism containing nucleic acid and associated with celluar proteins) or a filamentous virus (South Wood Report-1989). These agents are extremely resistant to heat (121^0 C for 1 hr), alkylating agents, organic solvents, common salt, ionising radiation and detergents.

In all spongyform encephalopathies, the incubation period is very long-measured in years and up to 8 years or more.

The onset of the disease is insidious requiring 1-6 months for the symptoms to get pronounced. Symptoms vary greatly in type and severity and since the Central Nervous System in involved, clinical signs take the form of altered behaviour, movement, posture and mental awareness. The lesions restricted to CNS include degenerative changes in the grey matter of brain stem. Discrete round and ovoid vacuoles or microcavaties formed in this location gives a spongy appearance in histological examination.

BSE is a slow progressive fatal disease of adult cattle (4-5 years age group) first recorded in England in November 1986 (Wells *et. al*-1987). However there are reports that it may also have occurred prior to this date.

Most of the herds (75%) have single cases and there is no breed or sex differences in susceptibility. Neither season nor pregnancy or lactation affect incidence.

Apart from cattle, BSE has also been recorded in a few Zoo animals and cats in England. The main cause of spread in these animals appears to be bovine meat and bone meal etc being used as source of protein in their feed. The offals viz Brain, Spinal Cord, Spleen,Thymus, Tonsils and intestine of infected animals are regarded as dangerous in this respect. Vertical transmission of the agent from cows to the progeny is also suspected.

BSE has been successfully transmitted to sheep (scrapie resistant and scrapie susceptible strains) goats, pigs and a wide range of laboratory animals from grain material of BSE positive cattle through oral and intra cranial routes. (Foster *et. al.* 1993)

Epidemiologically there is strong suggestion that initial cases of BSE in cattle were brought about by consumption of Scrapie infected feed (Wilesmith *et. al.* 1988) Increased population of sheep, increased incidence of Scrapie and altered rendering procedures adopted during

1970-80 (abandoning fat solvent process and rendering at much lower temperature which did not destroy Scrapie agent) might have precipitated BSE in cattle

Incidence of BSE in England was 2 per 1000 in 1988 and perhaps as a result of strict regulations (Bovine offal (probhitions) regulations) and other regulations regarding procedures in slaughter houses, condemnation and disposal of offals and branding of infected animals etc. introduced in 1988, the incidence came down to 1 in 1000 in 1990. The SEAC, (advisory committee on BSE to U.K. Govt.) has claimed that there is drastic reduction of BSE cases in animals born after above regulations were introduced and which are now in susceptible age group.

Apart from England BSE is recorded in Oman, Switzerland, Portugal, France and possibly Germany. However the number of cases reported from these countries during 1986 - 95 is very small (varying from 16 to 206) as compared to 1,60,000 cases in U.K. (Hillen brand k. Usher 1996) in spite of the fact that thousand of tons of possibly contaminated feed was sold from Britain to these countries during the said period. This has given rise to wide speculations.

Clinically early signs of BSE are increased alertness, anxious look and reduced milk yield. Later reluctance to move, altered gait (high stepping, incoordination), aggressive frenzied reaction to noise and touch, pole scratching, nose licking are noticed eventually leading to recumbency and death.

These nervous symptoms have to be differentiated from hypo - magnesemia, hypocalcemia, rabies, listeriosis, brain abscess, Histopathology of the brain tissue is fairly diagnostic.

Disease in Man

CJD effects people of 50 - 65 years age and dementia, cerebellar ataxia, cortical blindness, hypeaesthesia and loss of speech are symptoms with death reperserving in a month to 1 year. The incidence is 1 in a million and is fatal in 15% cases. It has been accidentally transmitted by corneal grafts and improperly sterilised electrodes.

GSS is usually fatal and symptoms include dementia, intellectual decline, memory loss and generalised seizures.

Kuru is confined to 'Fore' people in New Guinea and symptoms are helplnessness, ataxia, muscle tremors, emotional instability, joint pain and death in one year. This was transmitted by ritualistic ingestion of brain of dead relatives and is fast declining now. These human diseases have been very much under-diagnosed.

Zoonotic Importance of BSE:-

It has been suspected that CJD in Man is related to BSE and is contracted by consumption of beef from BSE infected cattle. This has lead to a world wide ban of beef and beef products originating from England bringing 860 million dollar beef export industry of that country to almost a halt.

Epidemiological evidence gave rise to the assumption that Scrapie agent " Jumped the (host) species " to cause initial cases of BSE in cattle. This further lead to the assumption that the agent " Jumped " again from cattle to man. However Eddy (1995) has disapproved this theory in view of the following facts viz 1. BSE could not be produced by experimentally injecting / feeding Scrapie material in cattle. 2. There are no reports of producing classical Scrapie in sheep by injecting BSE positive material. 3. There is no increase in the incidence of Scrapie in U.K. in spite of using 41,000 tons of feed containing bovine protein in sheep and cattle during same period.

Thus as per Franz Fischer, E.U. Agriculture commission, " there is no evidence that there is a link between BSE and human brain disease and there is also no evidence that there is a link " Many experts feel that chances of human infection, even if proved, are miniscule (Hillenbrand & Usher 1996)

Intesive research on the BSE agent and its

biology could settle the controversy, but with the present techniques it takes several years to complete such a study. Preliminary findings indicate that by using transgenic mice (containing prion protein gene) in place of present mouse model for CJD would reduce this period to 200 days. But to regain the consumer confidence which is so badly shattered, there is no way to shorten the period.

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Behavioural Reponses and Conception Rates in Oestrus Synchronised Buffaloes

M.R. Bhosrekar, Y.P. Phadnis, S.B. Gokhale and **B.R. Mangurkar**
BAIF Development Research Foundation, Uruli-Kanchan

Introduction

Buffaloes contribute to more than 50% of India's total milk production. In spite of its usefulness the buffalo was very much neglected in the past. However, since 1970 there has been a great interest in improving the productivity of buffaloes.

One of the main hurdles in improvement of buffalo production is its low reproductive efficiency. The factors influencing the reproductive performance are late maturity, long intercalving period, poor heat expression and seasonal breeding. In order to overcome these constraints and increasing the effectiveness of AI programme, oestrus synchronisation can form an integral part of buffalo breeding. The present work carried out in farmer owned buffaloes very vividly brought out these facts.

Material and Methods

A total of 403 non descript buffaloes (Bubalus bubalis) including 44 heifers were involved for studying the effects of oestrus synchronisation treatments on behavioural responses and conception rates during different seasons. Before including these buffaloes for this study observations on open days, body condition, parity and cyclicity were recorded. The distribution of buffaloes as per treatment and seasons was done as follows :

Seasons	PGF2 alpha (PG)	PRID PMSG PG (PRID)	Synchromate B + PMSG PG (Implant)	Total
Summer	33	80	39	152
Rainy	43	45	45	133
Winter	40	36	42	118
	116	**161**	**126**	**403**

The distribution of buffaloes as per open days was as follows :

	0 to 100 Days	101 to 200 Days	201 to 300 Days	301 & above Days	
Number	15	73	68	247	403

The parity wise distribution was as follows :

	1	2	3	4	5 and above	Heifers	
Number	58	67	100	65	69	44	403

Index terms : Buffalo, Oestrus synchronization, Conception rate.

185, 155 and 63 buffaloes had respectively good, fair and poor body condition, while 189 and 214 buffaloes were cyclic and acyclic respectively.

A protocol of 2 intramuscular injections with the interval of 11 days and AI on 72 and 96 hours from last injection was followed for PGF2 alpha (Lutalyse) treatment. For PRID and

synchromate B plus device was inserted in vagina and subcutaneously behind the ear respectively and kept in situ for 10 days. For synchromate B plus 2 ml of intramuscular injection of norgestomet and oestradiol valerate was given on the day of insertion. On day 8th of both the treatment an intramuscular injection of PMSG (400 Iμ) and PGF2 alpha (25 mg) was given and the device was withdrawn on day 10th. AI was done on 48 and 72 hours of withdrawal of devices. The ovulation time was determined by rectal palpation of ovaries at 6 hourly interval from 1st AI onwards 2 hourly interval from 2nd AI onwards in 63 buffaloes respectively under PG(18) PRID (24) and IMPLANT (21) treatment. Analysis of variance was carried out for conception rate taking main effects as treatment, body condition, seasons, open days, group, parity group and cyclicity.

Results and Discussion

In PG, PRID and Implant group respectively 72.4, 82.6 and 82.5 percent buffaloes expressed heat clinically showing sewlling of vulva and reddening of vulval mucus membrane. However, on rectal palpation 94.39, 98.13 and 97.61 percent buffaloes were recorded in heat based on open portio and toned uterine horns thus showing 22, 15.5 and 15.1 percent buffaloes in silent heat respectively. Buffaloes also showed seasonal variation in expression of heat symptoms viz. less intensity of oestrus in summer (80.0) as compared to rainy (83.1) and winter (82.6). The observations, coroborated with earlier findings by other researchers, though all the three treatments were not simultaneously used by them (1) (2).

Table 1 : Conception Rates (%) in Oestrus Synchronised Buffaloes in Relation to Seasons & Cyclicity

Seasons	Treatments					
	PGF2 alpha		PRID		IMPLANT	
	Acyclic	Cyclic	Acyclic	Cyclic	Acyclic	Cyclic
Summer	29.29 ± 10.0 (21)	25.75 ± 12.92 (12)	34.0 ± 7.9 (36)	32.50 ± 7.03 (44)	31.13 ± 9.71 (23)	38.13 ± 12.37 (16)
Rainy	50.50 ± 14.92 (12)	52.10 ± 9.03 (31)	56.96 ± 10.46 (23)	68.70 ± 10.06 (22)	55.0 ± 10.76 (22)	61.26 ± 10.3 (23)
Winter	42.25 ± 14.72 (12)	57.57 ± 9.42 (28)	54.31 ± 14.24 (13)	39.74 ± 10.3 (23)	50.50 ± 12.78 (16)	61.26 ± 9.78 (26)

Table 2 : Ovulation Time (Hours) from 1st AI in Oestrus Synchronised Buffaloes.

Cyclicity Status	Seasons			
	Summer Means ± SE	Rainy Means ± SE	Winter Means ± SE	Overall Means ± SE
Acylic	41.63 ± 2.08 (8)	29.00 ± 1.59 (12)	33.73 ± 1.42 (11)	33.94 ± 1.29 (31)
Cyclic	39.00 ± 2.89 (10)	34.17 ± 2.35 (12)	34.40 ± 1.12 (10)	35.75 ± 11.33 (32)
Overall	40.17 ± 1.83 (18)	31.56 ± 1.49 (24)	34.05 ± 0.89 (21)	34.86 ± 0.93 (63)

Rainy season and good body condition showed significantly higher ($P<0.01$ and $P<0.05$) conception rates in oestrus synchronised buffaloes while parity status open days groups ovarian cyclicity status, different synchronization treatments did not significantly affect the conception rate (Table 1). The average ovulation time recorded was 34.86 hours from Ist AI which is in close agreement with earlier work (5) on Nagpuri buffaloes. Seasons as well as body condition significantly influenced the ovulation time while parity group or cyclicity did not show any significant difference (Table 2).

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Readers' Column

1. **Dr. P.N. Singh**, Vety Officer, **Dhanbad**, Bihar - The 6th edition contains very important information regarding the problem of repeat breeders in cattle; Floxidin has very good results in inflammatory conditions as well as in chronic pyrexia. The efficacy of Tonophosphan is well proved.

2. **Dr. Sanjay Singhania**, **Rajnandgaon**, M.P. - The Blue Cross Book is beneficial to all veterinary clinicians actually engaged in the field. I am confident that the regular publication and supply of this book, would be a boon to Veterinarians.

3. **Dr. N.K. Basu**, **Calcutta** - I don't like this issue because it does not contain sufficient number of case reports for the practising veterinarian.

4. **Dr. H.L. Saxena**, **Pune** - Abstracts are good. But, there are too many articles on Floxidin. There should be other topics as well.

5. **Dr. S. K. Saini**, Vety Officer, **Ropar**, Punjab - It gives latest information about our profession as well as your products. I don't like this issue because it includes more trial reports of your products and less of general information. You should include articles of research and surgery also. Article on Mad Cow disease (BSE) will be welcome. Waiting for your next issue.

6. **Dr. S.C. Tripathy**, **Berhampur**, Orissa - I like this issue because of research articles on breeding - Efficacy of Floxidin - Floxidin is a wonder drug. Handy drug of choice for a practising veterinarian.

Tuberculosis at Nandankanan Biological Park - An Overview

A.T. Rao and L.N. Acharjyo

Department of Pathology, Orissa Veterinary College, Bhubaneshwar.

Tuberculosis (TB) - an ubiquitous and cosmopolitan chronic infectious disease caused by *Mycobacterium tuberculosis* is known to have a wide host range including wildlife species particularly in Zoological Gardens. The disease is worldwide in distribution though most predominant in temperate regions. Iyer (1937) while reporting the common occurrence of the disease in Indian elephants indicated that the disease is known since 2000 B.C. in India. Among Indian animals in captivity, Bombay Zoological Gardens in 1908 experienced a prolonged outbreak of TB which eventually infected many species including lamas, deer, sheep, antelopes, tapirs, coatis, binturongs and lesser pandas (Liston and Soparkar, 1924). Apart from monkeys of different types (Liston and Soparkar, 1924; Iyer, 1940; Nair and Murty, 1951; Chatterji, 1960; Basak *et. al.* 1976), TB has been reported in nilgai (Fox, 1923), bear and deer (Nair and Murty, 1951), spotted and hog-deer, gazelle and binturong (Mukherji and Chatterjee, 1958), giraffe (Rai and Nair, 1958) and rhinoceros (Mann *et. al.* 1981). The disease among animals and birds has been found to be the most common in majority of Indian zoos (Sengupta, 1974; Basak *et. al.* 1975; Rathore and Khera, 1982; Rao *et. al.* 1982; Baruah, 1983 Singh *et. al.*, 1986).

Materials and methods

Systematic necropsy examination of all the mammals, avians and reptiles which died at Nandankanan Biological Park and histopathological examination of tissues hitherto collected from suspected cases of TB have been carried out from 1967-1990 to gain information on the incidence and prevalence of tuberculosis at the zoo. About 1200 mammals, 2500 avians and 200 reptiles were screened for the purpose during this period. Formalin fixed tissues were processed by routine histological methods and stained by haematoxylin and eosin and Zeihi-Nielson's methods.

Results and discussion

The yearwise recorded cases, age, sex and organ involvement of TB in different zoo sepcies have been appended (Table 1 &2). The gross lesions in different species of ruminants and wild pigs which were more or less identical were characterised by multiple discrete encapsulated nodules of varying sizes in different organs. The smaller nodules had coalesced to form bigger ones, the incision of which often gave gritty sound. In 3 sambars and 2 spotted deer numerous ragged irregular cavities in lungs simulating vomicae in man and dog were seen. The pleura in some ruminants were markedly thickened due to fibrin strands/fibrosis as a result the lungs were frequently adhered to the chest wall. In a sambar and wild pig the udders were found to be firm and easy to cut with exeggeration of lobular architecture. Typical grape-like clusters were found on the peritoneum of a gaur.

In primates, the affected organs showed numerous soft creanmy-white nodules of varying sizes. In the avian species the livers were affected in all the cases. In the wallaby, liver, lungs and spleen were affected in which the greyish white tubercles could be enucleated easily simulating lesions in avians. Histological lesions in general were characterised by focal aggregation of reticuloendothelial cells with or without giant cells followed by caseation necrosis. The commonest nodular form

particularly in ruminants consisted of caseation necrosis with central calcification surrounded by epithelioid cells, macrophages, lymphocytes and langhans type of giant cells. The discrete nodules were encapsulated whereas there was extensive fibrosis around coalesced nodules. In avian species calcification was not observed. In wallaby, there was only epithelioid granulomas without caseation, calcification, encapsulation and giant cell formation. In 20 ruminants, there was exudative fibrinous pleuropneumonia. The udder of a sambar was affected with chronic organ tuberculosis while a wild pig has extensive area of caseation necrosis. The diagnosis of the disease was made on the basis of demonstration of acid-fast organisms morphologically akin to *Mycobacterium tuberculosis* in the affected tissues. Isolation and typing of the bacteria was not done.

Table 2 shows that spotted deer belonging to all ages were affected the youngest being 5 months old. In the mammalian species, the lungs were always involved suggesting repiratory route of infection while in the avian species, the liver was affected in all cases indicating possible alimentary route of the infection. Generalisation of infection was found to be frequent in wild pigs and primates perhaps due to their extreme susceptibillity to the disease. In the primates the lesions were found to be spreading in nature because of extensive areas of caseation necrosis, less fibrosis and infrequent giant cell response. Calcification and fibrosis were found to be common in wild ruminants and pigs indicating the signs of hosts resistance and the pathology resembles that seen in domestic cattle. This study showed that so far the carnivores and reptiles were free from the disease and the incidence of the disease in avian species considerably low.

Conclusions

Previous reports revealed that TB is widely distributed in animals and birds in different zoos of this country and this study confirms the earlier reports. The wide spread occurrence of the disease in zoo species has become an increased concern to Veterinarians, Zoo Directors and Scientists in related disciplines. Since there is neither any national programme to erradicate TB nor any mandatory reporting system accurate data is not available on the type(s) of Mycobacteria causing the disease in wildlife species. The inability to control the disease in valuable non domesticated animals has resulted in dramatic economic losses and failure to replace certain endangered species. The importance of Mycobacterial infections is further emphasised by public health hazard.

The diagnosis of the disease is often difficult in certain species because of slow progression and failure to recognise the clinical signs early in course of infection. Though tuberculin skin test procedures are somewhat useful in establishing a presumptive diagnosis of the disease, this test is not always reliable in some species. Moreover because of lack of facilities for capture operations in wildlife, this test is not routinely practised in Indian zoos. Confirmatory diagnosis has to be made only on the basis of necropsy and histopathological examination as has been done in this study and cultural examination and typing of bacteria wherever it is feasible.

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Table 1: Yearwise recorded cases of TB at Nandankanan

Year	Spotted deer	Barking deer	Sambar	Black buck	Four horned antelope	Hog deer	Gaur	Wild pigs	Primates	Avian	Marsupials (Wallaby)	Total
1967	—	—	—	—	—	—	—	—	1	—	—	1
1968	—	—	—	—	—	—	—	—	—	1	—	1
1969	—	—	—	—	—	—	—	—	—	2	—	2
1970 -1974	—	—	—	—	—	—	—	—	—	—	—	NIL
1975	—	1	—	—	—	—	—	—	—	1	—	2
1976	—	1	—	—	—	—	—	1	—	—	—	2
1977	—	—	—	—	—	—	—	—	—	1	—	1
1978	7	—	—	—	—	—	—	6	1	—	—	14
1979	4	—	—	—	—	—	—	1	—	—	—	5
1980	1	—	—	—	—	—	—	4	3	—	—	8
1981	—	3	—	—	—	—	1	2	—	—	—	6
1982	—	—	1	—	—	—	—	—	—	—	—	1
1983	1	1	1	—	—	—	—	—	—	—	—	3
1984	1	—	2	—	—	—	—	—	1	—	—	4
1985	2	2	1	3	1	—	—	—	—	—	—	9
1986	—	—	1	2	—	1	1	1	1	—	—	6
1987	1	1	—	—	—	—	—	—	—	—	—	2
1988	—	—	—	—	—	—	—	—	—	—	—	NIL
1989	—	—	—	—	—	—	—	—	—	—	1	1
1990	—	—	—	—	—	—	—	—	—	—	—	NIL
Total	**17**	**9**	**6**	**5**	**1**	**1**	**1**	**15**	**7**	**5**	**1**	**68**

Table 2 : Age, sex and organ involvement of TB in zoo species

Kind of zoo species	Number died M	F	Age group affected	Lung	Mediastinal/ Bronchial L.N.	Liver	Kidney	Spleen	Udder	Muslce	Pleura	Pricar-dium	Peritoneum
1. Spotted deer	11	6	5m-10 yrs	17	6/3	4	—	—	—	—	11	—	1
2. Barking deer	4	5	1-9 yrs	9	4/0	1	—	1	—	—	—	—	—
3. Sambar	3	3	5-12 yrs	6	3/1	—	1	—	1	—	6	—	—
4. Black buck	—	5	5-10 yrs	5	2/0	—	—	—	—	—	2	—	—
5. Gaur	1	—	12-13 yrs	1	1/1	1	1	1	—	—	1	1	1
6. Hog deer	—	1	1 yr	1	—	—	—	—	—	—	—	—	—
7. Four-horned antelope	1	—	8 yrs	1	—	—	—	—	—	—	—	—	—
8. Wild pigs	11	4	3-12 yrs	15	7/5	—	2	4	1	1	—	—	1
9. Primates	3	4	10-20 yrs	7	0/3	1	—	3	—	—	1	1	—
10. Wallaby	—	1	Adult	1	—	1	—	1	—	—	—	—	—
11. Lesser whistling teal	—	1	Adult	1	—	1	—	1	—	—	—	—	—
12. Pigeon	1	1	Adult	—	—	2	—	—	—	—	—	—	—
13. White Cockatoo	—	1	15 yrs	—	—	1	—	—	—	—	—	1	—
14. Grey Java sparrow	1	—	6 yrs	—	—	1	—	—	—	—	—	—	—

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Epidemiology and Economics of Bovine Pyrexia in Arids

Ashok Kumar and **B. P. Joshi**
Department of Medicine, College of Veterinary Science & Animal Husbandry, Mathura

Present day dairy farming is highly competitive agrobusiness and thus examination of costs and returns are important economic aspects of these enterprises. The diseases being a major source of losses among dairy animals, there is need to quantify the monetary drain from these channels. In such studies epidemiology and cost-benefit analysis seems to be significant tool (Radostits *et. al.*, 1994). The information thus elicited may form basis for an effective dairy health programs to be adopted.

Economic losses in terms of energy based approach and cost of animal disease may yield more accurate results for therapeutic decision making. Since bovine fever is of significant importance as a major stress in the developing countries of the tropics, the study was designed to work out of the disease in an energy based approach by calculating the additional digestible energy requirement to maintain the febrile episode. No such study seem to have been made in India, though losses per year from subclinical parasitosis Rs. 5000 crore (Anno.1993), rumen dysfunctions Rs. 500 crore (Joshi, 1995), mastitis Rs. 200 crore (Dutta, 1995), foot-and mouth disease Rs.2.5 crore (Chakrabarty, 1993), to cite a few examples, have been reported.

Materials and Methods

In total, 1098 bovine clinicopathies were examined during the year 1995 (January to December) in the College Clinic and 162 cases of all types of fever were diagnosed by routine techniques in buiatrics. Rectal temperature above 101°F with other signs was considered as febrile syndrome (Chakrabarty, loc. cit.). Overall and seasonal occurrence of fever in cattle and buffaloes was determined and expressed as percentages.

Epidemiological model for the estimation of the cost of bovine fever was drawn on the basis of Schewabe *et. al.* (1977) formula with minor modifications. For the calculation of accelerated digestible energy (DE) requirement and nutritional value of the concentrates, recommendations of Kearl (1982) and Ranjhan (1980) respectively were adopted. A standard of 350 kg was taken as an average body weight of Indian bovids. The details of calculations are elucidated in Table II.

Results and Discussion

Prevalence of bovine pyrexia in this arid region of the country according the frequency was 14.7%. Morbidities were 9.3% in cattle and 16.1% in buffaloes in one year. Average febrile rise was 2.1°F and 2.4°F, for a mean duration of 3 days, in cattle and buffaloes respectively. Higher incidence of febrile episode among buffaloes and more pronounced effect of high ambient temperature and humid environment during summers (26.5%) and rains (15.4%), could be due to the fact that these animals are more prone to heat stress (Cockrill, 1974).

Conservative estimates were made on the basis of the bovine population dynamics of the country, incidence of fever and degree, days rise of temperature. Published data indicated average D.E. requirement of 13100 Kcal in cattle and 13450 Kcal in buffaloes per day (Kearl, loc cit.), and each degree rise of fever required 7% of the total caloric requirement for its maintenance. From these estimates, for 115.6 and 86.4 million degree days, 106 and 81.34 billion Kcal of D.E. is required annually in cattle and buffaloes respectively. Converting to the requirement of concentrate and its cost, to maintain bovine fever per year, estimates

may be 74936 tonnes of feed, costing rupees 22.24 crore, not an insignificant burden on the already blighted ecosystem and around 31% shortage of fodder for cattle in the country.

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Table I. Prevalence of Bovine Pyrexia

Season	Cattle			buffaloes		
	total	fever	percent	total	fever	percent
summer (Mar-Jun)	62	6	9.7	204	54	26.5*
rain (July-Oct)	102	9	8.8	442	68	15.4
winter (Nov-Feb)	61	6	9.8	227	19	8.4
total	225	21	9.3	873	141	16.1

* Significant ($P<0.05$)

Table II. Energy Based Cost Analysis of Bovine Pyrexia.

Factor	**cattle**	**buffalo**
Population million, India (FAO, 1990)	197.3	75.0
Incidence pyrexia (%)	9.3	16.1
Average rise body temp. (F^0 per day)	2.1	2.4
Average duration of fever (days)	3	3
Total degree days (million)	115.6	86.4
Average D.E. requirement (per day for 350 kg. B.W. in Kcal)	13100	13450
D.E. required for each degree day rise in body temp. (7% of D.E., Kcal)	917	941.5
Total D.E. requirement for degree days fever (billion Kcal)	106	81.34
Average D.E. provided per gram concentrate (Kcal)	2.5	2.5
Concentrate required for enhanced D.E. due to fever (tonnes)	42400	32536
Cost of concentrate (300.00/Q) in rupees crore	12.72	9.7

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Management of Early Embryonic Death in Cows — A Field Report

T. Umakanthan
Veterinary Dispensary, Cumbum, Tamilnadu

The reproductive wastage is a major source of economic loss in animal production through extended calving seasons, shorter lactations, lower production and increased cost of AI (Roche, *et. al.*, 1981).

The basic factors resulting in repeat breeder syndrome or reproductive wastage have been suggested to be fertilization failure or embryonic mortality (Hawk, 1979). In general after Artificial insemination or Natural service 85-95 percent of recovered ova are fertilized in normal cows (Ayalon, 1978 and Diskin, *et. al.*,1980) and in Heifers fertilization rate is 90-95 percent following AI (Gordon, 1976). But comparatively the calving rates are much lower than normal and varied between 40-65 percent (Sreenan, *et. al.*, 1975 and Roche *et. al.*, 1978.)

This reflects that embryonic mortality assumes even greater importance in single ovulating species as a contribution to overall reproductive insufficiency (Roche, *et. al.*, 1985). Embryo loss accounts as the single biggest factor for almost half of the precalving losses (Moller Holtkamp 1995).

Hansel (1981) reported that the blastocyst is able to stimulate progesterone synthesis by the 10th day of pregnancy and possibly earlier. Cows suffering from early embryo loss generally have lower plasma and milk progesterone (Lamming, *et. al.*, 1989). Lamming, *et. al.*, (1993) have also shown that cows with low progesterone levels may suffer an enhanced luteolytic activity which could contribute to the rate of embryo loss. Umakanthan (1995) has observed that injection of Repositol progesterone has decreased the repeating problem upto 96% in longer period cows.

Embryonic deaths occurring at 8 to 16 days after breeding usually do not affect the normal cycle length of about 18-24 days, but embryonic deaths occurring at 16 to 25 days often result in longer periods between service and the next estrus (Robert, 1982).

Hence a field study was taken in cross bred cows, reared under increased ambient temperature, repeating itself to heat after 21 to 75 days and which have taken more than two artificial inseminations formed the group. Control animals were those reared under same condition and exhibited the like symptoms but no treatment with the hormone was given. The animals in oestrus with follicle in the ovary and clear discharge were the symptoms taken as criteria in this repeat breeding animals. No other medications like antibiotics infusion as pre or post insemination was carried out. Neither oral or parenteral treatment was done in the groups. Ayurvedic drugs, mineral and vitamins were not administered. In all the animals frozen semen (0.5ml) was used. Animals in the groups did not have apparent pathology of the reproductive tract on rectal exploration and vaginal examination.

The day of oestrus was taken as day one and AI was carried out on first and second day. On 4th, 14th, 24, 34, 44 and 54th day progesterone depot (Proluton Depot - German Remedies) 250 mgs were injected intramuscularly.

Pregnancy diagnosis per rectum was carried out from 60-90 days after AI. Of the 10 cows studied, 9 cows were pregnant and had completed the 4th to 54th day of proluton treatment schedule. One cow came into heat within 54 days of progesterone treatment. The treatment schedule was stopped, when on rectal

examination the cow was found non pregnant. Among the 5 control cows (untreated), one cow conceived

The dependence of uterus, during pregnancy as well for the sequential actions, on estrodiol 17 and progesterone has been convincingly demonstrated. The role of progesteronce has now been very much centered around its importance in the ovulation, attachment of embryo and in nurturing the embryo. Stress mediated more so by higher ambient temperature above the thermoneutral zone brings down the conception rate. Adverse effect of high environmental temperature on rate of fertilization, embryonic survival and altered hormonal status of the dam has been attributed as cause of lowered fertility (Roberts, 1986). The Repositol progesterone is effective to elevate blood levels of progesterone for 10 days because the finely divided particles precipitate in the tissue giving a slow release of the hormone.

Hence it is found that injection of Repositol progesterone at a constant interval-prevents the embryo loss in repeater cows.

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Role of Ectoparasites in the Transmission of Ringworm in Buffaloes

P. R. Patel, and **M.R. Dave**
Department of Medicine, Veterinary College, Anand, Gujarat.

Dermatophytosis (ringworm) is a primary skin disease, commonly caused by *Trichophyton verrucosum* in buffaloes. The disease is common in calves. The claves in India are also heavily flooded with various ectoparasites. Considering the commonness of the ailments, attempts have been made to study the role of ectoparasites in the transmission of ringworm in buffaloes.

Materials & Methods

Buffaloes (446 cases) brought to the Veterinary College Hospital, Anand and Ambulatory Clinics at Chikhodara, Bedva and Gamdi villages around Anand Town, with skin lesions were screened for ringworm by microscopic and cultural examinations. (Jungerman & Schwartzman, 1972), and grossly examined for the presence of ectoparasites. A total of 180 ectoparasites (67 ticks, 54 lice and 59 fleas) were collected randomly from buffaloes and cultured on Sabouraud's Dextrose Agar containing chloramphenicol and cycloheximide, for the isolation of dermatophytes, as per the method described by Buchvald and Klobusicky, 1974.

Results & Discussions

On microscopic and cultural examinations of skin scrapings and clinical examination of 446 buffaloes, 22.9% of buffaloes had ringworm lesion infected with *Trichophyton verrucosum*, 16.6% had both ectoparasites and ringworm lesions, 6.3% had ringworm lesions only without ectoparasite and 39.0% had only ectoparasites on the body. Of the 180 ectoparasites cultured, 5.5% ectoparasites yielded *Trichophyton verrucosum*.

The distribution of ectoparasites and dermatophyte infection in 446 cases of buffaloes is presented in the table. It shows that presence of ectoparasites has a significant ($P<0.01$) influence on the incidence dermatophytosis.

Of the 180 ectoparasites cultured, ten (5.5%) ectoparasites (2 ticks, 6 lice and 2 fleas) yielded *Trichophyton verrucosum* fungi. Kral &

Table : Distribution of Ectoparasites & Dermatophytes

Sr. No.	Number of buffaloes with	Ectoparasites					Total	Percent Infection
		Tick	Lice	Flea	Mite	Mixed		
1.	only ectoparasites	15	96	11	20	32	174	39.0
2.	ectoparasites & dermatophytes	2	24	6	17	25	74	16.6
3.	only dermatophytes	—	—	—	—	—	28	6.3
4.	No infection	—	—	—	—	—	170	38.1
	Total						**446**	

Schwartzman (1964) isolated *Trichophyton verrucosum* from lice and they considered lice as agents to spread ringworm in cattle.

Gibbons *et al.* (1970) isolated fungi from lice and suspected lice as a spreading agent of extensive outbreak of ringworm in calves. They also observed that after controlling lice infestation, the mycotic infection disappeared. Kamyszek (1997) isolated *Trichophyton verrucosum* fungi from 17% samples of ectoparasites from bulls and heifers.

Summary

On the basis of incidences of both the infections and cultural examination of ectoparasites, it is suggested that ectoparasites play an important role of transmission of ringworm in buffaloes.

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Efficacy of Oxyclozanide in Natural Cases of Amphistomiasis in Bovines

Sahadeb Sahu
State Animal Health Centre, Midnapore (West Bengal)

A field trial on Oxyclozanide was conducted in natural cases of amphistomiasis in bovines during the month of October'95. A total of fifteen cases (5 Crossbred, Jersey Cows, 3 Crossbred Holstein Cows, 2 deshi Cows, 2 buffalo Cows and 3 deshi bullocks were taken for trial. The animals were presented at State Animal Health Centre, Midnapore (West) with the complaints of inappetance, loose stools, with foul smell and bottle jaw condition.

All the animals were checked up physically and faecal samples were collected and examined microscopically by sedimentation and flotation methods. The samples were positive to amphistone ova (++ to ++). The animals were dosed with Oxyclozanide (TOLZAN-F®-Hoechst India Ltd.) @ 15mg/kg body weight per os. After 7 days and 15 days of each treatment, the faecal samples were again examined microscopically by aforesaid methods and were negative for any eggs of Amphistomes, although three animal owners complained of loose stools even 5 days after anthelmintic dosing, they were treated accordingly.

During this period none of the treated animals showed any discomfort and after 6-7 days of treatment the animals were cured completely and took feeds normally. The recovery of treated animals proved that the Oxyclozanide (TOLZAN-F®) was effective to control the natural cases of amphistomiasis, because no egg could be detected in faeces. The drug did not produce any side effects in treated animals. So, Oxyclozanide could be effectively and safely used in natural cases of amphistomiasis in bovines.

News

Dr. Mahesh Chandra Sharma, a Senior Scientist at IVRI, **Izatnagar**, was honoured with "Vigyan Shree Upadhi" Award (1995-96) for his outstanding and dedicated research and clinical work in the field of Veterinary Sciences in India and abroad. The award was given by his excellency - Governor of Uttar Pradesh, Shri Moti Lal Vohra at a distinguished gathering in the Raj Bhawan, Lucknow, on 27th February, 1996 The "Alankaran Samaroh" was organised by the Society for Environmental, Scientific and Agricultural Advancement. The award consisted of a Tamra Patra, with a cash of Rs.10,000/- and citation.

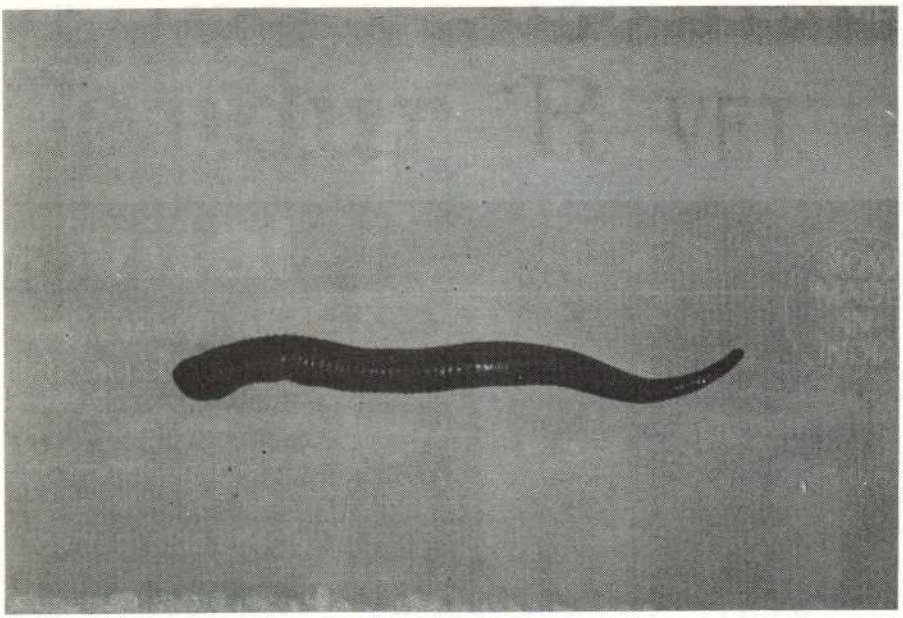
An Unusual Case of Rhinitis in Buffalo — A Case Report

T. Umakanthan
Veterinary Dispensary, Cumbum, Tamilnadu

A buffalo bull aged about two years presented a history that the animal was often scratching only the left side of the nasal bridge with the left fore hoof for three days which also affected its feeding.

On examination the animal was found to be normal in all habits. A 10x1 cm size inflammation was present on the middle of the left nasal bridge. No abnormality was noticed in the naris. While the point finger was inserted into the left nostril to find out any growth, the animal immediately sneezed, revealing a black coloured worm like mass which peeped out, and slipped back again.

The animal was given exercise by chasing and was cast to the right side suddenly, when the respiration became rapid and forceful. Through the left nostril a live, black coloured and serrated leech was slowly coming out, which was plucked slowly and forcefully as it was adhering fast to the wall of the nasal orifice. Later it was identified as a leech belonging to the genus Hirudinaria. Probably the leech might have got entry and entered into the nostril by contact while the animal was grazing.



A Clinical Trial of Tolzan ' F '

Ashok Kumar

Animal Health Consultant, Muzaffarpur, Bihar.

Introduction

Liverfluke or Fascioliasis is a common and wide spread disease of cattle, buffaloes, sheep and goats in Bihar. But North Bihar region is very much prone to Liverfluke and Amphistomiasis. The disease effects the animals in a big way specially just after the onset of monsoon and in the ending phase of the rainy season, resulting in heavy loses to farmers.

The disease sets out with early symptoms of intermittent diarrhoea with speedy body loss conditions which adversely affects the productivity of animal and ultimately causes death also.

Material and Method

A group of ten Cows and five Buffaloes which were suffering from Fascioliasis as diagnosed by clinical symptoms and were confirmed by parasitological examination, were taken into account.

All these animals were treated with Tolzan ' F' (oxyclozanide 3.4 % w/v) at the dose rate of 10 mg/kg B.W. Treatment was supplemented by calcium therapy and liver tonic. Signs of improvement were noticed in 48 hrs and complete recovery was observed in three days - in eight Cows and all the five Buffaloes. Remaining two Cows recovered in five days.

All the treated animals were kept under observation for another 20 days. Out of 15 animals - two Cows and one Buffalo again started loose motions and on pathological examination of stools Liverfluke infection could be detected. Tolzan ' F ' in dose of 10 mg/kg. B.W. was again repeated. All the three animals recovered fully and again did not show signs of the disease.

Simillarly a group of five Cows and five Buffaloes which were suffering from Amphistomiasis were treated with Tolzan ' F ' in the dose of 10mg/kg B.W. Treatment was supported with calcium therapy and B-complex liver extract. After three days treated animals were examined and found to be free of Amphistomes.

Results and Discussion

All the above animals treated against acute and chronic Fascioliasis and Amphistomiasis showed signs of recovery in a commanding manner. Out of 10 Cows which were taken for treatment two cows were pregnant. This treatment did not produce any adverse effects and after treatment the fetal development was to be found satisfactory. Also speedy recovery in productivity was found.

The area of Nothern Bihar has many rivers which becomes the source of Liverfluke and Amphistomiasis infections .

It is therefore suggested to drench the animals with Tolzan ' F ' just before the onset of the monsoon and again when water recedes from the area.

Abstracts

(1) Post operative analgesic and sedative effects of Carprofen and Pethidine in dogs

In a trial on 40 dogs undergoing various orthopedic procedures carprofen (4mg/kg S.c. pre operatively) produced better/equal analgesia and less sedation than pethidine (2 mg/kg pre operative + 3 mg/kg post operative). Carprofen was safe and caused no G.I. irritation.

Lascelles. B. Dx.; Buttersworth S.J. and Waterman A.E. (1994)
Vet. Record **134** : 187-191

(2) Transmission of BSE to Sheep and Goats

Bovine spongyform encephalopathy was experimentally transmitted to Sheep and Goats both by oral and intracerebral routes with homogenates of brains of cattle confirmed positive for BSE.

Incubation period varied from 440-940 days in Sheep and 506 to 1501 days in goats. Following intracranial route clinical signs included locomotor inco-ordination trembling without signs of pruritis. Progression of disease was rapid. With oral inoculation, lethargy, lead to recumbency in 1-2 weeks of onset of Symptoms lesions included wiodespread spongyform encephalopathy.

Foster J.D.; Hope J. and Fraser H (1993)
Vet. Record **133** :339-341

(3) A serological survey for bovine immuno deficiency like virus in Ontario dairy cattle and association between test results, production records and management practices

In a study of 928 cows from 256 herds, positivity by chemiluminescence western blot analysis to Bovine Immuno deficiency like virus (BIV) was 5.5%. No significant association was found between incidence of bovine leukemia Virus and BIV. Positive BIV test was associated with Milk Production below herd average.

Mc Nab W.B., Jacobs R.M., Smith H.E. (1994)
Can J. Vet. Res. **58** :36-41

(4) Effects of cattle tick infestation on bovine immune system

When six Herford cattle from tick free area were repeatedly infested experimentally with *Boophilus Microplus*, the percentage 'T' cells in blood were less than in controls at the end of second infestation. The effect was same in light and heavy infestations. The percentage of 'B' cells reduced after fourth infestation. Mitogen response was low in infested cattle than in controls.

Tick Saliva when injected into tick free cattle reduced mitogen response in the animals. Immuno suppression effect of ticks is a factor in pathogenesis of tick borne diseases.

Inokuma H; Kerlin R.L.; Kemp D.H. & Willadsen P. (1993)
Vet. Parasitology **47** :107-118.

(5) Calf diarrhoea accompanied with decrease of Serum tocopheral and Selenium concentrations in Japanese black cattle of a breeding farm

Serum Selenium and Vit E levels were low in calves with persistant diarrhoea. These responded positively to treatment supplementing Selenium and Vit E.

Ishikawa H. (1993) Tohoku J.
Vet. Clin **16(a)** :13-17

(6) Skin inflammation following Triclabendazole treatment of dairy cows

After treatment with Triclabendazole at 12.5

ml/100 kg Skin lesions were observed within three days mainly on teats (27.1% animals) and udder (16%). The percentage affected increased by 10th day after treatment. Other signs included reddening of eyes and nose, drooling increased excitability and skin (teat and udder) Sensitivity. Application of Sun cream (Sun protection factor 15+) significantly reduced development of subsequent lesions. Non pigmented part of skin was sensitive to photosensitization.

Martin P.A.J.; Davies R.H. and Mayberry C.J. (1993)
Vet. Rec. **133** : 300

(7) Biological control of Trichostrongyles in calves by fungus *Duddingtonia flagrans* fed to animals under natural grazing conditions

Certain microfungui are shown to prey on free living stages of parasitic nematodes (Waller & Larsen 1993; Gronvold *et. al.* 1993) The candidate fungi for use as biological control agent should withstand passage through alimentary tract of cattle, sheep & goats when fed orally and be able to colonise on dung and kill the trichostrongyloid larvae. After several experiments; *D.Flagrans* was selected as suitable fungus.

In the present work the fungus was fed to calves (fungal barley material as crib once a day) for 2 months of Season and it was noted that it lowered herbage infectivity and reduced acquisition of *ostertagia Sp* and *Cooperia Sp* later in the Season.. In addition it delayed onset of clinical disease. This was due to nematode destroying effects of fungus in dung excreted by fungus treated calves as evidenced by results of parallel *in vitro* assay of faecal larval cultures.

Larsen M.; Nansen P; Wolstrup J., Gronvold J., Henriksen S.A. and Zorn A. (1995)
Vet. parasitology **60** : 321-330

(8) Effect of probiotic supplementation on serum/yolk Cholesterol and egg shell thickness in layers

A study on three groups of WLH layers with dietary supplementation of 0, 100 and 150 mg probiotic/kg revealed that egg production improved by 5% and thin shelled egg percentage reduced from 18.6% in controls to 8.6% in the group receiving 100 mg/kg probiotic.

Serum cholesterol reduced from 176.5 mg/dl to 114.3 mg/dl (in group receiving 150mg/kg probiotics) and yolk cholesterol from 14.69 mg/dl to 11.28 and 11.37 g/dl (in both probiotic receiving groups) overall Mean cholesterol reduced in 10 weeks of observation (28-38 week age).

Mohan B.; Kadirvel R., Bhaskaran M. and Natrajan A. (1995)
British poultry Sc. **36** : 799-803

(9) Transplantation of spermatogonial stem cells

R. Brinster, Professor, School of Veterinary Medicine of University of Pennsylvania and his co workers have shown that Spermatogonial Stem Cells of an animal can be preserved by freezing, and further can be thawed and implanted in the testes of another animal (Surrogate) to obtain an almost inexhaustible supply of sperms of former in the Semen of Surrogate animal. They further showed that spermatogonial Stem Cells of rat implanted in testes of mice produced rat sperms in the Semen of mice. These path breaking discoveries (published in Nature 1996 and Nature Medicine 1996) have far reaching applications in Veterinary and Human Medicine and in preservation of endangered species.

Time International (1996) **147** (24) : 49

(10) Mitral Stenosis in Dogs

In a Study of 15 cases 5 were Newfoundlands and 4 were Bull terriers. Prominent findings were as below :

Ascertation : Cardiac Murmurs (left apical holosystolic, ejection over cardiac base and soft diastolic over left apical area)

Radiograph : Left atrial enlargement and pulmonary oedema.

ECG : Prolonged duration/increased amplitude of P waves ventricular enlargement. Atrial and ventricular premature polarizations

Eco Cardiography : Thickened mitral valve leaflets; parallel septal and parietal mitral valve

leaflet motion with incomplete leaflet separation during diastole;lack of leaflet closure during mid diastole; left atrial enlargement thickened mitral valve leaflets and diastolic doming of septal mitral valve leaflets into left ventricle.

Doppler Echo-Cardiography : Increased mitral inflow velocities and prolonged pressure half times.

Cardiac Catheterization : Trans mitral diastolic gradient prognosis was poor in all cases.

Lehmkutil L; Ware W.A. and Bongma J.D. (1994) J.Vet. Int. Med. **8** : 2-17.

(11) The relationship of Scrotal circumferences to histological and testicular parameters and sperm reserves (1995)

In 410 Bonsmara bulls of average 410 days age, Scrotal circumferences varied between 31 and 42 cms. Bulls with larger testicles (>36 cm Serotal circumference) had significantly greater Seminiferous tubule diameters, epididamal sperm reserves, less vacuolisation and higher sperms production that those with smaller testes (<36 cm)

Swanpoel F.J.C; Lubout P.C. and Christie J. (1995) J. Soc. Afr. Vet. Assn. **64** : 126-127

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