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January '95

Tribute To A Great Scientist

Dr. S.R. Rao (1909 - 1988)

Dr. S. Rammohan Rao was an eminent parasitologist of India. Born in 1909 in Andhra Pradesh, he obtained the degree of Doctor of Science in Zoology of Benaras Hindu University in 1938. After a short stint of service as Entomologist at Indian Veterinary Research Institute Mukteshwar and Sugarcane Research Institute Coimbatore he joined the Bombay Veterinary College in 1945. He founded the Department of Parasitology in this college and continued to Head the Department of Parasitology for the next 23 years till retirement. He was in John Hopkins Institute Baltimore U.S.A. as exchange Professor on Full Bright Travel grant during 1954-55.

Dr. Rao contributed to the science in the fields of coccidia, ticks, schistosomes, Amphistomes, oribatid mites, Trichinellosis and strongyle worms. His discovery of Hunterellus hookeri a hyper parasite of ticks and its use as a biological agent in tick control was greatly acclaimed by scientists all over the world.

Dr. Rao taught parasitology to two generations of veterinarians and guided 15 students for M.V.Sc. and 3 for Ph.D. degrees. He published more than 100 research papers and edited a book on "A decade of progress in Veterinary Science 1968-70" published by Indian Science Congress association.

Dr. Rao was Dean of Faculty of Technology of Bombay University. He was elected as president of medical and veterinary section of Indian Science Congress 1968. After retirement Dr. Rao was appointed Professor Emeritus for three years by University Grant Commission. When Dr. Rao passed away in 1988 at the age of 79, the entire Veterinary profession paid him rich tributes. The Bombay Veterinary College Alumni Association has instituted The " Dr. S.R. Rao Memorial lecture" every year in his memory.

Hydropericardium Syndrome (HPS) - An Emerging Problem And A Threat To Broiler Industry In India.

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Hydropericardium syndrome locally called as Leechi disease in broilers is an emerging problem and a major threat to the broiler industry in India, particularly in the northern parts of our country. During the month of April, 1994 the problem was noticed in some parts of Jammu poultry belt, later in the months of June and July similar problem was noticed in districts of Punjab and Delhi. Outbreaks of this disease syndrome has caused devastating mortality in the affected flocks to the extent of 60 to 80 per cent. The disease has failed to respond to the symptomatic treatment and prophylaxis resulting in virtual closure of several broiler farms in these areas. There have been similar reports of this devastating experience from neighbouring country - Pakistan during 1987 - 88. (Anjum *et al* (1989), Cowen (1989), Jaffery (1989) and Niazi *et al* (1989)).

Materials and Methods

The affected farms in the northern region were surveyed and the details relating to incidence, morbidity, mortality, the clinical signs and other relevant information were collected.

The materials such as blood, serum samples, liver, spleen, kidney, heart and bursa were collected for clinico-pathological studies. The tissues were also separately collected in 50 percent glycerol saline for virus isolation. The materials were processed routinely for histopathological examination.

Results and Discussion

The syndrome was found to be restricted mostly to the broiler flocks and occurred as a continuous problem in all the batches with increasing intensity of mortality losses in subsequent batches. The mortality experienced in the affected flocks ranged from 10 to 80 percent in the same area. The age group between three to five weeks was found to be more vulnerable with peak mortality during the fourth week. The disease was seldom recorded after sixth week. The onset of mortality was found to be abrupt without any clinical manifestation. The instances of mortality was found to be frequent among well nourished birds.

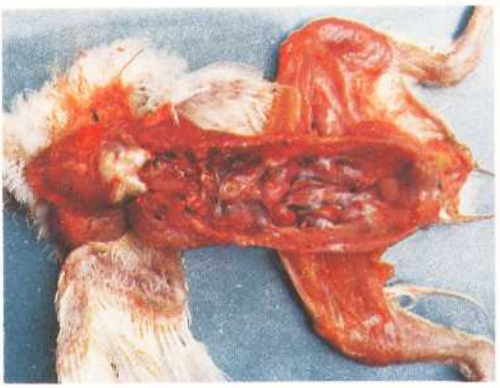
The haematological studies in the ailing birds indicated a significant decrease in total erythrocytes (1.70 million / cmm^3) and leukocyte counts (13.27 Thousand/mm^3) with extremely low haemoglobin values (5.12 g/100 ml.). The differential leukocyte counts showed an increase in heterophils, eosinophils and monocytes (48.2, 0.94, 1.28% respectively) with decrease in lymphocytes and basophils (55.15 and 1.21% respectively). Significant reduction in erythrocyte sedimentation rate and hematocrit values were also encountered, an observation very similar to those recorded by Niazi *et al* (1989) in spontaneous cases of HPS in Pakistan.

The findings of the present study also indicated an altered serum protein value with decrease in albumin values and this could be attributed to extensive hepatic and endothelial damage, leading to leakage of serum proteins.

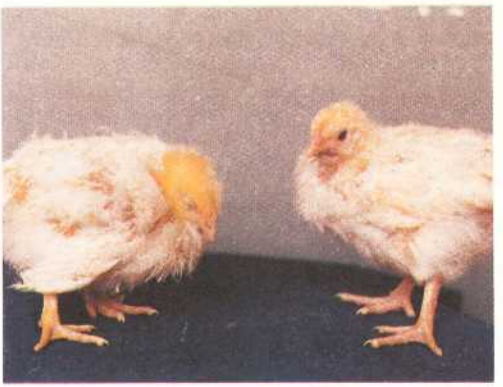
The postmortem examination in these cases in several farms revealed uniform manifestation of gross lesions. The carcass appeared pale, soft, moist and well nourished. The predominant lesion was observed in heart with typical hydropericardium containing 5 to 10 ml of straw coloured fluid. The pH of the fluid was 7.0 in these cases with the fluid showing tendency to

Typical lesions of hydropericardium

Pericardium showing straw coloured fluid



Involvement of other visceral organs such as lungs, liver, spleen and kidneys etc.



Experimental induction of (HPS). Note ruffled feathers and stunted growth

clot. The heart after removal of pericardial sac appeared reddish, glistening with white patches and occasionally with haemorrhagic spots. The lungs were congested and oedematous. The livers were moderately enlarged, tan-yellow, mottled with haemorrhages. In some instances rupture of liver with blood clots were also observed. The spleen appeared congested, mottled, atrophied in few and enlarged in some cases. Kidneys appeared pale, tumified, with prominent tubules. The bursa Fabricious appeared atrophic. The bone appeared pale. The mesentery of intestines were engorged with blood and intestines often showed mucoid enteritis. The abdominal fat was cloudy grey and in some cases appeared yellowish. Generalized anasarca was not a feature in this study.

The histopathological examination of heart revealed massive interstitial oedema varying degrees of congestion and haemorrhages, degenerative changes with proliferation of endothelial cells in the blood vessels. The liver showed congestion and haemorrhages, cellular degeneration with demonstrable basophilic or eosinophilic intranuclear inclusion bodies, indicative of involvement of adenovirus inclusion body hepatitis. The liver also showed infiltration of mononuclear cells and Kupfercell heperplasia. Spleen showed lymphocytolysis and atrophy of the follicles. The lungs showed varying degrees of congestion, haemorrhages and oedema. Catarrhal inflammation in parabronchiolar epithelium was also evident. Cellnests or cartilaginous masses with granulomas were also observed. The kidneys showed haemorrhages, degeneration of tubular epithelium and necrosis. The

bursa Fabricious showed degenerative follicles with varying degree of lymphocytolysis and vacuolations in the epithelium in some of the cases.

Conclusions

The findings of the present investigation indicated the involvement of several etiological agents and project it as a syndrome. In as much as the findings indicated the involvement of IBD, IBH, aplastic anaemia and aflatoxicosis resulting in a complex syndrome. The spread of infection from the neighbouring Pakistan could not be ruled out, since similar experiences have been recorded in the adjoining parts of the country.

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Efficacy of Butox (Deltamethrin) against *Psoroptes cuniculi* and *Sarcoptes scabiei* infection in rabbit.

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Abstract

Butox (Deltamethin) at 75 ppm and 50 ppm concentration, applied twice at 10 days interval was found to be very effective in curing the disease condition caused by *Psoroptes cuniculi* and *Sarcoptes scabiei*, respectively in rabbit. No sign of toxicity of this drug was observed in rabbit as well as in man handling the drug in these concentration.

Introduction

The infection of *Psoroptes cuniculi* is responsible for causing ear canker in rabbit. This is a very serious problem of rabbit in this region (Chandra and Ghosh, 1990). Moreover *Sarcoptes scabiei* infection in rabbit causes severe skin lesions on head and neck. This condition leads to slow growth and the fur skin becomes useless and there is a severe loss of wool production. Deltamethri Deltamethrin (Butox) is a drug marketed by Hoechst and is known to be very effective against mites (Mandal and Singh, 1988; Pathak *et al*, 1991). This drug is known to be less toxic. There is no published report on the effect of this drug against mite infestation in rabbits caused by *Psoroptes cuniculi* and *Sarcoptes scabiei*. So the present study has been undertaken to know the efficacy of this drug against rabbit mite infestation.

Materials and Methods

Forty rabbits suffering heavily from ear canker caused by *psoroptes cuniculi* as well as forty other rabbits suffering heavily from *sarcoptes scabiei* infection were selected for this experiment. These animals were Soviet Chinchilla and New Zealand White breeds. All the animals were above six months of age.

The animals of both the infective groups ie; with *Psoroptes cuniculi* as well as *Sarcoptes scabiei* were further grouped to apply different concentrations of drugs. As shown in Table I.

Table 1. Groups of Rabbit to study the efficacy of Butox against *psoroptes cuniculi* and *sarcoptes scabiei* infection.

Number of animals treated with various concentration of Butox

Type of infection	Group A 75 ppm	Group B 50 ppm	Group C 37.5 ppm	Group D N. Treated	Total No. of rabbits used
Psoroptes cuniculi	10	10	10	10	40
Sarcoptes scabiei	10	10	10	10	40

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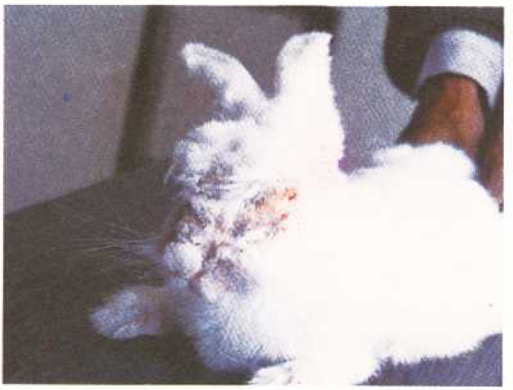
Rabbit (NW) heavily infected with ***Sarcoptes scabiei***



Rabbit heavily infected with ***Psoroptes cuniculi***

For the parasitological examination scrapings were collected from 5 places of measured area to assess the number of mites per cm2 area. The scrapings were put in 10% KOH for 24 hours for counting the number of mites by Stoll's technique (Pathak *et al* 1991)

Results

The clinical symptoms like itching, scratching and crust formation were reduced after 5 days of application of the first treatment with 75 ppm of Butox in cases of *Psoroptes cuniculi* and 50 ppm in *Sarcoptes scabiei*. After 10 days of 1st treatment the lesions were reduced to a considerable extent. On the 20th day after application of 1st treatment and 10 days after 2nd application, the lesions had completely vanished and new hair started growing . On the 30th day after treatment (first application) there was no sign or scar left in the area of infection.

But in a concentration below 75 ppm and 50 ppm against *Psoroptes Cuniculi* and *Sarcoptes scabiei* respectively, there was recurrence of the disease from 20 days after 1st application and 10 days after 2nd application of the drug.

In case of *Psoroptes cuniculi*, it was found that the average parasitic count of 189.9 $\pm$ 4.23 (ranging from 165-212) was reduced to nil on 20th day after application of 1st treatment and 10 days after 2nd treatment with a concentration of 75 ppm of Butox. But in other concentration, the infection continued at various level of counting throughout the experiment (Table II). Likewise in case of *Sarcoptes scabiei*, the average parasitic count of 204 $\pm$ 1.92 (ranging from 197 to 212) was reduced to nil on 20th day after application of 1st treatment and 10 days after application of 2nd treatment. On application of 37.5 ppm, the infection was continued along with the clinical symptoms and various levels of parasitic count. Seventy-five ppm was found to be equally effective like 50 ppm, concentration (Table III).

Discussion

Reports on the use of Deltamethrin against rabbit mange are not available. There are several information on use of this drug successfully against *Sarcoptes scabiei* infection in other animals like sheep, goat and camel (Mandal and Singh, 1988 ; Pathak *et al.*, 1991), but there is no information available on use of this drug against *Psoroptes cuniculi*.

The present investigation proved that 50 ppm Butox is sufficient to cure *Sarcoptes scabiei* infection and 75 ppm is sufficient to cure *Psoroptes cuniculi* lesions. Fifty ppm was also found to be successful in curing *Sarcoptes scabiei* in sheep, goat and camel (Mandal and Singh, 1988 ; Pathak *et al.*, 1991), but unlike the finding in other animals, in rabbit only two applications were sufficient to cure the disease instead of three applications.

Table II. Response of Butox at various concentrations to Ear Canker caused by *Psoroptes cuniculi*

Group with conc. of Butox	Number of rabbits	Number of mites/cm2 on various days after treatments			
		0 days	10 days	20 days	30 days
A					
Infected	10	189.90	82.10	-	-
Treated with		SD 13.36	SD 4.16	-	-
75 ppm		SE ± 4.23	SE ± 1.31	-	-
B					
Infected	10	192.10	117.10	166.40	197.20
Treated with		SD 8.47	SD 11.50	SD 8.49	SD 5.20
50 ppm		SE ± 2.67	SE ± 3.64	SE ± 2.83	SE ± 1.68
C					
Infected	10	188.30	195.80	213.60	217.10
Treated with		SD 11.64	SD 6.61	SD 8.90	SD 3.85
37.5 ppm		SE ± 3.68	SE ± 2.09	SE ± 2.80	SE ± 1.82
D					
Infected	10	194	203.50	210.40	211.10
non treated		SD 5.00	SD 6.84	SD 7.07	SD 6.92
		SE ± 1.58	SE ± 2.16	SE ± 2.13	SE ± 2.18

Table III. Response of Butox at various concentrations to mange in rabbit caused by *Sarcoptes scabiei*

Group with conc. of Butox	Number of rabbits	Number of mites/cm2 on various days after treatments			
		0 days	10 days	20 days	30 days
A					
Infected	10	201.70	174.10	-	-
Treated with		SD 5.16	SD 10.80	-	-
75 ppm		SE ± 1.63	SE ± 3.41	-	-
B					
Infected	10	204.20	83.40	-	-
Treated with		SD 6.07	SD 4.05	-	-
50 ppm		SE ± 1.92	SE ± 1.28	-	-
C					
Infected	10	199.40	69.20	194.50	204.60
Treated with		SD 4.98	SD 3.73	SD 7.12	SD 6.18
37.5 ppm		SE ± 1.59	SE ± 1.18	SE ± 2.25	SE ± 1.95
D					
Infected	10	196.80	203.90	207.90	208.60
non treated		SD 6.32	SD 4.11	SD 2.88	SD 3.83
		SE ± 2.00	SE ± 1.30	SE ± 0.91	SE ± 0.80

* Two treatments were given to all the treated groups with 10 days interval throughout the experiment.

In case of *Psoroptes cuniculi* higher requirement of concentration of 75 ppm, might have been due to the fact that heavy crust formation hampers this drug in low concentration to act with the mite population. As 50 ppm was sufficient to cure the *Sarcoptes scabiei* infection, there is no need to use 75 ppm, which was found equally effective.

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Efficacy Of Oxyclozanide In Amphistomiasis In Cattle In Tripura

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Amphistomiasis in cattle causes economic losses to dairy industry particularly in areas where cattle are allowed to graze in low lying pastures and a high mortality rate has been recorded as a result of this disease (Blood and Radostits, 1989). The agroclimatic condition of the north-eastern states of India is very much favourable for development of different parasites (Mahanta *et. al.* 1986). Saha and Das (1991) reported higher incidence of parasitic diseases in cattle of Tripura. They recorded amphistomiasis as one of the most common parasitic diseases in cattle. However, there is paucity of literature regarding the efficacy of any drug against these parasites under field conditions. The present trial was, therefore, undertaken to study the therapeutic efficacy of oxyclozanide against natural cases of amphistomiasis in cattle under field conditions.

Materials and Methods

Forty-six Jersey cross-bred adult cattle, the faecal samples of which were found positive for ova of Amphistome, were included in the present study. These animals showed clinical symptoms viz. inappetence, occasional diarrhoea, indigestion, rough coat and weakness. Tolzan F liquid (a product of Hoechst India Limited, Bombay) containing 3.4% W/V oxyclozanide was chosen as the chemotherapeutic agent. The drug was administered orally to each cow at the recommended dose rate (15 ml per 50 Kg body weight). The efficacy of the drug was assessed on the basis of disappearance of Amphistome ova in the faeces on subsequent microscopic examinations. Faecal samples of all the treated animals were re-examined on day 15, 30 and 60 post-treatment (PT) as per standard procedures (Sastry, 1983). As supportive therapy vitamin B-complex injections, oral rumenotoric and antidiarrhoeal drugs required depending upon the clinical symptoms were also given.

Results and Discussion

Out of 46 animals treated, 41 animals (89.12%) recovered as evident from the faecal examination on day 15 and 30 post treatment. Oxyclozanide is one of the few anthelmintics which are effective against amphistomiasis in cattle (Soulsby, 1982) and the drug is believed to act by uncoupling the oxidative phosp- horelation (Booth and Mc Donald, 1984). Earlier Chhabra and Ball (1976) recorded 100% efficacy of oxyclozanide in cases of amphist- omiasis in cattle.

Faecal examination on day 60 PT of the animal revealed presence of Amphistome ova in 4 more animals. This reappearance of ova in the faeces of the treated animals on day 60 might be attributed to reinfection due to grazing in the same contaminated pasture or development of the immature parasites might have survived the effects of oxyclozanide (Soulsby, 1982).

In the present study another dose of the drug might have produced better results on the day 60 PT as suggested by Rolfe and Boray (1986) who observed two doses of oxyclozanide gave consistent and better results against immature paraphistomes in cattle.

No untoward effect of the drug could be observed during the present study and the recovered animals showed remarkable improvement in respect of body condition.

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Amblyopia In Dog : A Case Report

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Amblyopia is a reduction in visual acuity, not dependent upon any visible changes in the eye (Helper, 1989). Its incidence of 2-2.5% of total human population is reported by Noorden (1980). Perusal of available literature indicates no record of this condition in dog, hence present case is reported.

Case History

A Boxer male aged 1.3 years was referred to College Hospital for congenital blindness. The animal was treated rationally for the blindness but remained refractory. Animal had no other complaint and was in perfect condition.

Clinical examination revealed a bilateral superficial corneal opacity. However, animal endeavoured to focus at the object by gazing through a narrow slit of transparent cornea at the irido-corneal region. Direct and consensual pupillary light reflex was normal. Fundoscopic examination did not reveal any detectable retinal anomaly.

Discussion

In the absence of retinal anomaly and any disorder of the visual pathway which could cause the defects of vision, it is likely that the defects of vision may be due to the absence of adequate symmetrical stimulation to the two eyes so that the binocular reflex cannot be developed. Disuse or understimulation of the retina is the primary cause of poor vision (Noorden, 1980). In the present case congenital corneal opacity might have prevented the retinal stimulation.

Congenital amblyopia was always regarded as being organic. When fullsib of the dog under consideration was examined for vision it was not found to be within normal physiological limit. Amblyopic human patients have low vision nystagmus, poor colour vision and defective photopic elements of their electroretinogram. These findings point to an irreversible, generally defective cone function. It is possible that the amblyope may be unable to integrate a form sensed through amblyopic eye into a meaningful percept.

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Etiopathogenesis of Gumboro Disease of Chicks

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Gumboro-Disease of chicks is known ever since the first report by Cosgrove (1962) who reported its incidence from Gumboro village of Delware in U.S.A. In view of the invariable involvement of kidney, the disease entity, was then named as "Avian-Nephrosis". In view of its infectious nature and bursal pathology it produces, the disease was subsequently known as "Infectious Bursal Disease" (Hitchner 1970) and was proved to be caused by Birna (IBD) virus (Calnek & Barnes 1992).

Widespread destruction of "B" lymphocytes in Bursa of Fabricius was observed to produce severe immunodepression of humoral immune response. As per the report from Ajinkya *et al* (1980) from Maharastra (India), its incidence came to light through its association with failure of New Castle Disease vaccinations and heavy mortality attributed to Ranikhet Disease.

From the analysis of the data from detailed study regarding etiopathogenesis in sixteen bursal disease outbreaks (Table 1); it was clear that Gumboro Disease on its own causes a limited mortality varying from ten to twenty per cent. Field outbreaks, however, were often complicated with superimposed infections such as New Castle Disease, Enteritis and/or Coccidiosis. The predominant postmortem lesions in such field outbreaks were often indicative of these complicating disease entities and etiopathogenesis of Gumboro Disease remained often masked.

During the exhaustive studies undertaken in two out of sixteen outbreaks (one in chicks of 4 to 5 weeks age and the other in growers of 11 weeks age), no other specific complicating factor was observed. The lesions and clinicopathological investigation therefore, in these outbreaks, pertain more or less entirely to Gumboro Disease.

In addition to the typical "Bursal" lesions (Plate 1) responsible for immunosuppression extensive damage to the kidney (Plate 2) was an invariable observation. It will not be out of place to mention that "Gumboro Disease" was earlier known as "Avian Nephrosis" and was reported as such in the literature.

Although typical bursal lesions (plate 3) could explain severe immunosuppression of humoral type, it fails to explain exciting etiopathogenesis of deaths due to "Gumboro Disease". It was therefore thought necessary to undertake detailed clinicopathological investigations in addition to histopathological study of tissues from bone, bursae and kindneys. These studies were found to be extremely useful in understanding typical etiopathogenetic relationship, in fatal cases of "Gumboro Disease"

Clinicopathological investigations included haematological studies such as haemoglobin estimation, total erythrocyte count, packed cell volume, mean carpuscular value, mean carpuscular haemoglobin, mean carpuscular haemoglobin concentration, total leucocytic count and differential leucocyte count (Table 2 & 3) ; to find out whether affected birds suffered from anaemia or otherwise if so, its severity.

Anaemia encountered was further identified as microcytic hypochromic type. In the absence of any evidence of chronic blood loss, the pathogenesis of microcytic hypochromic anaemia was attributed to impaired iron

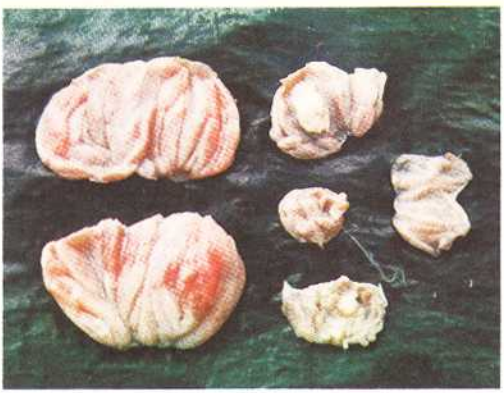
absorption. Observation of reduced packed cell volume, supported the theory of impaired assimilation of proteins together with the observation of stunted growth in the survivals.

Absolute leucocytosis was the invariable finding indicating secondary bacterial complications. Absolute lymphocytopenia at the same time was due to rapid and widespread destruction of "B" lymphocytes from bursa.

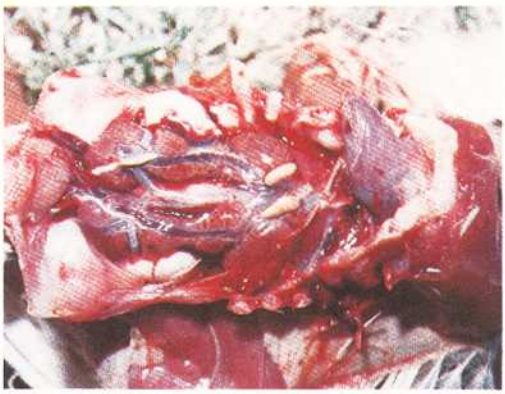
Elevation of blood phosphorus levels (Table 4) was an after effect of nephrosis wherein phosphate clearance is retarded. Persistent elevation of phosphorus in blood is responsible for excessive stimulation of parathyroids through transient hypocalcaemia (Coles, 1980 and Wardener, 1975).

Inadequate or delayed response by parathyroid glands is bound to result into progessive hypocalcaemia with more or less severe consequences. Fatal hypercalcaemia supervenes if parathyroid response is inadequate. If the response from parathyroids is adequate, extreme decalcification of leg bones - results into a peculiar type of lameness (Plate 4) (Sah *et al* 1988) in which the chicks are unable to support their weight on legs.

Treatment in time for maintenance if blood calcium levels ; helped to avoid fatal outcome. It has therefore an evidence to believe the claims of recuperative therapeutic action of homeopathic remedy name 'Calcor phos' (Jagtap *et al* 1993).



Bursa showing, haemorrahages, hypertrophy and subsequent atrophy (plate 1)



Photograph showing white chalky deposits on kidney surfaces with prominent ulcers (plate 2)

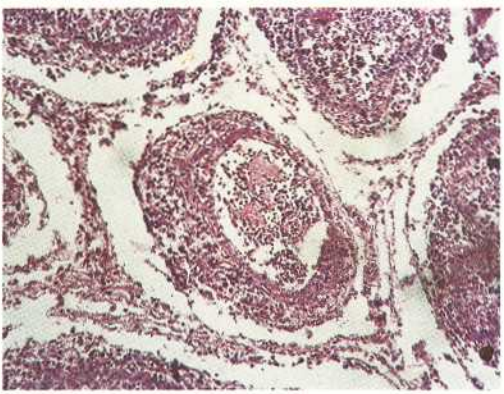


Plate showing typical follicular necrosis with heterophilic infiltration (plate 3)

Photograph showing a peculiar type of lameness in Gumboro affected chicks (plate 4)

Table 1 : Observations on morbidity and mortality pattern in bursal disease condition

Sr. No.	Place of outbreak	Month	Age in weeks	No.of birds in the flocks	Percentage of mortality	Duration of mortality	Complications in outbreak	Confirmation by Histo. exam./ Serology.
1.	Yeotmal	Nov.	9	2300	740/2300 (32.52 %)	4 days	Enteritis	H.P
	Yeotmal	Nov.	9	1400	253/1400 (18.17%)	4 days	Enteritis	H.P
2.	Yeotmal	Dec.	5	2075	348/2075 (17.18%)	4 days	Enteritis	H.P
3.	Amravati	Jan.	3	520	56/520 (10.76 %)	4 days	Enteritis	H.P
4.	Amravati	Feb.	18	520	90/520 (17.30%)	5 days	Coccidiosis	H.P
5.	Akola	Mar.	3	-	-	-	-	H.P
6.	Yeotmal	Mar.	8	7800	4852/7800 (62.62 %)	12 days	E.Coli. + Ranikhet	H.P Serology
7.	Nagpur	July	13	1259	233/1259 (18.00 %)	6 days	E.Coli	H.P Serology
8.	P.K.V. Akola	July	4/5	500	215/500 (43.00 %)	10 days	E.Coli + I.B.H	H.P Serology
9.	Amravati	Aug.	1	1000	200/1000 (20.00%)	7 days	E.Coli	H.P
10.	Amravati	Sep.	-	-	-	-		H.P
11.	Akola	Oct.	6	200	-	4 days		H.P
12.	Akola	Oct.	6	250	54/250 (21.6%)	4 days	-	H.P
13.	Akola	Oct.	3	200	90/200 (45.00%)	4 days	Enteritis	H.P
14.	Amravati	Oct.	9	-	89	3 days	I.B.H.	H.P
15.	P.K.V. Akola	Oct.	6	500	100/500 (20.00%)	10 days	I.B.H	H.P Serology
16.	Akola	Dec.	5	200	82/200 (41.00 %)	4 days	E.Coli	H.P. Serology

Mortality : Varied from 10 to 62 percent. Variation is because of secondary invasion of micro-organisms Mortality is highest in between 5 to 9 weeks of age.

Bursal lesions : Viz., haemorrhages, oedema, caseation, hypertrophy were observed.

Observations on Hemogram in Bursal Disease Condition

Table 2 A : Hematological values of bursal disease outbreaks in 4/5 weeks age (W.L.H.) chicks.

Bird No.	Hb gm/dl	T.E.C. m/mm3	P.C.V. %	M.C.V micro 3	M.C.H. uugm.	M.C.H.C. %
1.	7.8	1.98	24	121.2	39.39	32.50
2.	13.0	2.10	40	190.4	61.90	32.50
3.	6.4	2.30	26	113.0	27.82	24.60
4.	6.8	2.51	24	95.6	27.09	28.33
5.	7.2	1.96	23	117.3	36.73	31.30
6.	7.5	1.87	23	122.9	40.10	32.60
Mean	8.11	2.12	26.66	126.73	38.83	30.30
S.E.	1.22	0.120	3.31	16.34	6.31	1.61

Table 2 B : Normal Values by G.A. Sastry (1989).

	Hb gm/dl	T.E.C. m/cu.mm	P.C.V. %	M.C.V. cu/micro	M.C.H. uugm.	M.C.H.C. %
	6.83 to 11.3	2.18 to 4.12	27 to 42	127	37	29%

Table 3 A : Hematological values of bursal disease outbreaks in 12 weeks age (W.L.H) chicks

Bird No.	Hb. gm/dl	T.E.C. m/cu.mm	P.C.V. %	M.C.V. cu/micro	M.C.H. uugm.	M.C.H.C. %
1.	7.1	2.1	25	119.8	33.80	28.40
2.	6.3	1.97	23	116.7	31.97	27.39
3.	7.1	2.6	26	100.0	27.30	27.30
4.	7.4	2.3	27	117.3	32.17	27.40
5.	6.1	1.98	24	121.2	30.80	25.41
6	7.9	2.4	22	92.6	32.91	32.90
Mean	6.98	2.22	24.5	110.9	31.49	28.66
S.E.	0.338	0.126	0.935	6.07	1.141	1.84

Table 3 B : Normal Values

Hb gm/dl	T.E.C. m/cu.mm	P.C.V. %	M.C.V. cu/micro	M.C.H. uugm	M.C.H.C. %
6.83 to 11.3 by G.A. Sastry	3.02 by Lucas & Jamroj	27 to 42 by G.A. Sastry	127	37 by G.A. Sastry	29%

Table 4 : Observation on bio-chemical alterations in bursal disease condition

Sr. No.	Serum Phosphorus mg/dl	Serum Calcium mg/dl	Serum Alkaline Phosphatase K.A. unit
1.	8.42	8.51	10.12
2.	8.67	8.43	11.20
3.	7.28	8.73	10.90
4.	6.60	9.08	11.70
5.	8.38	9.20	13.80
6.	8.18	8.61	12.52
Average	7.9	8.76	11.70

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In Vitro Fertilization Of Cattle Oocytes

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Abstract

The ovaries of cattle were collected under sterile conditions within 15 minutes of their slaughter and transported to the laboratory. The follicles of 2-6mm dia were aspirated and screened under stereomicroscope. Good quality oocytes were washed thrice, and matured for 24 hours in, *in vitro* maturation (IVM) medium under 5% $C0_2$ in air with high humidity. Following maturation, the oocytes were fertilized using frozen thawed sperms capacitated with heparin. A high percent of maturity (85-100%) was obtained using modified techniques. The percentage of fertilization, cleavage to 2-4 cells, 8 cells and 16 cells to morula were 74, 53, 26 and 11 respectively.

Introduction

Walter Heape (1855-1929) was the first scientist to venture successful transfer of embryos in rabbits during 1891. He also contributed substantially to the science of artificial insemination and animal breeding (Heape, 1897). Later his followers, Marshall and Hammond (1946) continued the work of Heape. In the beginning, rabbits constituted the major species in the embryo work. The use of gonadotrophins for induction of ovulation was developed in the middle of the present century (Cole and Hart, 1930 ; Casida *et al.* , 1943). The culture media and techniques for embryo culture were developed at the same time (Warwick and Berry, 1949).

In the *in vitro* fertilization (IVF) system apart from the oocytes, the culture media and conditions, the quality of sperms used plays a great role. Most of the research on IVF is stressed towards improving culture media and conditions and their cryo-preservation. Therefore the present study was undertaken to produce cattle embryos in the laboratory using slaughtered animals, incorporating the latest culture systems and methodologies to obtain higher conversion of oocytes to embryos.

Materials and Methods

Media

For IVM : TCM 199 (Younis *et al.* 1989) was used with certain modifications.

For SPERM PREPARATION AND IVF: Tyrodes medium (Parrish *et al.* 1988) was used after modifying according to the requirement of sperm capacitation and fertilization.

OOCYTE COLLECTION : The ovaries of slaughtered cows were collected within 15 mins. of their slaughter under sterile conditions in normal saline containing antibiotics. The follicles of 2-6 mm were aspirated within 60 mins., screened under stereozoom microscope and classified according to Reddy *et al.* (1992a) into groups of ABC depending on their investments around them and grouped as 1,2,3 as per their diameter. The oocytes were washed thrice in IVM media and cultured for 24 hours in an incubator at 38.0 C. under 55% CO_2 in air with high humidity. The frozen semen was thawed, motile sperms were collected, washed in sperm preparation medium and capacitated by addition of heparin.

The *in vitro* matured oocytes were washed in IVF medium and 15-20 of them were placed in 50 microliter droplets of IVF media, 5-10 ul of 5 X 10^6 sperms per ml. was added to the droplets and were co-incubated for 24 hours. The fertilization was assessed by visualization of 2nd polar body, male and female pronuclei

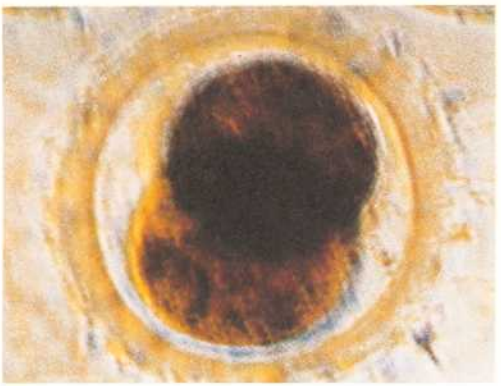

Fig 1

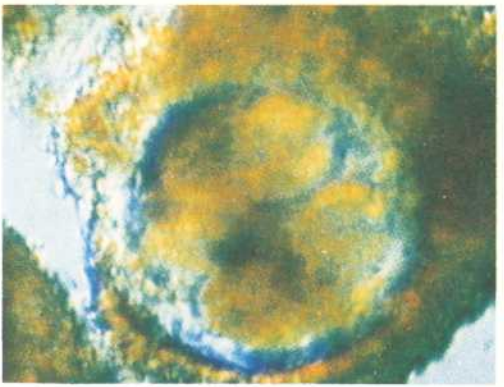

Fig 2

and/orinitiation of cleavage, and were cultured for 6-7 days.

Results and Discussion

When the class A1 and A2 oocytes were subjected to *in vitro* maturation, 85-100% maturation was observed which were comparable to those observed by other workers (Younis *et al.*, 1989; Shioya *et al.*, 1990). The fertilization rates were moderately high reaching 74%. Many zygotes were arrested at 2-4 cell stage and 66.66% of embryos developed to 8-16 cell stage (Fig. 1 & 2). The selection of oocytes played an important role in both maturation and fertilization. The presence of good cumulus complexes is essential to ensure fertilization.

The addition of serum in the culture media, apart from acting as a protein source, keeps the zona soft to ease the sperm entry. The addition of gonadotropic hormones to the media enhances the maturation of the oocytes. This simulates in vivo stimulation that occur under natural circumstances. The salts and biological contents of the media had great influence on both maturation, fertilization, embryogenesis and their maintenance in the laboratory.

It is concluded that once the system of IVM is standardized to a particular laboratory (Reddy *et al.*, 1992b) the embryo production can be much more cheaper, simpler and superior over superovulation and embryo collection in cattle (Reddy *et al.*,1992c). In our set up the IVM and IVF systems are standardized to meet the requirement of embryos of cattle and wherever necessary to apply the techniques for production of interspecies hybrids (Reddy *et al.*, 1991b) and to save the germplasm of endangered species (Reddy *et al.*, 1992d).

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Nobel Prize in Medicine (1994)

Two U.S. Scientists, Alfred G. Gilman and Martin Rodbell have been awarded the 1994 Nobel Prize in Physiology, Medicine for their 'G-Proteins and the role of these proteins in signal transduction in cells'. Dr. Alfred G. Gilman working at the University of Virginia and Dr. Martin Rodbell at the National Institute of Health in Bethesda found that G. Proteins act as signal transducers and convert them into relevant action. The G-Proteins receive multiple signals from the exterior, integrate them and thus control fundamental life processes in the cells.

Disturbances in the function of G-Proteins can lead to disease. The loss of salt and water in cholera is a direct consequence of the action of cholera toxin on G-Proteins. Some hereditary endocrine disorders and tumours are other examples. Furthermore, some of the symptoms of common diseases such as diabetes, alcoholism may depend on altered transduction of signals through G-Proteins.

The scientific contribution of Dr. Gilman and Dr. Rodbell has opened up a new and rapidly expanding area of knowledge on how cells receive information from outside and convert it into relevant action - i.e. how signals are transduced in cells.

Ref : Current Medical Scene - A CIPLA update on Therapeutics 1994, Vol **9** No.5, pp 1.

Heat Stress Induced Endocrine and Metabolic Responses in Pregnant Mammals

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Livestock production and health are threatened by climatic, physical and social stresses. Maximum production requires a balance between reproductive process and deleterious impact of environmental stressors. Intra-uterine growth retardation [IUGR] is the most common effect of enivronmental stress. The new born is dwarfed to as much as one half their expected size. Hence these young ones are especially vulnerable to biological and environmental stresses. The reason attributed to IUGR has been linked to sustained maternal heat stress late during gestation, resulting in decreased uterine blood flow due to hyper secretion of maternal vasopressin or oxytocin and that the reduced uterine blood flow shifts foetal metabolism from anabolic to catabolic pathway. This phenomenon has been observed in sheep, cattle, pig and rabbit. In humans maternal fever and hyperthermia are associated with premature delivery and low birth weight infants.

Maternal Response

Reduced placental weight during chronic heat stress is associated with decreased maternal to foetal transfer of oxygen and glucose and decreased production of placental lactogen and progesterone.

Suppression of placental function and subsequent foetal dwarfing is brought about by reduced uterine blood flow (UBF). During pregnancy the UBF increases 10 to 15 fold in sheep from 100 ml /min. at 40 day to 1000 to 1500 ml/min. near term. Ninety percent of this increase supplies the placental cotyledon with nutrients, oxygen & water for the foetus. As gestation progress there is a linear increase in placental blood flow and logarithmic increase is uterine uptake of O_2 and glucose which are highly correlated with foetal weight gain. Uterine blood flow decreases during heat stress in pregnant & non pregnant sheep. Maximum depression of UBF 50-60% is observed within 2 hours after 1 to 2 ^{0}C increase in rectal temperature. During acute heat stress decreased UBF is accompanied by decreased blood pressure and partial pressure of CO_2, increased respiration rate and plasma pH but there is no change in heart rate.

Sex hormones, catecholamines, PCO and pH regulate the uterine blood flow. Estradiol (E2) and Progesterone (P) antagonistically regulate UBF during pregnancy. E2 favouring UBF to the myometrium whereas P decreases the magnitude of E2 stimulated UBF.

The uterus possesses adrenergic receptors and UBF is depressed after non epinephrine and epinephrine infusions. Heat stress and epinephrine have additive effects in decreasing E2 stimulated increasing UBF.

Heat stress increases the respiratory rate (Panting) with concomitant rises in blood pH. (Respiratory alkalosis). The uterine vasculature have pH sensitive receptors and the decrease in UBF during acute heat stress may be a consequence of Alkalosis associated with panting in the pregnant ewes i.e UBF is decreased by bicarbonate induced alkalosis and increased by hyperpnoea.

Oxytocin and Vasopressin both decrease UBF during pregnancy. Vasopressin is synthesised in the Supra Optic nucleus of the hypothallamus. It is released from the neurohypophysis during elevated ambient temperature in addition to hyperosmolarity and hyporolemia. Elevated plasma Vaso

pressin depresses (ADH) UBF by stimulating contraction of the myometrium.

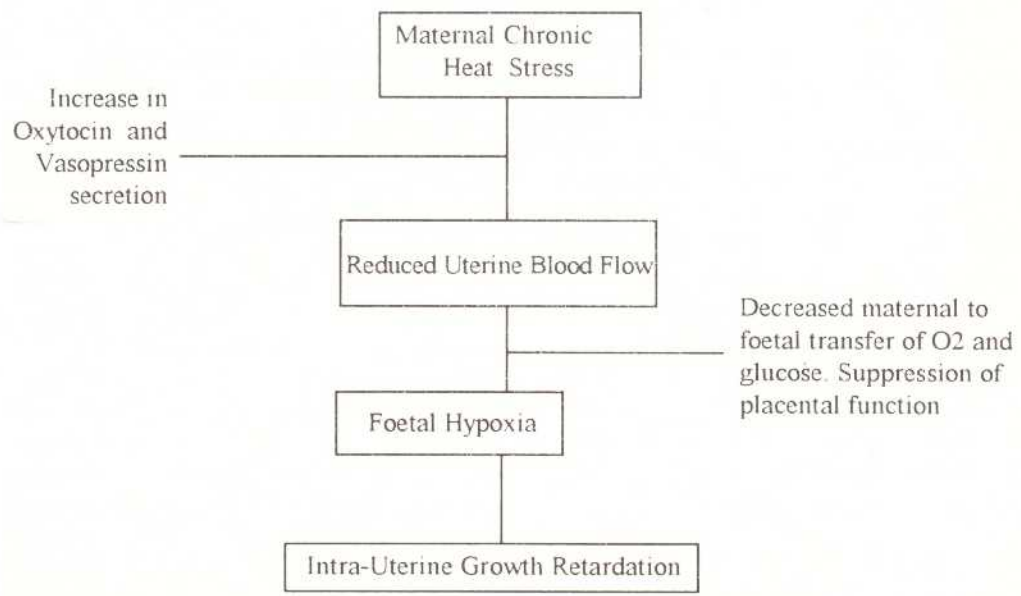
Oxytocin is synthesised in the para ventricular nucleus of the hypothallamus and is secreted from the neurohypophysis. Its secretion occurs during parturition in response to neutral inputs from cervix, uterus and mammary tissues. Oxytocin is also released during water deprivation & salt loading and is secreted in response to increased body temperature.

Foetal Responses

It was initially thought that depression of the foetal growth rate was uniform throughout the body and some report that it is a proportional miniature, however it is neither a true miniature nor achondroplastic dwarfs but is simply stunted. Extent of dwarfing is proportional to the duration of heat stress and the stage of gestation.There is a relative increase in body length, head size and kidney and adrenal weights. The liver, thyroid and thymus were disproportionately decreased in weight. The increased metabolic rate is due to the fact that the foetal temperature increases more than maternal temperature during heat stress.

Decreased UBF, regardless of etiology results in foetal hypoxia, foetal oxygen consumption and also availability is reduced to 50% during maternal heat stress. Hence the foetus must compensate or adopt to the reduced 0 availability. It is done by shifting its metabolic process to more efficient substrate fuel utilisation or increasing the efficiency of oxidative processes. Chronic heat stress induces protein degradation, shift in liver metabolism towards glucose production is accomplished by glucose sparing effects of adipokinesis and their synthesis of glucose from amino acids via gluconeogenesis. In intra-uterine growth retarded lambs during maternal heat stress the concentration of glucose-b-phosphate dehydrogenase is reduced. Thus heat stress have a significant impact on the genetic makeup of the developing foetus.

The deleterious effect of heat stress on pregnancy can be summarised as,



Pathology Of Staphylococcal Arthritis In Broilers

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Summary

Staphylococcal arthritis of the hock joint was found in 70 percent of the cases showing legweakness. The birds were having swollen joints, reluctance to move and severe loss of weight. There was decrease in plasma calcium and an increase in plasma alkaline phosphatase in staphylococcal infection birds. The bone calcium and phosphorous were severely decreased. Histopathology of the hock joint revealed inflammatory changes, necrotic degenerative and proliferative changes. Staphylococcal organisms were found in the perivascular region of narrow, deep cartilage and musculature.

Introduction

Legweakness is one of the emerging problems of poultry in fast growing broiler birds, causing a mortality of 15 - 20 percent in organised farms causing great concern to farmers and industry as a whole. In the present investigation. staphylococcal arthritis of the hock joint was found to be a common etiology in causing legweakness. This was studied by earlier workers in layer chicks in India (Suresh Prasad *et al*) . However, not much work has been done on the infectious bacterial arthritis in broilers in India. Therefore present investigation has been taken up to elucidate the incidence of staphylococcal arthritis in broiler. While studying the pathology of the disease simultaneous studies were carried out to know the changes in calcium (Ca) and phosphorous (P) in plasma and bone and alkaline phosphatase (ALP) levels in plasma.

Material and methods

The study comprised of a total of 16,500 birds from 10 different flocks aged between 3 to 5 weeks. Among them 2675 birds showed typical signs of legweakness. Ten birds with typical signs of legweakness and 5 normal penmates were obtained randomly from each flock. Body weights of the birds were recorded prior to exsanguination and blood was collected in heparin for plasma. The plasma samples were stored at -20 C. and later estimated for Ca, P and ALP using ERBA analyser 2010. The tibia of affected birds was collected for estimation of Ca and P in bone as per AOAC methods, 1985. The hock joints of the birds were collected under sterile condition and the joint fluid screened for bacterial isolation. The hock joint was subjected to histopathology using standard paraffin embedding technique. The relevant data pertaining to legweakness were subjected to test of significance and analysis of variance.

Results and Discussion

The incidence of legweakness was 16.22 percent in the population of 10 farms. The incidence ranged from 10.2 to 30% in different farms. The birds belonging to the age group of 3 weeks were found to have a higher incidence of legweakness (Suresh Prasad *et al* 1972; Griffiths *et al* 1985). On bacterial screening birds from seven farms were found to be positive for staphylococcal infection. Feed analysis did not reveal any deficiency of Ca and P in these farms. There was a decrease of 25 to 44 percent in weight in 3 to 5 week old birds when compared to normals. The observations on Ca, P and ALP in plasma are presented in Table 1. The average levels of Ca and P in staphylococcal infection birds was 9.05 ± 0.115 mg/dl and 6.146 ± 0.180 mg/dl respectively. In apparently healthy birds these were 11.136 ± 0.158 mg/dl and

6.694 ± 0.21 mg/dl respectively. The main levels of plasma Ca decreased significantly in affected birds compared to normals, but no significant change was observed in phosphorous. The decrease in Ca in affected birds was attributed to impairment in Ca absorption in the gut.

The ALP levels in staphylococcal infection birds and normals were 6912.95 ± 523.49 IU/L and 1552.31 ± 85.52 IU/L, respectively. The results showing high levels of ALP activity is adequately substantiated by earlier workers (Sanger *et al* 1966; Bell & Freeman, 1971) and this is attributed to increased activity of the hyperplastic osteoblasts in the lesions of infected birds.

The mean bone calcium and phosphorous in staphylococcal infection birds was 12.80 ± 0.127% and 5.82 ± 0.12% respectively. In apparently healthy birds it was 14.90 ± 0.163% and 7.02 ± 0.68% respectively. The significant decrease in bone Ca and P in infected birds is attributed to malabsorption and decreased accessability to feed and water due to immobility.

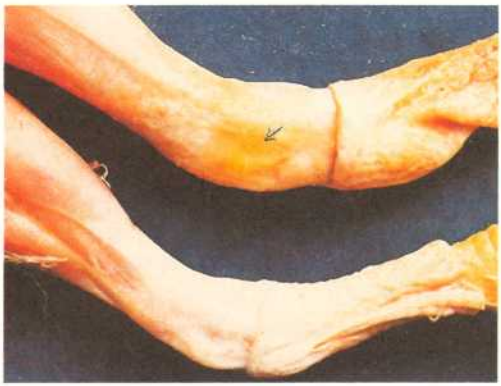
The staphylococcal infected birds appeared emaciated, reluctant to move, dull with ruffled feathers, greenish diarrhoea, and recumbency (Itakura *et al*, 1976) , Grossly, the changes seen were dry and dark red pectoral muscles, prominent keel bone, greenish diarrhoea (Emslie and Nade, S, 1985) . Hock joints were swollen on the posterior aspect, increase in joint fluid was noticed on opening the joint which was cloudy and viscid (Bell & Freeman 1971; Emslie and Nade, 1985)

Slight bending of the hock joints and legs below the metatarsus and swelling of the cartilaginous areas of the joint was found in some birds. On longitudinal sectioning of hock joints an abnormal development of the epiphyseal cartilage was seen at the proximal end of metatarsus and distal end of tibiotasrsus Minoru Maeda *et al* (1988) reported similar findings. In the present study dyschondroplastic lesions in the hock joints is attributed to inadequacy of dietary nutrients available to birds due to immobility.

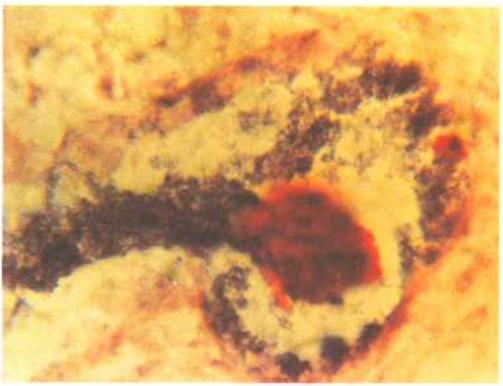
Histopathologically, joint capsule revealed edema, haemorrhages and fibrin deposition. Articular cartilage revealed inflammatory changes, proliferation of connective tissue and fibrosis. Deep cartilage revealed necrotic changes around vessels. Bone marrow revealed necrosis, cytolytic activity, atrophic degeneration and fatty change of marrow. Minoru Maeda *et al* (1988) reported similar findings. Mutalib *et al* (1983). Musculature showed inflammatory and degenerative changes .

Table 1 : Mean Values of Calcium, Phosphorous and Alkaline Phosphatase in staphylococcal infection and normal broiler birds.

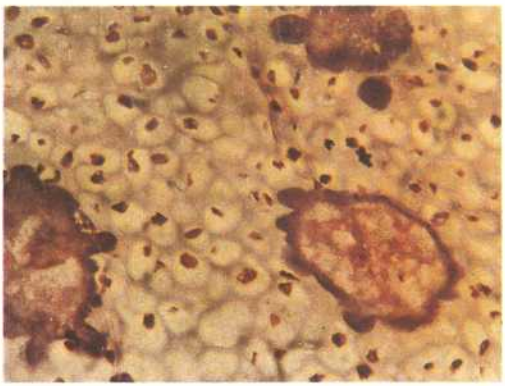
Parameters	Normal birds	staphylococcal infection birds
Calcium mg/dl	11.136 *	9.05*
	±	±
	0.158	0.115
Phosphorous mg/dl	6.964	6.146
	±	±
	0.21	0.18
Alkaline Phosphatase	1552.31 *	6912.95*
	±	±
	85.52	523.49



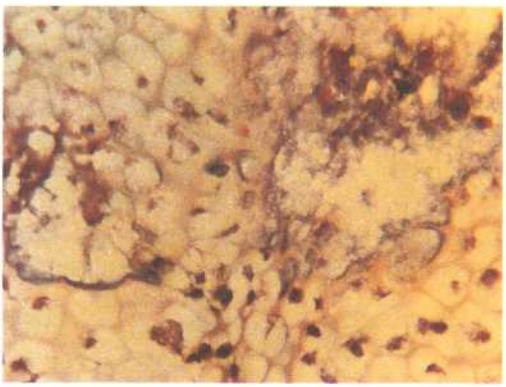
Swollen hockjoint with presence of yellow, viscid pus in the synovial cavity of the affected joint compare to normal



Section of hockjoint showing blood vessels stuffed with organisms in articular cartilage of metatarsus. H & E x 500



Section of hockjoint showing irregular multifocal areas of necrosis and presence of bacterial colonies in deep cartilage. H & E x 500



Section of hockjoint showing necrosis of deep cartilage with presence of organisms and occasional leucocytes. H & E x 500

Staphylococcal organisms were found in the joint capsule, articular cartilage, perivascular space in deep cartilage, muscle bundles and bone marrow (Itakura *et al.* 1976, Multalib, *et al* 1983 and Minoru Maeda *et al* 1988)

In the present study degenerative, inflammatory and proliferative changes were found to be a sequelae to staphylococcal infection.

Acknowledgement

I am thankful to Dr. Ramesh Babu, Scientist, IAH & VB for helping in bacteriological screening of the samples.

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Influence Of Ovarian Status And Lactational Stress On The Effect Of Norgestomet Treatment In Buffaloes

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Anoestrus and suboestrus are the major reproductive disorders in buffaloes in India. According to Rao and Rao (1979) effective synchronization of Oestrous was possible with norgestomet in cycling buffalo cows. Singh *et al* (1983) reported that the norgestomet treatment in anoestrous buffaloes induced oestrous better than PRID. Rao and Sreeman Narayana (1983) assessed conception rate in anoestrous buffaloes after inducing oestrum with norgestomet and PMSG. The present investigation is undertaken to find our whether the norgestomet treatment with PMSG can be effectively used for induction of oestrum in true anoestrous and suboestrous conditions during lactation and dry period.

Materials and Methods

Twenty nine anoestrous buffaloes of livestock Research Station, Kattupakkam belonging to the Tamil Nadu Veterinary and Animal Sciences University at 3-10 months after Calving were selected for this study. The animals were fed with adequate green fodder with required concentrates. The animals were divided into four groups as lactating suboestrum (n-4) and lactating true anoestrum (n-6) and dry suboestrum (n-4) and dry true anoestrum (n-7). Suboestrus and true anoestrus were confirmed by palpation of the ovaries per rectum two times at 12 days interval. The buffaloes were given a 9 day treatment with subcutaneous ear implant containing 6 mg of Norgestomet (Intervet. Holland) with intramuscular injection of 5 mg estradiol valerate + 3 mg norgestomet at the time of implantation and 500 IU PMSG when the implant was removed. Oestrus detection was carried out by external observation and by twice daily rectal examination of genitalia after the removal of implant. The intensity of induced oestrus was assessed based on the physical signs as per Rao and Rao (1979). Inseminations were done twice at 12 hours interval about 12 hours after the onset of oestrum with frozen semen. All the animals were kept under observation after AI and those exhibiting next cyclical oestrus were inseminated again. Two buffaloes in each group were observed as control without any treatment and any control exhibiting oestrus was also inseminated. Pregnancy was detected by rectal examination at 45 days after the last AI.

Results and Discussion

All the suboestrous and anoestrous buffaloes treated with norgestomet showed oestrus. The onset and pattern of induced oestrus for all the four groups are given in Table I. Oestrus was exhibited by all the animals within 36-72 hours after the removal of ear inplant. This was in accordance with the findings of Rao and Sreeman Narayana (1983) in non cycling buffaloes treated with norgestomet during low breeding season whereas Singh *et al* (1983) recorded only 58.33 and 77.77 percent in their two experiments after the treatment with norgestomet and PMSG. The duration of induced oestrum was 21 and 22.5 hours in lactating and dry suboestrous buffaloes and it was 15.33 and 15.43 hours in lactating and dry true anoestrous buffaloes. Regarding intensity of oestrus it was intense in 25 percent and normal in 75 percent of dry and lactating suboestrous buffaloes whereas 28.5, 43.4 and 28.5 and 16.71, 50 and 33.3 percent buffaloes exhibited intense, normal and weak oestrous signs in dry and lactating group of true

anoestrous buffaloes respectively. No suboestrous animals exhibited weak oestrum. The differences in the time of onset, duration and intensity between suboestrous and true anoestrous buffaloes might have been due to the difference in progesterone level at the time of giving norgestomet implant and oestradiol level at the time of induced oestrous. In control two dry suboestrous buffaloes exhibited normal oestrous and all other control animals have notexhibited oestrous during the study.

Regarding, conception rate (Table-II), 3 animals in suboestrous group and 5 in true anoestrous group conceived with the first AI and each 2 in both the groups conceived by second AI. The overall conception rate was 62.5 percent in suboestrous group and 54.3 percent in anoestrous group. In control group out of 2 buffaloes inseminated only one conceived with the first AI. This conception rate was more or less in accordance with the conception rate of 57.1 percent observed by Rao and Sreeman Narayana (1983) in non cycling buffaloes.

The conception rate according to the milk production status of the treated buffaloes irrespective of their type of anoestrum at the time of treatment is presented in Table-III. Conception rate of 63.64 percent in dry buffloes was more than 50 percent in lactating buffaloes. Slightly low conception in lactating buffaloes might be due to lactational stress. This observation was similar to the observations done by Mulvehill and Sreenan (1978) in postpartum cows.

It is concluded that Norgestomet with 500 IU PMSG treatment is effective in inducing oestrus in suboestrous and true anoestrous buffaloes during lactation and dry period and the conception rate was better in suboestrous buffaloes (62.5%) than in true anoestrous buffaloes. It is also observed that the conception rate was better in dry buffaloes (63.64%) than in lactating buffaloes (50%).

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Table - I

Criteria	Status of the buffaloes	No. of animals	Time of onset of estrous (in hours)	Duration of estrous (in hours)	Nature of Induced Oestrous		
					Intense	Normal	Weak
Suboestrous	Lactating	4	48 (36-60)	21 (18-24)	1	3	0
	Dry	4	45 (36-48)	22.5 (18-24)	1	3	0
True Anoestrous	Lactating	6	56 (36-72)	15.33 (12-18)	1	3	2
	Dry	7	49.7 (36-60)	15.43 (12-24)	2	3	2

TABLE - II

Criteria	Dry/lactating suboestrous buffaloes	Control	Dry/Lactating true anoestrous buffaloes	Control
No. treated	8	4	13	4
No. exhibited estrous and inseminated	8	2	13	---
No. pregnant with I AI	3	1	5	---
No. pregnant with II AI	2	---	2	---
Percentage of conception over 2 AI	62.5	25	53.57	---

TABLE - III

Criteria	Suboestrous / true anoestrous lactating buffaloes	Control	Suboestrous / true anoestrous dry buffaloes	Control
No. treated	10	4	11	4
No. exhibited estrous and inseminated	10	1	11	1
No. pregnant with I AI	3	---	5	---
No. pregnant with II AI	2	---	2	---
Percentage of conception over 2 AI	50	---	63.64	25

An Appeal

All the members of the Veterinary Profession are invited to contribute their papers for publication in the Blue Cross Book and share their experience with their brethren. The Papers should not have been published elsewhere and should generally not exceed six to eight pages.

- Editor

A Brief Review On Brucellosis Research

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Introduction

Bombay with high humidity and rainfall area creates ideal condition for brucellosis. Importing of cattle stock in the past is root cause of today's high prevalence of brucellosis all over the country. The improved strain was *Brucella abortus* biotype 1 which dominates the present brucellosis scenario. The indigenous strain *Brucella abortus* biotype 3 is almost extinct now.

The author was engaged in brucellosis reasearch for nearly a decade (1981-89). This paper is a concised review of this work.

Incidence and Seasonal Distribution

A survey on incidence of brucella-abortion in cows and buffaloes was conducted based on culture results. All strains were *B. abortus* biotype 1 with minor biochemical and physiological variations (Das and Paranjape, 1987 & 88).

Animals	Out of	% Prevalence
Buffaloes	18 / 76	23.68
Cows	21 / 31	67.74

Seasonal distribution of abortion in buffaloes is shown in the table below. The profile was similar in cows.

Monsoon	Winter	Summer
30%	11.76%	21.05%

Species Difference

Specific species difference was detected between buffaloes and cows aborting due to brucellosis infection (Das et al., 1990).

Sr.No	Species Difference	Buffalo	Cow
1.	Occurrence	Low	High
2.	Stage of abortion	Early (50%)	Late
3.	CO_2-Independent *B. abortus*	More frequent	Less frequent
4.	Naturally occurring rough strains (in-vivo)	Nil	4
5.	Difference in SAT titre between acute & convalescent phase sera	Narrow	Wide

A group of buffalo - and crossbred - calves vaccinated with *B. abortus* strain 19 exhibited following differences in the immune response (Das and Mulbagal, 1982 & 83 ; Das, 1984 and Das and Patil-Kulkarni 1984).

Sr. No.	Species Difference	Buffalo Calves	Cow Calves
1.	Peak Concentration of IgM	10th day	15th day
2.	Peak Concentration of IgG	15th day	30th day
3.	Kinetics of antibody titre	Quicker and shorter in persistence (3 months)	Slower and longer in persistence (6 months)
4.	Persistence of agglutinins non-agglutinins	Non-agglutinins persisted longer	Agglutinins persisted longer
5.	Appearance of IgGI non-agglutinins	15th day	5th day
6.	Disappearance of IgGI and IgG2 non-agglutinins	120th day	180th day
7.	CFT and CCAT titres	Shorter in persistence	Longer in persistence

Immuno-Serological Observations in Field Cases

Immunoglobulin profile and their relationship with supplementary serological tests were studied in randomly selected aborting cows and buffaloes from a known brucella-positive herd having a history of third trimester abortion storm (Das and Mulbagal, 1985).

1. There was no specific species difference observed.
2. IgM was much lower in concentration as compared to IgG1 and IgG2.
3. IgG2 was higher in concentration than IgG1
4. The predominance of IgG was clearly indi cated in IgG specific serological tests like HIT, MET and ABGT in terms of resistance to heat & 2-mercaptoethanol, and high titres of non-agglutinating antibodies.
5. Non-agglutinating IgG2 titres were higher than that of non-agglutinating IgG1 titres.

Bacteriological-/ Sample Collection - Techniques

Early diagnosis by culture results (Das *et al.*, 1990)

1. Take direct impression smears of placental lesions/cervical mucus/vaginal discharge/ foetal spleen, liver, heart blood & abomasal contents.
2. Stain by modified acid-fast technique and look for organisms retaining carbol fuchsin.
3. Inoculate onto Brucella selective medium(Himedia) and incubate at 37 deg. C with 10% CO_2 atmosphere for 36 hours.
4. Detect milky white brucella colonies with a bacteriologic loop.
5. Stain with Gram's and modified acid-fast method
6. If suggestive of brucella morphology, perform plate agglutination test with monospecific A and M antisera.

A modified method to collect cervical swabs for brucella isolation (Das *et al.*, 1987)

Conventional tampon was modified in such a way that it collected mucus specifically from cervical region. The procedure minimized contaminating microflora greatly and facilitated isolation of brucella in both selective and nonselective media as compared to the conventional tampon.

Isolation of brucella from aborted foetuses (Das et al ., 1990)

Usually abomasal contents and heart blood are the materials of choice when brucella are to be isolated from an aborted foetus. In our investigation, apart from these two specimens, lung, liver, and spleen were also collected for brucella isolation. The results are as follows:

Organs / Materials	Out of	Brucella Recovery %
Liver and Spleen	10 / 13	76.9
Abomasal contents and heart blood	3 / 13	23.07

Serological Techniques

Modified quick tests for IgG detection (Das et al., 1983)

Conventional mercaptoethanol test and heat inactivation test are read after 24 and one hour respectively. The latter has many labourious steps. Both the tests were modified in such a way that the procedures could be completed within half an hour with easy steps. These tests were named as follows:

1. Rapid mercaptoethanol test (RMET)
2. Rapid heat inactivation test (RHIT)

Modified plate and rapid methods of anti-bovine globulin test (ABGT) (Das, 1984)

ABGT are of three types viz. tube, plate and rapid methods performed with anti-gamma globulin. Antisera against subclasses of IgG (IgG1 & IgG2) have been tried only in tube method. The author used such antisera in the plate and the rapid variant of ABGT. These two methods consume lesser time as compared to the tube method in detecting incomplete antibodies associated with a particular subclass of immunoglobulin.

Comparison of Brucella-stabilized antigen plate test (BSPT) with Rose Bengal plate test (RBPT) (Das and Paranjape, 1987 a)

BSPT is a serological kit manufactured by Preco, USA. The sensitivity of this kit was compared with the standard RBPT:

S. No.	Criteria	BBPT	RSPT
1.	Sensitivity	Less	More
2.	Reaction time	30 - 90 Sec.	Immediately or within 30 sec.
3.	Colorimetric Contrast	Poor	Good
4.	Hemolysed sera	Interfere with the visibility of agglutination reaction.	Do not interfere

Immunization / Vaccine

Das **et al.** (1986) isolated a strain of Versinia enterocolitica serotype 0:9 - biotype 3b from aborting buffaloes which serologically cross-reacted with anti-*B. abortus* serum. An attempt was made to find out if it also had the property of cross-protectivity against brucellosis.

A preliminary field trial with a simple heat killed vaccine prepared with this strain showed encouraging results by protecting pregnant buffaloes against abortion due to brucellosis (Das and Paranjape, 1992).

Brucellosis in Breeding Bulls

Misleading clinical features:

Unusual lesions (pustular inflammation all over the penis with oedematous swelling leading to phimosis) associated with penis of breeding bull suspected to be a case of IBR/IPV, yielded Brucella abortus biotype 1 (Das and Deopurkar, 1988). A combination of long acting tetracycline and streptomycin for 21 days cleared the infections.

Serological survey

EDTA-Standard tube agglutination test (EDTA-SAT) was employed in the survey-cum-diagnosis of brucellosis among breeding bulls (Das and Paranjape, 1988). Following were the striking features :

1. Low titre sera in general contained EDTA-sensitive antibodies - Nonspecific reaction.
2. Seroprevalence dropped down from 17.81% (By conventional SAT) to 8.21% (By EDTA-SAT)
3. Results of EDTA-SAT correlated well with the clinical status of animals.

Summary

Prevalence of brucellosis is greater in cows than in buffaloes in Bombay. Monsoon records highest incidence of brucellosis in both the species. Specific species difference between the two is observed in aborted and in vaccinated animals. Calfhood vaccinal antibodies in the buffalo serum disappear much faster than in the cow serum, thereby resolving possibilities of their interference in the serological diagnosis of brucellosis.

Diagnosis by culture results in 36 hrs; modified tampon improving brucella isolation from cervical mucus and newer informations on the distribution of brucella in foetal organs are efficacious adjuncts to brucella-bacteriology.

Modified quick tests viz. rapid mercaptoethanol test and rapid heat inactivation test to detect IgG antibodies and modified plate and rapid anti-bovine globulin test to detectincomplete antibodies belonging to subclasses of IgG are updates in brucella-serology. In bulls, EDTA-standard tube agglutination test that detects only brucella-specific agglutinins, is a valuable serological test in the diagnosis of brucellosis.

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Efficacy of Deltamethrin (Butox) against *Haematopinus eurysternus* infestation in cattle

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Introduction

Sucking lice *Haematopinus tuberculatus* infestation in buffalo is very common in India. These lice remain on the body of the host anchoring tightly with their powerful claws. On the other hand *H. eurysternus* infestation in cattle is comparatively uncommon. In a missionary cattle farm at Mondouri near the University, twelve cross bred cows were found to be affected with H. eurysternus, the short-nosed cattle louse. The lice did not infect the whole body but almost exclusively were found on the soft skin surrounding the vaginal orifices (Fig. 1). The economic losses are likely to occur due to discomfort, irritation and toxicosis, on account of the bite of these lice in this highly vascularised vital area. They were not easily removable with fingers, because of their tightness with the skin with the powerful claws. In these affected animals a large number of eggs and a few larvae were found only in the tuft of hairs of tails. Nowhere other than this site the eggs were deposited by the gravid females. During observation for a few days no female lice were found inside the hair tuft. The gravid females are nocturnal in their laying habits, and come down through the tail stem directly to the tuft of hairs and lay their eggs fixed to the hairs by means of small terminal clasps (Fig. 2). When these eggs hatch the larvae migrate upwards to the vaginal site, moult to nymph and adult stages and remain fixed just surrounding the vaginal orifices.

Deltamethrin has been tried successfully to control different ectoparasites of cattle (Banerjee and Sangwani, 1990). But there seems to be no report on efficacy of Butox against the lice H. tuberculatus infestation. Therefore, in the present study an attempt was made to assess the effective concentration of Butox (Hoechst India Limited, Bombay) which will destroy the eggs larvae and adults of the lice at a time, as control measure against these lice on that particular vulnerable site is rather difficult for application of various acaricides.

Materials and Methods

A total of 12 affected animals were divided into three unequal groups (I,II and III) of 5, 5 and 2 animals respectively. The affected parts surrounding vagina and the tails with hairs of groups 1 and 2 were thoroughly dipped with 12.5 and 25 ppm aqueous solution of Butox for a period of 5 minutes for a single day. Two animals of the control group were dipped in distilled water for 5 minutes. They were observed for a period of 15 days post treatment to evaluate the efficacy of the treatment.

Results

The animals of group II(25 ppm application) showed complete elimination of infection within 24 hours surrounding the vagina. No live lice were found to remain attached on treated places. The eggs remained attached but they did not hatch within the next 15 days and on observation they were found to be dead.

The animals of group I (12.5 ppm application) showed almost similar results to group II in case of adult lice but for the eggs of which 20% hatched, the larvae did not develop and died without further moulting.

The two animals of the control group showed normal lousy behaviour as previous to the experiment.

Figure 1 showing Haematopinus eurysternus lice

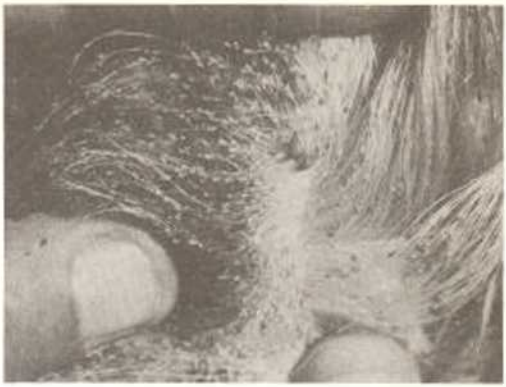

Figure 2 showing the infection of tail hair with eggs of Haematopinus eurysternus lice

Discussion

A rare cattle louse *H. eurysternus* infestation surrounding only the vaginal orifices and the laying of eggs only in the tuft of hairs were recorded and treated with 12.5 and 25 ppm of Butox. Both the concentrations were found to be 100% effective against the adults as well as eggs though 20% of eggs treated with 12.5 ppm hatched but did not develop further.

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In continuation of page 3, "The Blue Cross Book", No. 3 .....

Prof. Lagerlof was an ardent disciplined person and a strict teacher. He followed as well as preached personal hygiene and introduced a special "Protective clothing" for field workers in Animal Reproduction which has been reflected in high standards of personal hygiene in this discipline.

Credit goes to him for creating an independent chair of Professor of Gynaecology and Obstetrics, Andrology and Artificial Insemination in Veterinary Colleges in India and abroad and now there exist independent departments in most of the Veterinary Colleges.

With the assistance from the F.A.O and S I D A, he successfully organised " International Postgraduate Courses in Animal Reproduction". Such courses were held every alternate year in 1954-55, 1957, 1959,1961,1963, 1965,1967 and 1969 when he worked as Director of the Courses. He would meet and follow up with his students during the next year and solve their problems at personal and Government levels a unique characteristic of a good teacher. These Courses are being continued even after him.

Prof. Lagerlof suddenly passed away on 9th November 1970 when still active till the last day. He will always be remembered as the pioneer in Animal Reproduction and his voluminous contribution will serve as a source of inspiration and guidance to the Veterinarians in the years to come.

Biological Control Of Ixodid Ticks Using A Leguminous Plant *Stylosanthes Scabra* (Vogel)

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Despite the urgent need to restrict the indiscriminate use of insecticides and to adopt different biocontrol programmes for effective control of ticks, very few biocontrol agents to date, have become popular. Therefore a nutritionally important easily cultivable legume, *Stylosanthes scabra* has been selected for the present study since it is known to secrete sticky substance (fig. 1 & 2) responsible for immobilisation and killing of larval ticks.

Material and Methods

A. Collection and maintenance of different stages of ticks

Engorged female ticks of *Boophilus microplus, Hemaphysalis intermedia* and *Rhipicephalus sanguineus* were collected from cattle, sheep and dogs respectively. Individual ticks were placed in different petri dishes for egg laying. Eggs laid were transferred to glass tubes tied with muslin cloth and rubber band and kept for hatching. Larvae and nymphs raised on rabbits by the ear bag method were preserved in dessicator containing saturated potassium chloride solution at room temperature.

B. Cultivation of *Stylosanthes scabra*

Seeds of *Stylosanthes scabra* were sown and grown in 20 cm. (top) diameter pots. 1.5 g of urea and 0.7 g of superphosphate were added at the time of sowing and every 4 months and the pots were watered daily. The plants thus grown (Fig. 3 and 4) were utilised in the present experiments.

Thin muslin cloth bags measuring 40 x 40 cm with purse string at one end were prepared. A small opening was also made at the other end of the bag to facilitate the introduction of larvae or nymphs. Such a bag was inserted over the *S.scabra* plant and the purse string end was tied at the bottom. Small glass vials containing identified larvae or nymphs of ticks were introduced through the small opening and closed with a thread followed by adhesive plaster (Fig. 5). The cloth bag used in the experiment prevented larval escape and helped them to be confined and climb almost all parts of the plant. After different periods of larval/nymphal exposure Viz. 24, 48 and 72 hours, the plant along with the cloth bag was incised just above the roots and transferred to an enamel tray. This tray was again placed in a water trough to prevent live larval / nymphal escape. Later the cloth bag was opened and dead larvae and nymphs were counted and recorded. Three replications of the experiment was conducted using 3 to 10 months old plants of *S. scabra.*

Results

The percentage larval mortality of ticks at different periods of exposure on *Stylosanthes scabra* of various ages is shown in table. 1

Boophilus microplus :

Though the percentage of mortality varied at different periods of exposure on plants (table 1). It was found that the age of the plant had marked influence ($P<0.01$) on the mortality of the larvae. The highest percentage of mortality was observed on 6th month (53.95 ± 1.12) followed by seventh month (53.18 ± 1.18) and lowest (9.90 ± 0.61) on 10th month old plants (table 2.). There was no significant difference in the mortality of larvae when exposed to different periods of exposure.

Haemaphysalis intermedia

A lowest larval mortality was observed in comparion with *B. microplus* and *Rhipicephalus sanguineus* (Table 1) and it could be seen from the anova table 1, that the larval mortality was significant during the different periods of exposure ($P<0.05$) and also to the different ages of plant ($p<0.01$). Highest mortality was seen at 6th month (7.73 ± 0.31) followed by 7th month (7.67 ± 0.31) and lowest (3.33 ± 0.18) at 3rd month (Table 2). The larval mortality rate was 5.78 ± 0.45 when larvae were exposed to 72 hours, 5.68 ± 0.57 at 48 hours and 5.17 ± 0.43 at 24 hours (Table 3).

Rhipicephalus sanguineus

The results indicated the varied percentage larval mortality (table 1) and significant differences ($p<0.01$) were seen in the larval mortality when exposed to different ages of the plant and different periods of exposure (Anova Table 1). Highest mortality was observed at 7th month (33.97 ± 1.42) followed by 6th month (32.53 ± 0.90) and the lowest (8.02 ± 0.32) was seen at third month (Table 2). A mortality of 20.66 ± 2.80 was seen when larvae were exposed for 48 hours, 20.36 ± 2.43 at 72 hours and 18.31 ± 2.80 at 24 hours (Table 3).

The study also revealed that the overall mortality of larvae was significantly influenced by the tick species, age of the plant and periods of exposure ($p<0.01$). Highest mortality (31.64 ± 4.61) and lowest (7.49 ± 0.79) on 10th month plants (Table 4)

Acaricidal effect of *S. scabra* on different tick nymphal stages.

The varied mortality of nymphs is shown in the table 5, and fig. 6. Significant variation ($p<0.01$) was observed between nymphs of different tick species when exposed to the *S.scabra* plants. Significant differences were also seen in the mortality of nymphs when exposed to different ages of the plant.

Discussion

Stylosanthes is a small genus comprising of about 30 species and the main centre of distribution is in the tropics and sub-tropics of south and central America (Mohlenbrock, 1957). *S. scabra* is one of the species which produce sticky secretion possessing acaricidal property (Sutherst et al., 1982). Besides., *S. scabra, S. viscosa and S. guianensis* also have similar acaricidal properties (Sutherst et al., 1988)

The present study indicated that the secretion of the 6th month plant possessed highest trapping and killing effect. A similar observation was reported by Sutherst et al. (1982) who found maximum mortality of *B. microplus* larvae on flowering (approximately 6 months) *S. scabra* and *S. viscosa*. However, the trapping ability of S. scabra on the larvae of *H. intermedia* and *R. sanguineus* was not reported as has been done in the present investigation.

The significant differences in the larval mortality was observed in *H. intermedia* ($p<0.05$) and *R.sanguineus* ($P<0.01$) when exposed to various periods of exposure (Anova table 1). The study indicated the highest mortality in *B.microplus* and lowest in *H.intermedia*. The trapping and killing effect of *S. scabra* on *H.intermedia* and *R.sanguineus* larvae appears to be the first since there are no other published reports.

The study also showed that there was high mortality of larvae on the 6th and 7th month plants and less on 3rd and 10th month plants. This could be possibly due to more of secretions and sticky branches of the 6th and 7th month plants and less secretion on younger (3rd month) and older plants (10 months) ,as had been reported by Sutherst et al., (1982). The mortality of various ages of ticks may be attributable either to the inhalation of vapour from the secretion or inability of larvae to escape from the sticky secretion. It may also be due to the contact toxicity of chemical substances present in the secretion. The varied

percentage mortality of larvae of ticks in the present study may perhaps be due to the variation in enzyme and enzymatic activities of the ticks.

Maximum trapping and killing of larvae was observed by 6th and 7th month old plants, hence the acaricidal effect of these plants on nymphal stages of *B.microplus*, *H.intermedia* and *R. sanguineus* was investigated for the first time since no such published reports are available on nymphal mortality.

S.scabra appears to have a potential practical utility for the control of different species of ticks of domestic animals since it is an important forage legume suitable for cultivation in tropical and subtropical countries because of it's low water requirement, drought resistance, resistance to anthracnose disease and fire (Gardener, 1980), ability to grow on low phosphorus soils (Gillard, 1982). The legume can be cut every three to four months (Venugopal et al, 1983). Because of its dual activity of being a highly nutritious legume and acaricide, it is therefore recommended that this legume be cultivated around livestock farms, stables, kennels etc. when effective control of ticks is envisaged.

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Table 1: Percentage mortality of larvae of ixodid tick on *Stylosanthes scabra* plants

Period of exposure (hours)	Age of the plant (months)							
	3	4	5	6	7	8	9	10
B. microplus								
24	20.85	21.60	41.05	54.95	53.95	42.35	22.15	8.30
48	22.55	20.90	43.10	50.85	53.45	40.45	31.55	9.90
72	20.40	23.60	43.85	56.05	52.15	41.50	30.35	11.50
H. intermedia								
24	3.25	3.50	5.15	7.00	7.55	6.45	5.00	3.45
48	3.35	2.95	7.10	8.55	7.65	7.75	4.70	3.35
72	3.40	4.25	6.15	7.65	7.80	7.55	5.85	3.60
R. sanguineus								
24	7.70	8.45	19.55	30.30	31.45	25.30	16.90	6.80
48	8.25	9.95	21.45	34.90	37.05	26.90	18.10	8.65
72	8.10	10.80	21.10	32.40	33.40	27.60	17.55	11.90

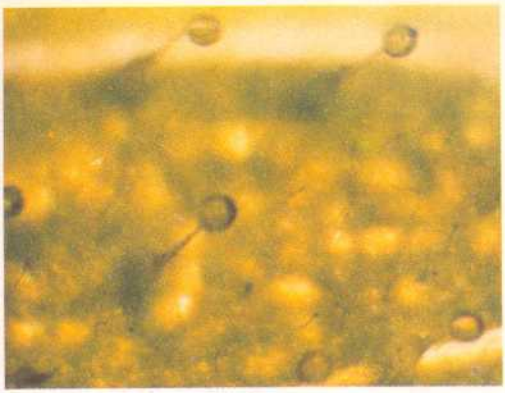

Fig.1 Droplet secretions on ***S. scabra*** *leaf*

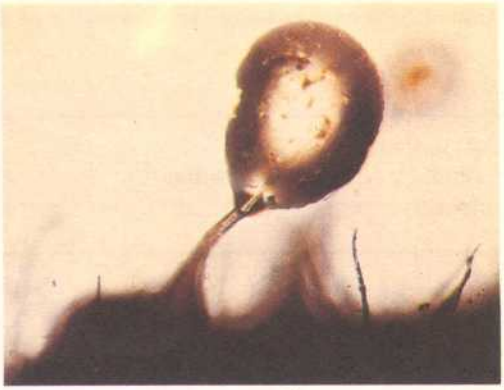

Fig.2 ***S. scabra*** *stem showing small droplet secretion*

Fig.3 Six months old ***S. scabra*** *plant*

Fig.4 ***S. scabra*** *of different ages*

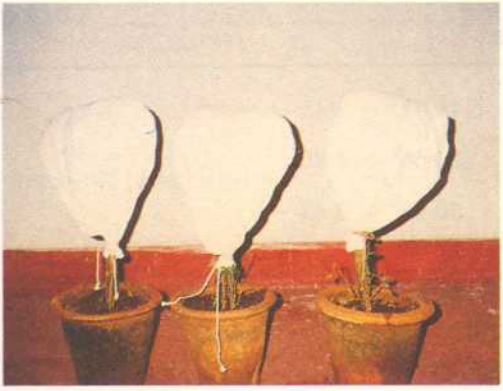

Fig.5 Tick stages on ***S. scabra*** *plants secured in cloth bags*

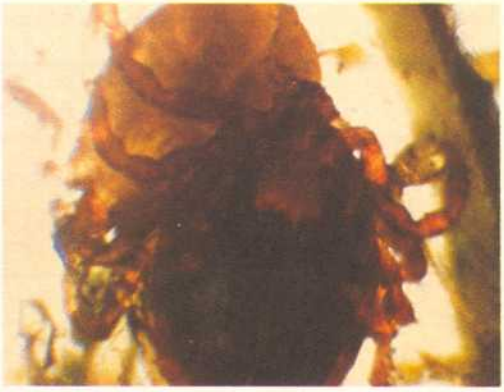

Fig.6 Immobilized and killed nymph of ***H. intermedia*** *on* ***S. scabra*** *leaf*

Table 2: Percentage mean larval mortality of different species of tick on *S.scabra* plants

Age of the plant (months)	*B. microplus* Mean ± S.E.	*H. intermedia* Mean ± S.E.	*R. sanguineus* Mean ± S.E.
3	$21.27^{a} \pm 0.51$	$3.33^{a} \pm 0.18$	$8.02^{a} \pm 0.32$
4	$22.03^{a} \pm 1.07$	$3.57^{a} \pm 0.26$	$9.73^{a} \pm 0.48$
5	$42.67^{b} \pm 1.13$	$6.13^{b} \pm 0.44$	$20.70^{b} \pm 0.71$
6	$53.95^{c} \pm 1.12$	$7.73^{c} \pm 0.30$	$32.53^{c} \pm 0.90$
7	$53.18^{c} \pm 1.80$	$7.67^{c} \pm 0.31$	$33.97^{c} \pm 1.42$
8	$41.43^{b} \pm 1.14$	$7.25^{c} \pm 0.30$	$26.60^{d} \pm 0.82$
9	$28.02^{b} \pm 2.36$	$5.18^{b} \pm 0.23$	$17.57^{e} \pm 0.49$
10	$9.90^{d} \pm 0.61$	$3.47^{a} \pm 0.20$	$9.12^{a} \pm 1.01$

Means bearing any one common superscript in column do not differ with each other.

Table 3 : Percentage mean mortality of larvae of ixodid ticks at different periods of exposure to *Stylosanthes scabra* plants.

Period of exposure (hours)	*H. intermedia* Mean ± S.E.	*R. sanguineus* Mean ± S.E.
24	$5.17^{a} \pm 0.43$	$18.31^{a} \pm 2.45$
48	$5.68^{b} \pm 0.57$	$20.66^{b} \pm 2.80$
72	$5.78^{b} \pm 0.45$	$20.36^{b} \pm 2.43$

Table 4 : Percentage mean mortality of larvae of ixodid ticks on *Stylosanthes scabra* plants

Age of the plant (months)	Mean ± S.E.
3	$10.87^{a} \pm 1.85$
4	$11.78^{a} \pm 1.90$
5	$23.17^{b} \pm 3.67$
6	$31.41^{c} \pm 4.61$
7	$31.61^{c} \pm 4.59$
8	$25.09^{d} \pm 3.43$
9	$16.91^{e} \pm 2.39$
10	$7.49^{f} \pm 0.79$

Means bearing any one common superscript in column do not differ with each other.

Table 5 : Percentage mortality of nymphs of ixodid ticks on *S. scabra* plants

Species of ticks	Period of exposure (hours)					
	6 month plant			7 month plant		
	24	48	72	24	48	72
B. microplus	42	50	51	38	50	44
H. intermedia	22	30	32	22	32	38
R. sanguineus	23	32	34	16	26	36

ANOVA Table 1 : Relationship between the age of the *S. scabra* periods of exposure on larval mortality

Source	df	MSS	F
B. microplus			
Period of exposure	2	12.62	1.17 ^{NS}
Age of the plant	7	1158.54	144.87 **
Period of exposure x age of the plant	14	10.59	0.98 ^{NS}
Error	24	10.76	
H. intermedia			
Period of exposure	2	1.71	4.81 *
Age of the plant	7	22.11	62.01 **
Period of exposure x age of the plant	14	0.57	1.61 ^{NS}
Error	24	0.36	
R. sanguineus			
Period of exposure	2	26.17	8.98 **
Age of the plant	7	659.49	226.16 **
Period of exposure x age of the plant	14	3.21	1.10 ^{NS}
Error	24	2.92	

* ($P \leqslant 0.05$)

** ($P \leqslant 0.01$)

NS Not significant

Clinical Report On The Treatment Of Canine Babesiosis

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Babesiosis is an important tick-borne haemoprotozoon disease of domestic as well as pet animals. Although babesiosis has been well studied in cattle there are only a few reports on babesiosis infection in dogs. The present paper describes the clinico-pathological and therapeutic studies on canine babesiosis.

History : The dogs were brought to the College Hospital with complaints of haemoglobinuria, vomition and inappetence.

Clinical observations: The clinical observations recorded in dogs naturally infected with Babesia and the therapeutic regimen evaluated is presented in Table 1, Clinico-therapeutic observations.

Table 1

Sr. No.	Breed	Age in year	B.S.examination / clinical pathology	Clinical Observations	Therapeutic regimen with outcome of disease.
1.	Pomeranian	1.6 Yrs.	*Babesia canis* DLC-N-92 % L-7% M.1%	Body tempe-ature 105.6° F concentrated urine, hepato-spleenomegally, severe tick infestation.	Inj. Berenil @ 10 mg/kg b.wt.
			Repeat B.S. after one week revealed B.canis.		Repeat Berenil
			Repeat B.S. after one month still revealed B. canis.		Inj. T.M. 150 mg I / V, However, patient succumbed.
2.	German Shepherd	8 Yrs.	*B.canis*	Dark brown coloured urine, inapp-etence 103.6° F	Inj. Berenil @ 10 mg / kg Inj. Livobex 0.5 ml /I/M daily Responded favourably.
3.	Alsatian	9 Yrs.	*B. gibsoni*	Inappetence shifting oedema of the dependent parts of hind legs. Hepato-megaly prosta-tomegaly Hae-moglobinuria.	Inj.Berenil @ 10 mg/kg. Inj. Livobex 0.5 ml I / M. Responded favourably to the treatment.

Sr. No.	Breed	Age in year	B.S.examination / clinical pathology	Clinical Observations	Therapeutic regimen with outcome of disease.
4.	Spitz Cross	6 Yrs.	*B.canis* DLC-N-91% L-6%, E-1% and M-2%	Persistent haemoglo-binuria body temp. 102°F pollar, vomit-ion moderate hepatomegaly, corneal opacity in one eye. Further developed icterus.	Inj. Berenil @10 mg / kg Inj. Livobex 0.5 mg daily Inj. Fructodex 20% succumbed.
5.	Cocker Spaniel	4-6 Yrs.	*B.canis*	Haemoglo-binuria body temp. 102°F persistent hae-moglobinuria.	Inj. Berenil @ 10 mg/kg b.wt Inj. T.M. 200 mg I/V for prolonged period. Responded favourably.

Discussion

It is evident from table 1 that out of 5 dogs studied only one was infected with *B. gibsoni.* However, Davies (1962) claimed it to be responsible for most cases of canine piroplasmosis in India. It is also observed that babesiosis is not an uncommon disease of dogs. The disease appear to affect young as well as adult dogs without discrimination of age, sex or breed. However, Abdullani et al. (1990) recorded *B. canis* infection in 70% of dogs less than 1 year of age.

The more common symptoms observed in the present study were variable appetite, weakness, anaemia, haemoglobinuria and are in accordance with the findings of Bansal and Gautam (1981). Only one dog manifested symptoms of shifting oedema of the dependent parts of the hind legs probably because of the obstruction to the lymphatic drainage by an enlarged prostate. Nair et al. (1979) noted lameness of hind leg in 3 cases. Only one animal developed corneal opacity. This appeared to be due to oedema of iris. The enlargement of spleen was palpable in one and of liver in 3 cases.

Haematological investigations revealed neutrophilia and both these cases died despite judicious treatment. Abdullani (1990) reported neutrophillic leucocytosis in 4 dogs and the death was attributed to hyperacute disease.

Atilola and Dipealu (1983) reported deaths in untreated cases of *B. canis* infection when degree of parasitoemia exceeded 5% compared to B. gibsoni exceed 40%.

All the dogs were treated with Berenil @ 10 mg / kg b.wt. Of the 4 cases treated for *B. canis* infection, one responded with single dose, one responded to combination of Berenil and Oxytetracycline (Teramycin) Awaz *et al.* (1984). Successfully treated *B. canis* infection by Berenil @ 10 mg/kg b.wt. He reported decline in parasitemia by next day of treatment. Berenil 3-6 mg / kg has also been found effective by Chaudhary (1978), Nair *et al.* (1979) and Bansal and Gautam (1981). In the present study B. gibsoni responded favourably to single dose of Berenil.

From the results of this study *B. canis* appears to be more pathogenic as compared to *B. gibsoni.*

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Squamous Cell Carcinoma At The Site Of Left Horn In Polled (Dehorned) Cow

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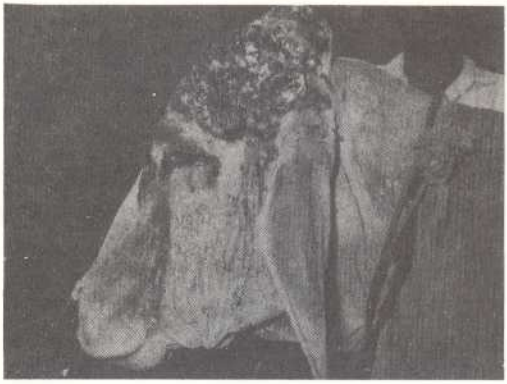
Horn Cancer, a squamous cell carcinoma is the commonest malignant disease reported in working bullocks, less frequently in cows and rarely in bulls (Kulkarni, 1953; Naik *et al*; 1969 and Naik 1993). The site of origin of horn cancer has been extensively investigated and found to be in the mucous membrane lining the horn core cavity which is an extension of frontal sinus (Heranjal et al; 1980; Naik *et al*; 1988). Etiological factors are of physical, chemical and biological (viral) nature. Among them trauma to the horn due to striking of yoke, tying the leathering rope at the base of horn or pairing and painting the horns were suspected causes. It has therefore been recommended to evolve polled cattle by calf hood dehorning to get rid of horn cancer in cattle. (Naik and Balkrishan, 1963). However growth of a squamous cell carcinoma at the site of horn in polled cow is quite interesting and has not been recorded so far.

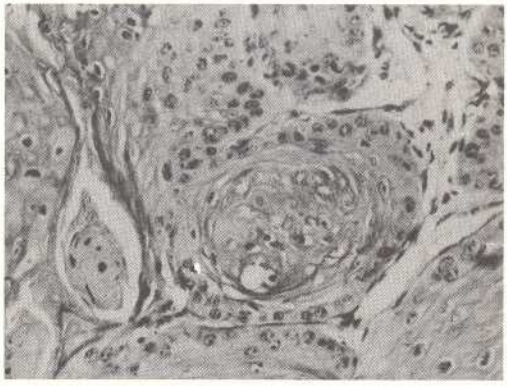
History :

A polled cow which was a cross between Kankrej and Gir breed about 8 years old, yielding 14 litres of milk per day had developed a massive cauli-flower like growth (Fig. 1) at the site of the left horn. The growth was amputated surgically under local anaesthesia and pieces of tissues were fixed in Bouin's fixative for histo-pathological studies. The tissues were processed and sections stained by H E technique. They revealed a typical squamous cell carcinoma with cells having large nuclei, scanty cytoplasm and a large number of mitotic divisions, besides epithelial pearls with keratinization in some area (Fig. 2).

The animal after operation was treated with a course of chemotherapy but the animal lost condition quite rapidly and succumbed to the disease. During this time there was a noticeable protruding growth at the site of right horn also (see Fig. 1). revealing the extension of disease all along the frontal sinus to the right horn site as well.



Polled cow with lesions of horn cancer



Histological section of the growth (Squamous cell carcinoma) showing affected epithelial pearl having large nuclei high mitotic figure

This case is reported to record a case of horn cancer in a dehorned or polled cow. This further proves that the horn cancer can occur even in polled animals from the mucous membrane lining the frontal sinus which extends into the horn core.

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Micrometry Of Preculture And Postculture Buffalo Follicular Oocytes

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For successful fertilization and embryonic development, conditions during *in vitro* maturation of oocyte are believed to play a key role. Morphologically oocyte maturation is the full expansion of the cumulus cells surrounding the oocytes. Additionally, extent of investment around the oocyte, stage of ooplasm and stage of nuclear development might be used to identify oocytes most likely to mature in vitro. The ability of oocytes to mature in vitro is shown to be related to their morphology (Mikhilenko *et al*, 1983). The objective of the present study was to determine the diameters of buffalo follicular oocytes before culture and after 24, 48 and 72 h postculture in different media.

Materials and Methods

To measure the diameter of oocyte, it is very necessary to remove the cumulus cells surrounding the oocytes. A method for dissociation of cumulus cells described by Kinis (1989) was followed. Oocytes were incubated for about two minutes in 3% sodium citrate solution. This helps in removal of cumulus of oocytes by repeated pipetting with fine glass pasteur pipette. The diameter of buffalo follicular oocytes preculture and postculture was measured at random with the help of micrometer. (Culling, 1969).

Results and Discussion

Micrometry of oocytes

The mean diameter of preculture and postculture oocytes at 0, 24,48 and 72 hours postculture in M-199, Ham's F-10 and DPBS were measured and are presented in table-1.

Table-1: Mean diameter of preculture and postculture oocytes in different media.

Period of Culture	Statistics	Medium			Total
		M-199	Ham's F-10	DPBS	
0 hours	No of oocytes (n)	15	8	7	30
	Average diameter (U)	147.85	149.32	149.48	148.44
	S.E.	1.66	2.43	1.32	0.44
24 hours	No. of oocytes (n)	13	7	7	27
	Average diameter (U)	147.09	148.82	146.84	147.58
	S.E.	1.40	1.87	2.19	0.62
48 hours	No. of oocytes (n)	8	7	7	22
	Average diameter (U)	149.32	147.50	150.80	149.21
	S.E.	1.45	1.21	1.38	0.95

Period of Culture	Statistics	Medium			Total
		M-199	Ham's F-10	DPBS	
72 hours	No. of oocytes (n) Average Diameter (U) S.E.	7 152.13 2.56	7 150.80 0.93	7 148.16 1.43	21 150.36 1.17
Total	No. of oocytes (n) Average diameter (U) S.E	43 149.10 1.11	29 149.11 0.68	28 148.49 0.83	100 148.90

It is seen from the above table that irrespective of media, average diameter of oocytes at 0, 24, 48 and 72 hours were 148.44 ± 0.44, 147.58 ± 0.62, 149.21 ± 0.95 and 190.36 ± 1.17 u, respectively. Similarly, irrespective of culture period in M-199, Ham's F-10 and DPBS were 149.10 ± 1.11, 149.11 ± 0.68 and 148.49 ± 0.83 u respectively.

Overall mean diameter of oocytes (including zone pellucida) respective of media and culture period was 148.90 u. Effect of diameter of oocytes in M-199, Ham's F-10 DPBS at 0, 24, 48 and 72 hours are presented in table-2.

lar oocytes as 194.34 and 139.24 u, respectively (including zone pellucida) showing non significant difference. Hafez (1979) observed the diameter of intrazonal vitellus of the egg to be in the range between 80-200 u in farm animals. Overall diameter of preculture and 43 hours post culture humun oocytes reported by Kiyoshi *et al.* (1985) is 116 ± 1.4 u Motlik and Fulka (1986) also reported diameter of follicular oocytes in cattle (excluding zone pellucida) as 110 u. It is seen from the investigation made by above workers that there is no significant difference between preculture

Table-2: Analysis of variance for diameters of oocytes between 0, 24, 48 and 72 hours in three different media.

Source	DF	SS	MSS	F
Between hours	3	0.22	0.0733333	0.02 ^{NS}
Between media	2	11.76	5.88	2.03 ^{NS}
Error	6	17.37	2.8933333	
Total	11	29.34		

NS = Non Significant

The mean diameters of oocytes between different media and culture periods remained almost same.

Present observations are in accordance with Jadhav (1988) who reported the preculture and postculture mean diameter of buffalo follicu- and postculure diameter of oocytes as also observed in the present study.

Summary

The measurements of diameters of buffalo follicular oocytes were done after removing cumulus cells, by incubating oocytes in 3% sodium citrate solution for two minutes and

thereafter repeated pipetting with fine glass pasteur pipette. Diameter of oocytes at 0,24,48 and 72 hours in three different media viz. M-199, Ham's F-10 and DPBS remained unchanged. Overall mean diameter irrespective of media and culture period was 148.90 u.

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