STUDIES ON BUFFALO CALF MORTALITY WITH PARTICULAR REFERENCE TO ASCARIASIS

BY

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B V Sc. & A.H

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CERTIFICATE

Mr. R. SRINIVASA RAO has satisfactorily prosecuted the course of research and that the thesis entitled **STUDIES ON BUFFALO CALF MORTALITY WITH PARTICULAR REFERENCE TO ASCARIASIS** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Dated: 21-6-1985

(Dr. A.A. GAFFAR)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled, **STUDIES ON BUFFALO CALF MORTALITY WITH PARTICULAR REFERENCE TO ASCARIASIS** submitted in partial fulfilment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr.R.SRINIVASA RAO** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. Published part has been fully acknowledged. All the assistance and help received during the course of the investigation have been duly acknowledged by him.

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(**R. SRINIVASA RAO**)

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DECLARATION

I, **R. SRINIVASA RAO** hereby declare that the thesis entitled, **STUDIES ON BUFFALO CALF MORTALITY WITH PARTICULAR REFERENCE TO ASCARIASIS** submitted to Andhra Pradesh Agricultural University for the Degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part there of has not been published earlier in any manner.

Date :

(R. SRINIVASA RAO)

LIST OF ABBREVIATIONS

AST	:	Aspartate amino transferase (Formerly SGOT)
SGOT	:	Serum glutamic oxaloacetic transaminase
ALT	:	Alanine amino transferase (Formerly SGPT)
SGPT	:	Serum glutamic pyruvic transaminase
ALP	:	Alkaline phosphatase
^{o}C	:	Celsius degree
B	:	Basophils
2,4 DNPH	:	2,4-Dinitrophenyl hydrazine
Cmm	:	Cubic millimeters
dl	:	Decilitre
DLC	:	Differential leucocyte count
E	:	Eosinophils
EDTA	:	Ethylene diamine tetra acetic acid
EPG	:	Eggs per gram
^{o}F	:	Fahrenheit degree
Fig.	:	Figure
g	:	gram
Hb	:	Haemoglobin
IU	:	International units
KA	:	King Armstrong Unit
Kg	:	Kilogram
l	:	Litres
L	:	Lymphocytes
lbs	:	Pounds
M	:	Monocytes

mg	:	Milligram
ml	:	Millilitre
mt	:	Minute
N	:	Normality
N	:	Neutrophils
nm	:	Nanometers
No	:	Number
OD	:	Optical Density
pH	:	Negative logaritham of Hydrogen Ion Concentration
P.C.V.	:	Packed cell volume
R.B.C.	:	Red Blood Corpuscles
SSPT	:	Sodium sulphite precipitation test
S/c	:	Sub cutaneously
SE	:	Standard error
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count
ug	:	Microgram
viz.	:	Namely
V/s	:	Versus
%	:	Per cent
$\pm$	:	Plus or Minus
<	:	Lesser (Less than)
>	:	Greater than

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ABSTRACT

Studies were carried out to analyse the causes of buffalo calf mortality with particular reference to ascariasis in relation to incidence, epizootiology, haematology, blood chemistry and therapeutic drug trial. In order to find out various causes of mortality, old records of the organised dairy farms were scrutinized for the last five years. In the present study 30.0 per cent of mortality was due to pneumonia alone followed by 18.0 per cent due to enteritis, 16.0 per cent due to septicaemia, 8.0 per cent due to aflatoxicosis, 6.0 per cent each due to cirrhosis of liver and nephritis and remaining 2.0 per cent due to coccidiosis.

To find out the incidence of ascariasis, faecal samples of 309 buffalo calves were screened. Of them, 93 were positive for ascarid ova giving an overall incidence of 30.09 per cent of infection.

In the present study, incidence in calves upto the age of 30 days was 42.5 per cent, between 30 to 60 days 33 per cent, between 60 to 90 days 27.27 per cent, whereas only 4.08 per cent incidence was reported in the calves aged above 90 days.

Similarly out of 309 calves examined, 120 were males and 189 were females. Of them, 35 males (29.16%) and 58 females (30.68%) were found positive for ascarid infection.

Toxocara vitulorum infection in relation to breed was also studied in the graded murrah buffalo calves, murrah calves and non descript calves which revealed an incidence of 30.16, 30.43 and 29.76 per cent, respectively. The Chi-square test revealed significant influence of age (χ^2 = 24.28 > 11.34) and insignificant influence of sex (χ^2 = 1.24 < 6.63) and breed (χ^2 = 0.0075 < 9.21) on ascariasis infection.

The clinical findings recorded in the infected calves were poor body coat, unthrifty condition, alopecia, loose faeces with foul smell, some times diarrhoea followed by constipation, dehydration, sunken eyeballs and pale conjunctiva. Body temperature was normal in many cases and slightly less than normal in few cases. In advanced cases, recumbency and coma was noticed with weak and feeble pulse.

The haematological studies of the affected calves showed low P.C.V., low haemoglobin content, erythrocytopenia and leucocytosis. The differential leucocyte count showed neutrophilia, eosinophilia and lymphopenia.

The biochemical estimations showed significantly low serum glucose and significant increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP). The serum immunoglobulins in the infected calves were less than 5 mg in 14 (77.77%) calves and between 5-15 mg in 4 (22.23%) calves.

The therapeutic trials were conducted using an old drug, Piperazine adipate (orally) and the latest parenteral drugs Ivermectin and Levamisole hydrochloride. Of all the 3 groups, the results with the levamisole hydrochloride were encouraging when compared with the other two drugs. The Ivermectin though effective was very costly whereas the Piperazine though cheaper could cause complications due to faulty drenching.

INTRODUCTION

CHAPTER I

INTRODUCTION

The calf morbidity and mortality are the major hazards of the dairy enterprise all over the world which cause loss of the future livestock of dairy industry. In India and South-east Asia, ascariasis, a disease of young animals in general and of buffalo calves in particular is an important cause of mortality (Blood and Radostits, 1989) resulting in the attendent loss in production and a disturbance in the breeding programme.

The Toxocara vitulorum infection in calves is acquired by transfer of larvae through the colostrum feeding and thus the infection is seen early in the calfhood, mature worms being present by 10 days and eggs are passed in faeces within 2 to 3 weeks of birth. During this period the migration of larvae either through liver or through lung may result in liver enlargement or in lung oedema. The affected calf will have stunted growth, poor coat, alopecia, diarrhoea (steatorrhoea) and anaemia.

The review of literature revealed very scanty information on the subject of ascariasis in buffalo calves. Therefore, considering the pathogenic effect of this parasite on the health of the calf and consequent economic losses to the farmers, it was considered desirable to assess the incidence of this parasite in

different age groups of buffalo calves so that a broad based control programme might be launched.

Since, the migrating larvae in the infected calves cause anaemia, liver fibrosis and lung oedema, it was proposed to study the haematological changes and the liver enzyme profile. Since the immunodeficient calves may be more prone for diarrhoea, an attempt was also made to study the immunoglobulin status of calves.

Thus the present study was undertaken with the following objectives.

1. To study the causes of calf mortality

2. To study the incidence of ascariasis in buffalo calves in relation to age, sex, breed and serum immunoglobulins

3. To study haematological changes in respect of TEC, TLC, DLC, Hb and PCV.

4. Biochemical profiles in respect of serum glucose, AST, ALT and ALP.

5. To evaluate the comparative efficacy of anthelmintic drugs.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 CAUSES OF MORTALITY

Tripathi (1967) observed that ascariasis in calves is of quite common occurence in India and is often responsible for high mortality among them especially in buffalo calves.

Singh and Singh (1972) reported bronchopneumonia, interstitial Pneumonia with cuffing and acute or chronic enteritis (Catarrhal or Mucohaemorrhagic) in 34 buffalo calves out of 65 on post mortem examination.

Oxender et al. (1973) conducted a survey of 77 michigan dairy herds and reported a calf mortality of 17.7 per cent between the birth and 60 days of age. Still birth accounted for 6.4 per cent, death between birth and 14 days of age 8.5 per cent, and death between 2 weeks and 2 months of age 2.8 per cent. Mortality ranged from 16.1 per cent for the herds less than 50 cows to 34.9 per cent for herds of more than 200 cows. They also reported diarrhoea in 70 per cent of the herds and pneumonia in 14 per cent of the herds as the major health problems in young calves.

Singh and Singh (1973) studied the influence of season, sex and birth weight on the calf mortality and

reported an higher incidence of mortality in calves born during winter than those born during the summer or rainy season. They observed a higher mortality rate in male than in female calves. They also reported that the calves weighing 7 kg less than the normal birth weight (20 kg) died with in the first month of birth.

Verma and Kalra (1975) conducted post mortem examination of buffalo calves and reported the presence of E. coli, Salmonella, Klebsiella, Coryne bacterium, Pasteurella, Streptococci, Staphylococci, Citrobacter, Proteus, Bacterium alkaligenes, Neoascaris vitulorum, Eimeria and Moneizia and stated that Pneumonia, enteritis, pneumoenteritis, pyosepticaemia, hepatitis, nephritis, as causes of death in calves.

A study conducted by Bali et al. (1979) revealed 4 to 60 per cent of calf mortality between 1969 and 1978, where the mortality was slightly higher in females than in males and was higher in weaned (43%) than in unweaned (22%) calves. They concluded the causes of mortality as enteritis (27%), pneumonia (21%), pneumoenteritis (15%), coccidiosis (5%) and foot and mouth disease (4%).

Greene (1979) indicated enteritis or septicaemia as the cause of neonatal mortality (occuring between birth and three weeks of age) in 81 (70%) out of 116 neonatal deaths.

In an epidemiological survey of Neonatal calf mortality in Haryana Bos taurus cross breds, conducted by Kaushik et al. (1980), it was found that the overall mortality was 18.2 per cent (133 of 728) where the incidence was higher in males (25.5%) than in females (13.9%) and they attributed the causes of mortality to bacterial diseases (59.42% of mortality in males than 50% in females) and organic diseases such as pneumonia, enteritis, jaundice, hepatitis, pleuritis and nephritis (31.88 and 34.37%).

Rao and Nagarcenkar (1980) studied the calf mortality in cross bred dairy cattle between birth and six months of age and reported that the percentage of mortality was largely due to respiratory diseases in the birth to one month age group while diseases affecting the digestive system resulted in more deaths in the three to six months age group. Digestive and respiratory diseases caused mortality rate of 34.3 per cent and 31.4 per cent respectively in calves that died up to six months of age.

Srivastava and Sharma (1980) investigated the causes of calf mortality in Zebu and buffalo calves during their first year of life. The most common causes of deaths observed by them in Zebu and buffalo calves respectively were Gastroenteritis, including colibacillosis and colisepticaemia, 47 per cent and 43 per cent, pneumonia

and lungs congestion 17.8 per cent and 18 per cent haemorrhagic septicaemia, 2.8 per cent and 1.3 per cent and urinary tract disease 3.4 per cent and 2.3 per cent and they reported a little difference in the mortality rates between male and female calves and between the different seasons.

Williams et al. (1980) observed a greater mortality (39%) in calves of 11-17 days of age where the commonest diseases associated with mortality were coliform gastroenteritis, salmonellosis, viral pneumonia with secondary bacterial infection and Escherichia coli septicaemia.

Khera (1981) observed 55.79 and 51.72 per cent of mortality in cattle and buffalo calves respectively during their first month of life where the largest proportion of deaths were due to digestive system diseases including Escherichia infection (32.21 and 49.66% respectively) followed by respiratory diseases (22.74 and 20.46% respectively), debility and anaemia (6.95 and 6.90% respectively) and septicaemia, pyrexia and toxaemia (4.42 and 5.75%).

Blood et al. (1983) indicated anaemia as a cause of death in cow calves suffering from ascariasis.

Ulaganathan (1984) stated that the two major health disorders affecting calves are enteric and

respiratory where the enteric disorders in the form of neonatal diarrhoea primarily affecting calves in the first two weeks of life and the respiratory disorders are more apparent in older calves of 6 to 8 weeks age due to infectious bovine rhino tracheitis.

Lakshmi Prasad (1991) recorded higher mortality rates in buffalo calves (0-3 months age group) during winter months and reported that gastrointestinal disorders and respiratory disorders were the main causes of mortality.

Patil et al. (1991) reported an overall mortality rate of 15.13 per cent in cow calves and 32.17 per cent in buffalo calves where the mortality was highest in the month after birth for buffalo calves (33%) and from first to third month in the cow calves (26.1%). They also reported a higher mortality rate in females (21.8 and 37.3%) than in males (8.3 and 27.5%) in cow calves and buffalo calves respectively. They attributed enteritis and pneumonia to more than half of the deaths occuring in each species.

While studying the factors affecting calf mortality in Kankrej, Siddiquee et al. (1991) noticed that the mortality was higher among male calves than in female calves and was lower in summer than in winter or the monsoon and they reported causes of mortality (as

percentage of total mortality) as tympany (30.4%), gastroentritis (21.5%), pneumonia (13.9%), urinary disorders (17.7%) and congenital abnormalities (1.3%).

2.2 ASCARIASIS

2.2.1 Incidence

According to a survey conducted by Vaidyanathan (1949) positive findings of eggs of Ascaris vitulorum were greatest-both in Bos bubalis and Bos taurus from 31-40 days.

Nagappapai (1955) in his observations reported an incidence of 37.9 per cent of Neoascaris vitulorum in buffalo calves and 6.3 per cent in cow calves.

Patnaik and Pande (1963) in their survey in one month old buffalo calves and a histological study of the lesions have shown that the species in order of frequency and relative pathogenicity are the intestinal nematodes, Neoascaris vitulorum, Strongyloides papillosus, Paracooperia nodulosa, Cooperia laterouniformis and Bunostomum phlebotomum and Setaria labiatopapilosa from the peritoneal cavity.

The most serious disease in buffaloes was Neoascaris vitulorum infection of buffalo calves in Malaysia (Anon, 1972).

Chauhan et al. (1974) detected the presence of larvae of Neoascaris vitulorum and Strongyloides papillosus in the milk of buffaloes from 7-8 days postpartum.

Presence of infective larvae of Toxocara vitulorum in milk and colostrum was recorded by Thienpont et al. (1977).

Bhatnagar et al. (1980) recorded an incidence of 27.5 per cent of Neoascaris vitulorum in buffalo calves less than 6 months old, comprising 42 per cent of those 16 to 30 day old, 34 per cent 1-3 months old and 4 per cent of those 3 to 6 months old whereas 20 calves less than 15 days old and 162 between 6 months and 10 years old were not infected. They also reported seasonal occurence of infection as 32, 25, 36 and 18 per cent in rainy, post monsoon, winter and summer season respectively.

Baruah et al. (1981) in his field trials reported that 52.2 per cent of 1151 buffalo calves in Hissar (India) were found to have Neoascaris vitulorum eggs in their faeces where the eggs appeared from 11 days after birth and the prevalence of infection peaked at 77.1 per cent in 46-53 day old calves and was lower (35.7%) in 81 to 90 day old calves.

Banerjee et al. (1983) revealed the presence of Neoascaris vitulorum larvae in the colostrum/milk samples

of 21 recently calved buffaloes out of 274 examined which accounted for 7.66 per cent incidence.

Sukhapesna (1983) studied the incidence of parasites in swamp buffalo calves in Thailand and reported a 100 per cent Neoascaris vitulorum infection in them.

Christopher (1984) reported that ascariasis infection in calves is one of the most common diseases and the incidence was more in buffalo calves compared to cow calves.

Gupta et al. (1985) studied the epidemiology of Helminth infection in calves of Haryana state, and found that 15.2 per cent out of 1626 female calves and 29.1 per cent out of 2411 buffalo calves were infected with Neoascaris vitulorum and they opined that the prevalence of infection showed a negative correlation with age of the host.

Hariprasad (1985) conducted faecal examination of buffalo and cattle calves of different age groups and reported that 15.12 per cent of calves were positive for ova of Toxocara vitulorum where the incidence was higher in buffalo calves (18.22%) when compared to white cattle calves (4.8%) and they also noticed a higher rate of incidence in the calves of 16-30 days age group.

Gupta (1986) observed an incidence of 81.8 per cent of Neoascaris vitulorum in buffalo calves under 3

months of age and also detected the infection in buffalo calves as young as 6 days but the majority were detected on or after 19 days. In his study no infection of calves with Neoascaris vitulorum was detected beyond 90 days of age and reported that the maximum patency of N. vitulorum is 60 days.

Swain et al. (1987) examined faecal samples from 170 buffalo calves below 6 months of age, which revealed 67.65 per cent of N. vitulorum infection where the infection rate was severe (93.75%) in 1-2 months old calves and the incidence declined sharply to 31.25 per cent in calves above three months of age.

Barbosa and Correa (1989) discussed the incidence and transmission of Toxocara vitulorum infection in buffalo calves and reported that the 58 per cent calves were positive in the first week of life. 87.5 per cent in the second week, 96 per cent in the third and 100 per cent in the fourth week and he opined that the calves have been infected through the placenta and few through the milk.

Kenyon et al. (1989) while studying health in draught animals observed Toxocara vitulorum infection in 7 out of 14 buffalo calves and stated that the infection is associated with diarrhoea in Indonesia.

Muangyai (1989) reported the main parasitic diseases affecting buffaloes as toxocariasis, strongyloidiasis,

schistosomiasis, fascioliasis, paramphistomiasis, moneiziasis, mange, pediculosis, coccidiosis and surra.

Roberts (1989) demonstrated the transmission of Toxocara vitulorum infection from the cow to the calf via milk during the first 2 days after birth which decreased to 53 per cent by 6 days, 10 per cent by 8-9 days and 2 per cent from day 10 onwards.

Gupta and Chhabra (1990) conducted a survey on intestinal parasitic infections in young buffalo calves and showed that Toxocara vitulorum was the predominant infection (41.2%) followed by Eimeria spp (27.1%) Strongyloides spp (25.9%), strongyles 3.8 per cent and Moneizia spp (2.6%). They also examined 46 calves over 6 months of age and found that Strongyloides papillosus was the main parasite and no single calf is found to be infected with Toxocara vitulorum infection.

Gupta and Paul (1990) reared fifty, 10 to 15 day old calves under usual husbandary practices in eastern Haryana and found that the buffalo calves were invariably infected with Toxocara vitulorum, Strongyloides papillosus, Strongyles, (mostly Haemonchus contortus and Trichostrongylus) and coccidia and they also reported that incidence and severity of Toxocara vitulorum was highest in calves upto 2 months of age.

Lau (1990) reported ascariasis as the commonest disease under one of the aged calves in Amazonian Brazil.

Agyei (1991) detected Toxocara vitulorum ova in the faeces of calves as early as 2 days after birth which reached to a maximum patency after 2 months and the fall in Toxocara ova output coincided with a raise in that of Haemonchus spp, Trichostrongylus spp and Oesophagastomum spp.

Chartier et al. (1991) on their anthelmintic trials found Toxocara vitulorum as the dominant infection in calves between the age of 1 to 3 months and the gastro intestinal strongyles from 3 to 18 months.

Pradhan et al. (1991) studied the age wise distribution of parasites in suckling calves which revealed that Neoascaris (Toxocara) vitulorum, Trichuris and Strongyloides were the commonest parasites in the age group of 0-60 days.

Sahoo et al. (1991) studied the incidence of helminthiasis in buffalo calves and reported that the prevalence of ascariasis was greatest in calves of 2-3 months old. They have also examined 318 buffalo calves under 6 months of age out of which 129 were infected with Toxocara vitulorum infection.

2.2.2 CLINICAL FINDINGS

Nagappapai (1955) reported that calves suffering from ascariasis had shown the symptoms of unhealthy coat, scraggy coat, alopecia over the ears, head and neck, conjunctivitis, aphtha and also observed that urine was high coloured and dung in early cases showed greyish colour indicating disorder of the liver. He also observed diarrhoea and normal or slightly subnormal body temperature. Pyrexia and severe colic were also observed.

Christopher (1984) discussed the ascariasis infection in calves and reported the clinical findings as loss of appetite, constipation in early stages, diarrhoea and dysentery in later stages with severe abdominal pain. He also recorded the pathological lesions as cirrhosis, pneumonia and some times obstruction of intestinal passage leading to colic and death.

The observations made by Pandey and Mishra (1985) in N. vitulorum affected calves were weakness, pale conjunctiva, prominent ribs, unthriftiness, constipation and slight elevation in the heart, pulse, and respiration rates.

Abeydeera and Roberts (1991) studied the liver tissue responses of buffaloes to experimental infection with Toxocara vitulorum and noted the lesions varying from

cellular infiltrations in early stages to eosinophilic granulomata with degenerating larvae in later stages of infection.

2.3 FAECAL EXAMINATION AND EPG

Akhtar et al. (1982) treated buffalo calves (20-60 days old) with natural N. vitulorum infection with piperazine (88 mg/kg) and recorded the percentage reductions in EPG on 3rd day as 82±15, 90.2±3 and 91.3±2.3 per cent and on 7th day as 88±16, 97±3 and 98±2 per cent in light, moderate and heavy infections respectively.

Hariprasad (1985) examined faecal samples of buffalo calves infected with N. vitulorum and recorded epg count of 1,94.00.

Lau and Singh (1985) made a study on N. vitulorum infection in naturally infected buffalo calves and recorded the faecal egg counts in infected animals as 3000 eggs per gram when compared to the healthy control calves (10 eggs per gram).

2.4 HAEMATOLOGICAL STUDIES

Pautrizel et al. (1949) reported the presence of high quantities of histamine in ascarid worms which may be a contributory factor in the genesis of histamine release.

In an experimental study on ascariasis, Panebianco (1955) recorded mild eosinophilia which increased on reinfection and he could not find any change in R.B.C., lymphocyte and monocyte counts.

El-Abdin et al. (1975) studied the haemogram in buffalo calves infected with Neoascaris vitulorum and reported a significant decrease in RBC numbers and marked neutrophilia, eosinophilia, basophilia and lymphopenia when compared to the healthy control calves.

Baruah et al. (1979) in their haematological studies of calves suffering from N. vitulorum reported an increased haematocrit, erythrocyte count, and haemoglobin content than in the control calves.

Yadav (1984) made use of Nilverm (Tetramisole) at the rate of 10 mg/kg body weight orally in buffalo calves (20 days to 6 months old), naturally infected with T. vitulorum and after 7 days of treatment he noticed an increase in the Hb. concentration from 9.5 to 11.5 g/100 ml of blood as compared to untreated control where the Hb concentration decreased from 10.4 to 7.8 g/100 ml of blood.

Hariprasad (1985) reported that haematological studies in ascarid infected calves revealed a low

haemoglobin, P.C.V. and erythrocytic count, but with slight higher MCH value in the infected calves.

Lau and Singh (1985) studied haematological changes in suckling buffalo calves naturally infected with N. vitulorum and reported a significantly lower numbers of erythrocytes, decreased Hb content and cell volume, leucocytosis, lymphocytosis and eosinophilia whereas the MCH, MCV, monocyte, basophil and neutrophil counts remained unchanged.

Pandey and Mishra (1985) in their study on Neoascaris vitulorum infection in cow calves reported a low PCV (20-24%), Haemoglobin (6.8-7.4 g/dl) and TEC (4.01-4.52 millions/ml) in all ailing calves and they also reported that there was no significant difference in the pre and post treatment values of MCH, MCHC, TLC, DLC, reticulocyte count and osmotic fragility of erytrocytes of affected calves.

2.5 BIOCHEMICAL STUDIES

El.Abdin et al. (1975) made biochemical studies on buffalo calves infected with Neoascaris vitulorum and showed a significant increase in the levels of serum alkaline phosphatase, Serum glutamic pyruvic transaminase and serum oxaloacetic transaminase when compared to the healthy control calves.

Baruah et al. (1979) observed a low serum glucose content in N. vitulorum infected calves.

Davidson et al. (1981) made a study on serum IgG values and found that calves with lower IgG levels suffered with severe respiratory disease and had high morbidity and mortality than those with high IgG.

Pyne and Maitra (1982) made biochemical studies in blood of male buffalo calves aged between 8 and 14 months of age and observed the mean concentrations of aspartate aminotransferase (73.18±4.6 Karmen Units/ml), alanine aminotransferase (18.35±2.3 Karmen units/ml) in the serum.

Hariprasad (1985) made biochemical studies and observed a low blood glucose content in the buffalo calves infected with Neoascaris vitulorum.

Pandey and Mishra (1985) reported no change in the blood glucose values in N. vitulorum infected calves which was with in the normal range as compared with the healthy control.

2.6 THERAPY

Lee (1955) reported that piperazine adipate at a dose rate of 0.1 or 0.2 g per lb body weight is effective against immature and mature Ascaris vitulorum infection in zebu calves and the eggs disappeared from the faeces

within 5 days of treatment. He also stated that the drug has no effect on developing egg.

Tewari et al. (1966) indicated piperazine adipate (200 mg/kg body wt.) as an effective drug for treating ascariasis in calves.

Tripathi (1967) in his clinical trials with piperazine citrate (Antoban) indicated that the drug given in a single dose of 0.5 g per 10 lb body weight was 86.36 per cent effective against ascariasis in calves.

Thienpont et al. (1977) recommended levamisole at a dosage of 5 mg/kg body weight against Toxocara vitulorum infection in calves.

Manuel and Rugay (1979) reported 100 per cent efficacy of Levamisole against Neoascaris vitulorum in naturally infected cattle, where it was administered as an injectable solution (6.12 mg/kg) or as a drench (8 mg/kg).

While comparing the efficacy of Tetramisole (ICI) and Uvilon (Bayer) against Toxocara (Neoascaris) vitulorum infection in buffalo calves, Hossain et al. (1980) stated that seven days after treatment with tetramisole at 7.5 mg/kg body weight or piperazine at 220 mg/kg body weight the Ascaris vitulorum egg counts in the faeces of 12 buffalo calves were reduced to 99 and 96 per cent respectively.

Akhtar et al. (1982) suggested that santonin at the rate of 15 mg/kg has an efficacy similar to piperazine at 88 mg/kg for the treatment of ascariasis in buffalo calves.

In a field trial, Bhasker Singh (1982) used tetramisole (Nilverm) orally at the rate of 15 mg/kg body weight against ascariasis in buffalo calves and observed a drop in faecal egg counts (EPG 400) on 15th day of treatment as compared to the egg counts on the day of treatment (EPG 1200 to 78000).

Sukhapesna (1983) reported that tetramisole at 7.5 mg/kg b.wt. and piperazine citrate at 220 mg/kg body weight were effective against Neoascaris vitulorum infection in buffalo calves.

Yadav (1984) administered Nilverm (tetramisole) at the rate of 10 mg/kg body weight, orally to 8 buffalo calves (20 days to 6 months old) with natural Toxocara vitulorum infection and observed a reduction in EPG (99.5%) after 7th day of treatment and clinical recovery after 12th day of treatment.

Prasad (1985) treated ten buffalo calves and eight Bos taurus calves (1.5 to 4 months of age) with Neoascaris vitulorum infections (1350-1550 EPG faeces) using piperazine hexahydrate (220 mg/kg body wt.), tetramisole (15 mg/kg body weight) Fenbendazole (10 mg/kg body

weight) and 17 days later he found that the faecal egg counts had been reduced by 96, 98 and 100 per cent respectively.

Gupta (1986) opined that the Neoascaris vitulorum infection in calves could be controlled by treating them 2 to 3 days after birth and thereafter fortnightly until the calves were 2 to 3 months old.

Gill et al. (1989) observed Ivermectin (200 µg/kg body weight) as an effective drug in treating infections of Neoascaris vitulorum, Trichostrongylidae, Oesophagostomum and Bunostomum which were eliminated within one week of treatment.

Roberts (1989) studied the efficacy of levamisole (7.5 mg/kg body wt.) and piperazine (200 mg/kg body wt.) on immature and mature stages of Toxocara vitulorum infection in buffalo calves and recorded that the levamisole and piperazine were effective at 97 and 42 per cent respectively against immature worms and 83 per cent and 57 per cent respectively against mature worms. He also recommended the treatment of 10-16 day old calves with an anthelminitic which is effective against immature parasites.

Shastri (1989) studied the therapeutic efficacy of Ivermectin (200 ug/kg body wt.) for treatment of

Toxocara vitulorum infection in 10 buffalo calves (2 months old) and found a total reduction of EPG in 5 calves whereas the other 5 calves had egg counts reduced by 90.83 to 97.26 per cent.

Uysal (1989) noticed a reduction (99.50%) of faecal egg counts in Toxocara (Neoascaris vitulorum) infected calves (20-60 days old) after treating with ivermectin (0.2 mg/kg body weight) subcutaneously.

While assessing the therapeutic efficacy of Febantel, piperazine adipate and tetramisole, Gupta and Chhabra (1990) stated that Febantel (7.5 mg/kg body weight) was highly effective against T. vitulorum, S. papillosus and strongyle infections. Piperazine adipate 200 mg/kg body weight was effective selectively against Toxocara vitulorum infection whereas the Tetramisole 40 mg/kg showed a poor efficacy against T.vitulorum infection.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 SELECTION OF ANIMALS

In order to find out the various causes of calf mortality in buffalo calves the old records of the organised dairy farms were scrutinised for the last five years. The information in relation to age, sex and breed of the calf was also collected in a seperate proforma designed for the purpose. The calves with natural infection of ascariasis were taken up for the present study to study the clinical signs, haematobiochemical parameters and the therapeutic drug trials.

3.2 CLINICAL FINDINGS

The clinical signs were recorded in the calves naturally affected with ascariasis.

3.3 COLLECTION OF CLINICAL MATERIALS

Faecal samples were examined to know the incidence of ascariasis in buffalo calves and the samples were also collected for the detailed investigation.

3.3.1 Preparation of glassware

All glassware (Borosil make) were soaked overnight in detergent water and then washed under running tap water. Later they were rinsed with single glass

distilled water, air dried, paper packed and sterilised in hot air oven at 160°C for one and half hour.

3.3.2 Faecal examination and EPG

Faecal samples, about 10 grams each, were collected in clean dry test tubes from individual animals and examined microscopically, to find out the ascarid ova. Faecal egg count (EPG) was carried out (Stoll, 1923) to find out the intensity of ascarid infection. For this purpose, three grams of faeces was transferred to a 45 ml test tube. The test tube was then filled to 45 ml mark by adding distilled water. The tube was then closed with a rubber stopper and shaken to give a homogenous suspension of the faecal material. After shaking sufficiently the mixture was sieved into another test tube and 0.15 ml of the well mixed suspension was drawn off with a graduated pippette and placed on a clean glass slide covered with coverslip and examined under the low power (10x) microscope. The total number of eggs was counted and the number multiplied by 100 to give number of eggs per gram of faeces. Average of such three counts was taken in each case. EPG was also done on the treated calves to know the therapeutic efficacy of the drugs.

3.3.3 Haematological studies

3.3.3.1 Collection of blood and serum samples

The blood samples about 5 ml each were collected from jugular vein into sterile vials containing an anticoagulant, Ethylene Diamine tetraacetic acid (EDTA), a disodium salt, added at the rate of 1 mg per ml of blood for the estimation of total erythrocyte count (TEC), total leucocyte count, differential leucocyte count (DLC), Haemoglobin (Hb) and packed cell volume (PCV).

For collection of serum, the blood samples were collected into clean and sterile test tubes without adding any anticoagulant and allowed to clot completely in slanting position, undisturbed for half an hour and the serum was seperated for the estimation of serum glucose, Aspartate aminotransferase (AST), Alanine amino transferase (ALT), Alkaline Phosphatase (ALP) and Serum immunoglobulins.

The blood parameters were studied before starting the treatment (Day-0) and during (Day-14) and after the treatment (Day-21).

3.3.3.2 Total Erythrocyte Count

Total erythrocyte count was carried out by employing a Haemocytometer slide and erythrocyte diluting fluid and the count was expressed as millions/µl (Schalm et al., 1975).

3.3.3.3 Total Leucocyte Count

Total leucocyte count was done by employing a haemocytometer slide and leucocyte diluting fluid and the count was expressed as thousands/µl (Schalm et al., 1975).

3.3.3.4 Differential Leucocyte Count

Thin blood smears were prepared on clean glass slides and stained with Leishman's stain. The differential count was expressed as percentage of cells (Schalm et al., 1975).

3.3.3.5 Packed Cell Volume (PCV)

PCV was estimated by using microhaematocrit method as described by Schalm et al. (1975).

3.3.3.6 Haemoglobin concentration

For estimation of haemoglobin, Sahli's Acid haematin method, according to the procedure of Schalm et al. (1975) was followed and the values were expressed as g/dl.

3.3.4 Biochemical studies

3.3.4.1 Serum glucose

The serum glucose was estimated by using a kit supplied by Span Diagnostics Private Limited,Udhna (Surat), India and photoelectric calorimeter and the

values were expressed as mg/dl. The procedure is described in Appendix A.

3.3.4.2 Aspartate Amino Transferase (AST)

The AST activity was determined by method of Reitman and Frankel (1957), by employing a kit supplied by Span Diagnostics Private Limited, Udhna (Surat), India and photoelectric colorimeter, and the results were expressed in units/ml. The procedure is described in Appendix-B.

3.3.4.3 Alanine Amino Transferase (ALT)

The ALT activity was determined by method of Reitman and Frankel (1957) by using a kit supplied by Span Diagnostics Private Limited, Udhna (Surat), India and Photoelectric colorimeter and the values were expressed as units/ml. The procedure is described in Appendix-C.

3.3.4.4 Alkaline Phosphatase (ALP)

The ALP activity was determined by kind and kings method, by using a kit suipplied by Span Diagnostics Private Limited, Udhna (Surat), India and Photoelectric colorimeter and the values were expressed as KA units.

3.3.4.5 Serum Immunoglobulins

The serum immunoglobulins were estimated by using sodium sulphite precipitation test (Pfieffer and Mc Guire, 1977).

3.4 THERAPY

The calves found positive for ascarid ova were randomly divided into three groups, each containing 6 animals and were given treatment as mentioned below :

Group I : Comprised of healthy calves which served as control group.

Group II : Animals were administered piperazine adipate orally at the dose rate of 200 mg per kg body weight as a single dose.

Group III : Calves were given a single dose of Ivermectin at the rate of one ml per 50 kg body weight diluted with equal quantity of distilled water and given sub cutaneously in the neck region.

Group IV : Animals were injected levamisole at the dose rate of 7.5 mg per kg body weight as a single dose sub cutaneously in the neck region.

The efficacy of these drugs was assessed based on clearance of the parasitic ova from faeces and EPG and improvement in the clinical signs along with the blood parameters. The calves affected severely and showing the clinical signs of dehydration were also given supportive therapy of Ringer's lactate and liver extract parenterally.

3.5 STATISTICAL ANALYSIS

The data was processed as per the procedure of Snedecor and Cochran (1967). The analysis of variance was done to find the significance of difference.

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RESULTS

CHAPTER IV

RESULTS

To list out the causes of mortality in buffalo calves for the last five years, the mortality registers of the organised dairy farms were scrutinised.

4.1 CAUSES OF MORTALITY

The retrospective studies of causes of mortality revealed that 30 per cent of the mortality was due to pneumonia alone followed by 18 per cent due to enteritis, 16 per cent due to septicaemia, 8 per cent due to aflatoxicosis, 6 per cent each due to cirrhosis of liver, nephritis and 2 per cent due to coccidiosis. For remaining 14 per cent of mortality no cause could be attributed. The details of causes of mortality is represented in Table 1 and Fig.1.

4.2 ASCARIASIS

In the present study the buffalo calves affected with ascariasis were studied in detail.

4.2.1 Incidence

In the present study faecal samples from 309 buffalo calves were examined and of them 93 were positive for ascarid ova, giving an overall incidence of 30.09 per cent.

Table 1: Causes of mortality in buffalo calves

S.No.	Cause	No.of calves died	Percentage of mortality
1.	Pneumonia	15	30.00
2.	Enteritis	9	18.00
3.	Septicaemia	8	16.00
4.	Aflatoxicosis	4	8.00
5.	Cirrhosis of liver	3	6.00
6.	Nephritis	3	6.00
7.	Coccidiosis	1	2.00
8.	Miscellaneous causes	7	14.00
	Total	50	100.00

Table 2: Incidence of Ascariasis in relation to age

S.No.	Age group	No. of calves		Incidence (%)
		Examined	Positive	
1.	Upto 30 days	94	40	42.55
2.	30 - 60 days	100	33	33.00
3.	60 - 90 days	66	18	27.27
4.	Above 90 days	49	2	4.08
	Total	309	93	30.09

$P<0.01$; $X^2 = 24.28 > 11.34$.

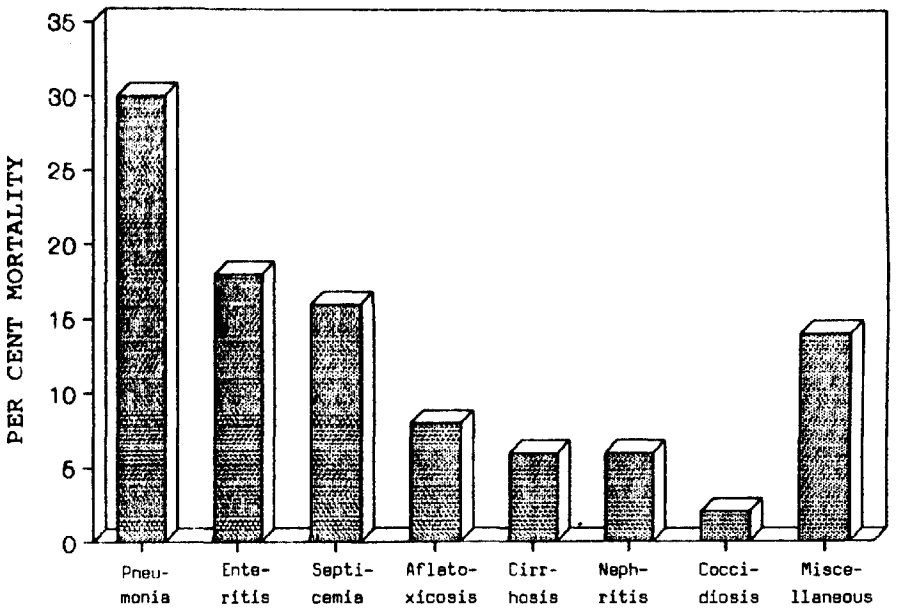

Fig.1: CAUSES OF MORTALITY IN BUFFALO CALVES

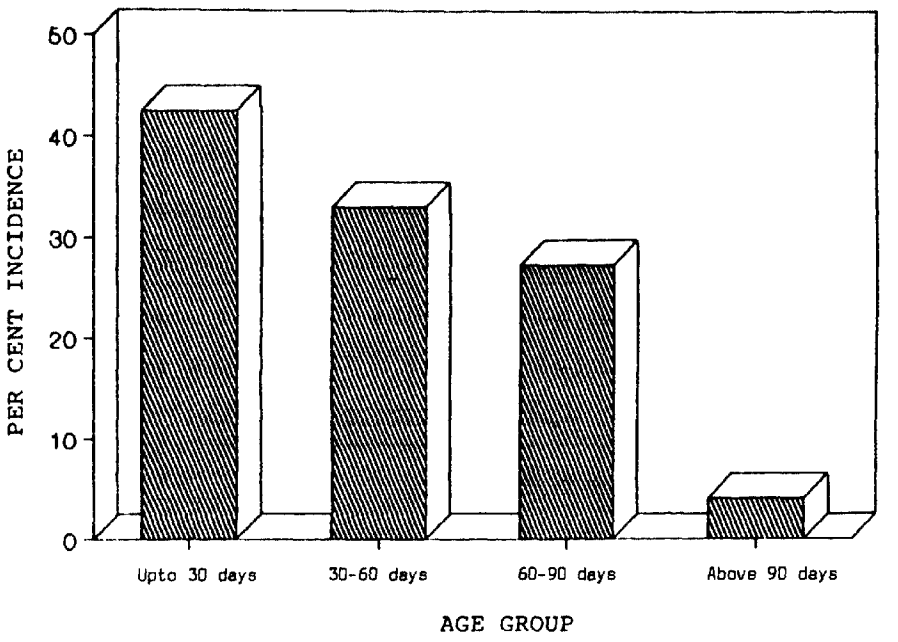

Fig.2: INCIDENCE OF ASCARIASIS IN RELATION TO AGE

4.2.1.1 Incidence in relation to age

Buffalo calves upto the age of 5 months were screened to study the incidence in relation to age. The data is projected in Table 2 and Fig.2. Calves upto the age of 30 days showed incidence of 42.55 per cent (out of 94 samples, 40 were positive). In the age group of 30-60 days the incidence was 33 per cent (out of 100 samples 33 were positive). In the age group of 60 to 90 days, incidence was reported to be 27.27 per cent (out of 66 samples 18 found positive) and in the age group of above 90 days only 2 calves out of 49 were positive giving an incidence of 4.08 per cent.

The chi-square test of the age-wise data indicated high correlation of age with incidence of ascariasis in buffalo calves (X^2 = 24.28 > 11.34; $P<0.01$). The most vulnerable age being 1st month of life, which had the highest incidence of the ascaris infection.

4.2.1.2 Incidence in relation to sex

In the present study out of 309 calves examined 120 were males and 189 were females. Of them 35 males with incidence of 29.16 per cent and 58 females with 30.68 per cent incidence were observed. The details are projected in Table 3 and Fig.3.

There was insignificant difference between the male and female calves statistically (X^2=1.24 < 6.63;

Table 3: Incidence of Ascariasis in relation to sex

S.No.	Sex	No. of calves		Incidence (%)
		Examined	Positive	
1.	Male	120	35	29.16
2.	Female	189	58	30.68
	Total	309	93	30.09

$P>0.01$; $X^2 = 1.24 < 6.63$

Table 4: Incidence of Ascariasis in relation to breed

S.No.	Breed	No. of calves		Incidence (%)
		Examined	Positive	
1.	Graded murrah	179	54	30.16
2.	Murrah	46	14	30.43
3.	Non descript	84	25	29.76
	Total	309	93	30.09

$P>0.01$; $X^2 = 0.0075 < 9.21$

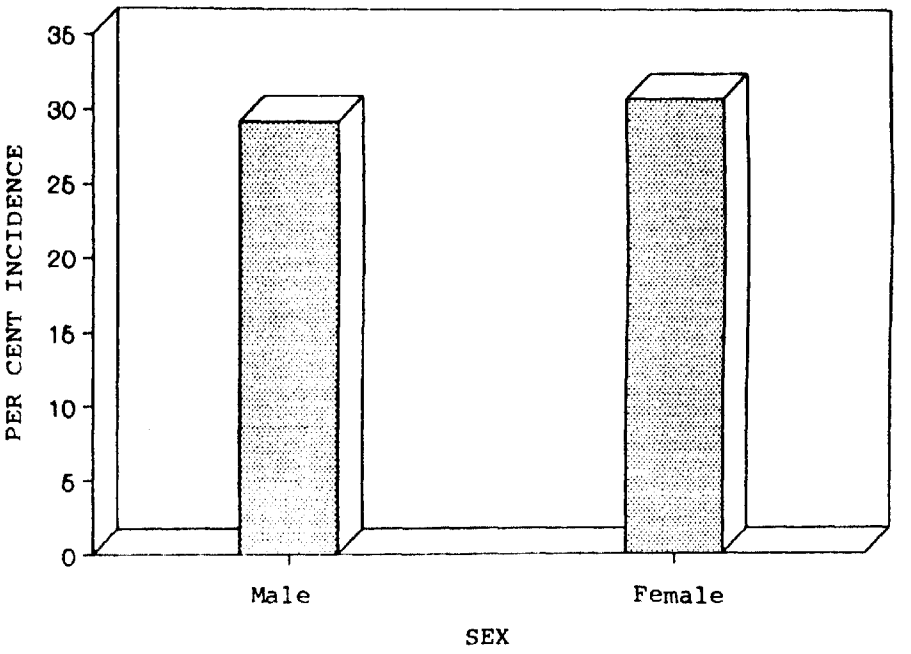

Fig.3: INCIDENCE OF ASCARIASIS IN RELATION TO SEX

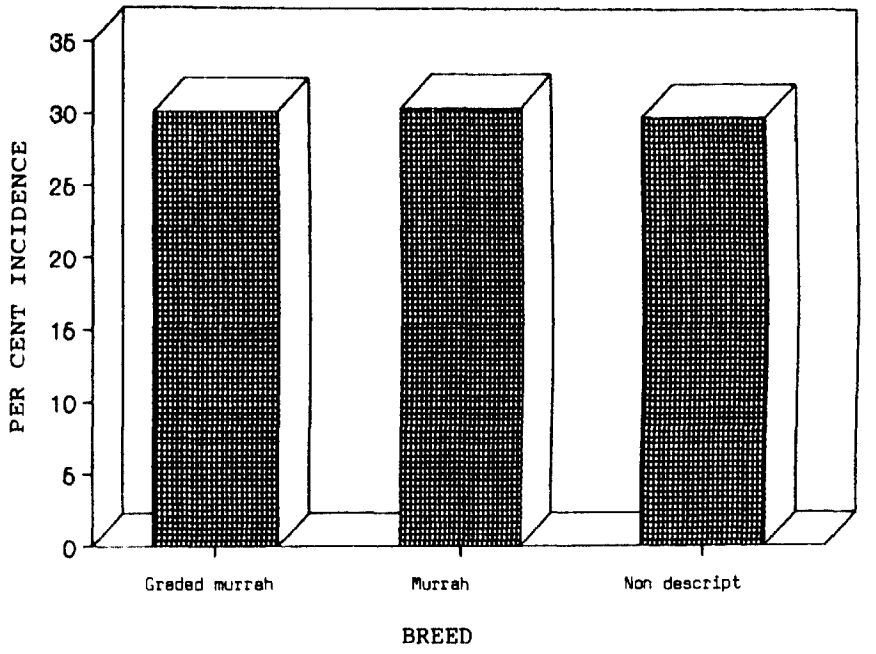

Fig.4: INCIDENCE OF ASCARIASIS IN RELATION TO BREED

P>0.01) indicating that both the sexes were more or less equally susceptible to ascaris infection.

4.2.1.3 Incidence in relation to breed

Out of 309 faecal samples examined 179 were from graded murrah buffaloes and of them 54 were positive with an incidence of 30.16 per cent. 46 samples were from murrah breed and 14 of them were positive giving an incidence of 30.43 per cent whereas remaining 84 samples belonged to non descript breed and of them 25 were positive with an incidence of 29.76 per cent. The details of breed wise data is shown in Table 4 and Fig.4.

The statistical analysis of the data showed insignificant influence of breed on ascariasis infection (X^2=0.0075 < 9.21; P>0.01).

4.2.2 Clinical findings

The calves found positive for ascariasis have invariably shown the following symptoms such as poor body coat, unthrifty condition, alopecia over the ears and neck region, loose faeces with foul smell, some times diarrhoea followed by constipation, dehydration, sunken eye balls and pale conjunctiva. Body temperature was normal in many cases and slightly less than normal in few cases. In advanced cases recumbency and coma was noticed with weak and feeble pulse.

4.3 HAEMATOLOGICAL STUDIES

4.3.1 Total erythrocyte count (TEC)

The results of TEC are presented in table 5. The mean values of TEC on day 0, 14 and 21 in animals of group I were 8.85±0.107, 8.81±0.116 and 8.81±0.112 millions/µl, respectively. There was no significant variation in TEC till the end of the experiment.

In animals of group II the mean values of total erythrocytic counts were low on day 0 and increased on day 14 and 21 post treatment. The mean values of TEC on day 0, 14 and 21 in this group were 6.75±0.125, 8.15±0.192 and 8.31±0.138 millions/µl respectively.

The TEC of group III animals was low on day 0 and increased to nearly normal levels by day 14 and 21. The mean values of TEC were 6.82±0.130, 8.27±0.131 and 8.50±0.112 millions/µl on day 0, 14 and 21, respectively.

In group IV animals the mean values of TEC were low on day 0 and increased by 14 and 21 days. The mean values of TEC were 6.78±0.124, 8.31±0.147, 8.70±0.141 millions/µl on day 0, 14 and 21 respectively. The difference in TEC between these groups was found to be significant ($P<0.01$).

Table 5: ESTIMATION OF TOTAL ERYTHROCYTIC COUNT (millions/µl)

Characteristics	Day 0	After treatment	
		Day 14	Day 21
Group I Mean ± SE	8.85±0.107	8.81±0.116	8.81±0.112
Group II Mean ± SE	6.75±0.125	8.15±0.192	8.31±0.138
Group III Mean ± SE	6.82±0.130	8.27±0.131	8.50±0.112
Group IV Mean ± SE	6.78±0.124	8.31±0.147	8.70±0.141

ANOVA OF TOTAL ERYTHROCYTIC COUNT

	d.f.	S.S.	M.S.S.	F
Between groups	3	13.30	4.43	19.77**
Between days	2	22.92	11.46	51.16**
Error	66	14.82	0.224	
Total	71			

** Significant at 1% level

4.3.2 Total leukocyte count (TLC)

The results of TLC are presented in table 6. In group I animals the mean values of TLC on day 0, 14 and 21 were 7.62±0.143, 7.55±0.123 and 7.47±0.161 thousands/µl respectively.

The TLC of group II animals was high on day 0 and decreased to normal by day 14 and 21. The mean values of TLC on day 0, 14 and 21 were 8.28±0.126, 7.83±0.128 and 7.72±0.120 thousands/µl, respectively.

In group III animals the mean values of TLC were high on day 0 and decreased to normal by 14 and 21 days. The mean values of TLC were 8.30±0.139, 7.84±0.129, 7.71±0.104 thousands/µl, respectively.

The mean TLC counts in animals of group IV were high on day 0 and came to normal by 14 and 21 days. The TLC mean values on day 0, 14 and 21 were 8.32±0.128, 7.65±0.125 and 7.66±0.122 thousands/µl, respectively. The differences in the TLC between these groups was found to be significant ($P<0.01$).

4.3.3 Differential leukocyte count

The mean absolute values of differential leukocyte count are presented in table 7. In group I animals the mean absolute values of neutrophil count on day 0, 14 and 21 were 2.14±0.048, 2.17±0.075, 2.16±0.095

Table 6: ESTIMATION OF TOTAL LEUKOCYTE COUNT (thousands/µl)

Characteristics	Day 0	After treatment	
		Day 14	Day 21
Group I Mean ± SE	7.62±0.143	7.55±0.123	7.47±0.161
Group II Mean ± SE	8.28±0.126	7.83±0.128	7.72±0.120
Group III Mean ± SE	8.30±0.139	7.84±0.129	7.71±0.104
Group IV Mean ± SE	8.32±0.128	7.65±0.125	7.66±0.122

ANOVA OF TOTAL LEUKOCYTE COUNT

	d.f.	S.S.	M.S.S.	F
Between groups	3	1.95	0.65	6.31**
Between days	2	3.29	1.64	15.92**
Error	66	6.83	0.103	
Total	71			

** Significant at 1% level

Table 7: ABSOLUTE VALUES OF DIFFERENTIAL LEUKOCYTE COUNTS* (X $10^3/\mu l$)

Cell type	Day	Group I	Group II	Group III	Group IV
Neutrophils	0	2.14±0.048	3.05±0.095	2.96±0.186	2.99±0.140
	14	2.17±0.075	2.50±0.073	2.39±0.185	2.36±0.235
	21	2.16±0.095	2.19±0.118	2.10±0.128	2.20±0.098
Lymphocytes	0	4.90±0.116	4.48±0.159	4.49±0.117	4.49±0.133
	14	4.80±0.151	4.70±0.117	4.86±0.148	4.73±0.160
	21	4.79±0.135	5.00±0.121	5.05±0.117	4.91±0.160
Eosinophils	0	0.332±0.058	0.578±0.046	0.649±0.039	0.624±0.049
	14	0.353±0.034	0.392±0.047	0.352±0.034	0.342±0.045
	21	0.297±0.043	0.305±0.044	0.312±0.070	0.318±0.034
Monocytes	0	0.242±0.033	0.165±0.030	0.193±0.33	0.207±0.041
	14	0.226±0.02	0.236±0.045	0.232±0.041	0.216±0.037
	21	0.212±0.024	0.222±0.049	0.246±0.040	0.230±0.044

* Mean + SE values

thousands/µl respectively. The mean values of neutrophil count in group II animals on day 0, 14 and 21 were 3.05±0.095, 2.50±0.073 and 2.19±0.118 thousands/µl, respectively. In group III animals the mean values of neutrophil count were 2.96±0.186, 2.39±0.185 and 2.10±0.128 thousands/µl on day 0, 14 and 21 respectively. The mean values of neutrophil count in group IV animals were 2.99±0.140, 2.36±0.235 and 2.20±0.098 thousands/µl on day 0, 14 and 21 respectively.

The mean absolute values of lymphocyte count in group I animals on day 0, 14 and 21 were 4.90±0.116, 4.80±0.151 and 4.79±0.135 thousands/µl respectively. In group II animals the mean absolute values of lymphocyte count on day 0, 14 and 21 were 4.48±0.159, 4.70±0.117 and 5.00±0.121 thousands/µl respectively.

The mean values of lymphocyte count in group III animals on day 0, 14 and 21 were 4.49±0.117, 4.86±0.148, 5.05±0.117 thousands/µl respectively. In group IV animals the mean values of lymphocyte count were 4.49±0.133, 4.73±0.160 and 4.91±0.160 thousands/µl respectively.

In group I animals the mean absolute values of eosinophil count on day 0, 14 and 21 were 0.332±0.058, 0.353±0.034 and 0.297±0.043 thousands/µl respectively. The mean values of eosinophilic count in group II animals on day 0, 14 and 21 were 0.578±0.046, 0.392±0.047 and

0.305±0.044 thousands/µl respectively. In group III animals the mean absolute values of eosinophilic count on day 0, 14 and 21 were 0.649±0.039, 0.352±0.034 and 0.312±0.070 thousands/µl respectively. The mean values of eosinophilic count in group IV animals on day 0, 14 and 21 were 0.624±0.049, 0.342±0.045 and 0.318±0.034 thousands/µl respectively.

The mean values of monocyte count in group I animals on day 0, 14 and 21 were 0.242±0.033, 0.226±0.02 and 0.212±0.024 thousands/µl, respectively. In group II animals the mean values of monocyte count on day 0, 14 and 21 were 0.165±0.030, 0.236±0.045 and 0.22±0.049 thousands/µl, respectively. In group III animals the mean values of monocyte count were 0.193±0.33, 0.232±0.041 and 0.246±0.040 thousands/µl respectively. The mean values of monocyte count in group IV animals on day 0, 14 and 21 were 0.207±0.041, 0.216±0.037 and 0.230±0.044 thousands/µl respectively.

4.3.4 Packed cell volume (PCV)

The results of PCV are presented in table 8. The mean values of PCV in group I animals on day 0, 14 and 21 were 37.66±0.42, 37.83±0.30 and 38.16±0.30, respectively.

The PCV values in group II animals on day 0 were low and increased gradually by day 14 and 21. The mean

Table 8: ESTIMATION OF PACKED CELL VOLUME (%)

Characteristics		Day 0	After treatment	
			Day 14	Day 21
Group I	Mean ± SE	37.66±0.42	37.83±0.30	38.16±0.30
Group II	Mean ± SE	33.33±0.33	35.66±0.61	36.50±0.42
Group III	Mean ± SE	33.50±0.42	35.50±0.764	36.33±0.76
Group IV	Mean ± SE	33.00±0.36	34.83±0.70	36.50±0.56

ANOVA OF PACKED CELL VOLUME

	d.f.	S.S.	M.S.S.	F
Between groups	3	112.82	37.60	21.00**
Between days	2	76.77	38.38	21.44**
Error	66	118.40	1.79	
Total	71			

** Significant at 1% level

values of PCV on day 0, 14 and 21 were 33.33±0.33, 35.66±0.61 and 36.50±0.42 respectively.

In animals of group III, the mean values of PCV were low on day 0 and increased gradually on day 14 and 21. The mean values of PCV on day 0, 14 and 21 in this group were 33.50±0.42, 35.50±0.76 and 36.33±0.76, respectively.

In group IV animals the PCV values were low on day 0 and increased gradually by day 14 and 21. The mean values were 33.0±0.36, 34.83±0.70 and 36.50±0.56, respectively.

The difference in PCV values between these groups was noticed to be significant ($P<0.01$).

4.3.5 Haemoglobin concentration

The results of haemoglobin concentration of all the four groups are presented in table 9. The mean values of haemoglobin concentration of group I animals on day 0, 14 and 21 were 13.70±0.15, 13.63±0.14 and 13.60±0.11, respectively.

In group II animals the mean values of haemoglobin concentration were low on day 0 and increased by day 14 and 21. The mean values of haemoglobin concentration on day 0, 14 and 21 were 10.23±0.26, 11.83±0.32 and 11.83±0.27, respectively.

Table 9: ESTIMATION OF HAEMOGLOBIN CONCENTRATION (gm/dl)

Characteristics		Day 0	After treatment	
			Day 14	Day 21
Group I	Mean ± SE	13.70±0.15	13.63±0.14	13.60±0.11
Group II	Mean ± SE	10.23±0.26	11.83±0.32	11.83±0.27
Group III	Mean ± SE	10.56±0.09	11.96±0.18	12.13±0.15
Group IV	Mean ± SE	10.33±0.12	12.16±0.18	13.26±0.29

ANOVA OF HAEMOGLOBIN CONCENTRATION

	d.f.	S.S.	M.S.S.	F
Between groups	3	71.37	23.79	51.45**
Between days	2	30.12	15.06	32.57**
Error	66	30.52	0.46	
Total	71			

** Significant at 1% level

The mean values of haemoglobin concentration in animals of group III were low on day 0 (10.56±0.09) and increased to normal by day 14 and 21 (11.96±0.18 and 12.13±0.15).

In group IV animals the mean values of haemoglobin concentration were 10.33±0.12, 12.16±0.18 and 13.26±0.29 on day 0, 14 and 21 respectively. The haemoglobin concentration was low on day 0 and increased gradually by days 14 and 21.

The difference in haemoglobin concentration between these groups was found to be significant ($P<0.01$).

4.4 BIOCHEMICAL STUDIES

4.4.1 Serum glucose

The results of serum glucose level of four groups are presented in table 10. In animals of group I the mean values of serum glucose level on day 0 and 14 were 103.81±2.30 and 106.35±2.57 mg/dl.

The mean values of serum glucose level in group II animals on day 0 and 14 were 60.08±1.62 and 98.53±1.69 mg/dl. The serum glucose level was low on day 0 and increased on day 14.

In group III animals the mean values of serum glucose level on day 0 and 14 were 61.25±2.17 and

Table 10: ESTIMATION OF SERUM GLUCOSE LEVEL (mg/dl)

Characteristics	Day 0	After treatment Day 14
Group I Mean ± SE	103.81±2.30	106.35±2.57
Group II Mean ± SE	60.08±1.62	98.53±1.69
Group III Mean ± SE	61.25±2.17	101.68±0.91
Group IV Mean ± SE	60.19±1.71	104.95±1.63

ANOVA OF SERUM GLUCOSE LEVEL

	d.f.	S.S.	M.S.S.	F
Between groups	3	5236.22	1745.41	17.52**
Between days	1	11939.62	11939.62	119.82**
Error	43	4284.79	99.65	
Total	47			

** Significant at 1% level

101.68±0.91 mg/dl, respectively. The mean serum glucose level was low on 0 day and later increased by 14th day.

The mean values of serum glucose level in group IV animals on day 0 and 14 were 60.19±1.71 and 104.95±1.63 mg/dl. The serum glucose level was low on day 0 and increased by 14th day. The difference in serum glucose level between these groups was significant ($P<0.01$) statistically. D2744

4.4.2 Aspartate amino transferase (AST)

The results of AST activity of four groups are presented in table 11. In group I animals the mean values of AST level on day 0 and 14 were 52.66±1.11 and 52.33±0.8 units/ml, respectively.

In group II animals the AST activity was increased on day 0 and became normal by 14 day. The mean values of AST activity on day 0 and 14 were 74.33±2.55 and 58.66±1.97 units/ml.

The mean values of AST activity in group III animals on day 0 and 14 were 74.33±3.81 and 57.0±2.23 units/ml indicating the AST activity was high on day 0 in comparison with day 14.

In group IV animals the AST activity was high on day 0 and later decreased by 14 day. The mean values of AST on day 0 and 14 were 78.33±2.89 and 55.66±0.96

Table 11: AST ACTIVITY (RF units/ml)

Characteristics	Day 0	After treatment Day 14
Group I Mean ± SE	52.66±1.11	52.33±0.80
Group II Mean ± SE	74.33±2.55	58.66±1.97
Group III Mean ± SE	74.33±3.81	57.00±2.23
Group IV Mean ± SE	78.33±2.89	54.66±1.68

ANOVA OF AST ACTIVITY

	d.f.	S.S.	M.S.S.	F
Between groups	3	1700.25	566.75	11.20**
Between days	1	2433.90	2433.90	48.10**
Error	43	2175.75	50.60	
Total	47			

** Significant at 1% level

units/ml respectively. The difference in AST activity between four groups was noticed to be significant ($P<0.01$).

4.4.3 Alanine amino transferase (ALT)

The results of ALT activity of four groups were presented in table 12. In group I animals the mean values of ALT activity on day 0 and 14 were 16.66 ± 0.98 and 16.33 ± 0.95 units/ml.

In group II animals the ALT activity was high on day 0 and decreased on day 14. The mean values of ALT on day 0 and 14 were 27.0 ± 1.52 and 20.0 ± 1.36 units/ml.

The mean values of ALT activity in group III animals on day 0 and 14 were 27.33 ± 1.33 and 18.66 ± 0.66 units/ml. The mean ALT activity was high on day 0 and decreased by day 14.

In animals of group IV the mean ALT values on day 0 and 14 were 29.66 ± 1.20 and 17.66 ± 0.95 units/ml, respectively. The mean ALT activity was high on day 0 and later decreased by 14th day. There is significant difference in ALT levels between the groups ($P<0.01$).

4.4.4 Alkaline phosphatase (ALP)

The results of ALP levels of all the four groups are presented in table 13. The mean values of ALP level

Table 12: ALT ACTIVITY (RF units/ml)

Characteristics	Day 0	After treatment Day 14
Group I Mean ± SE	16.66±0.98	16.33±0.95
Group II Mean ± SE	27.00±1.52	20.00±1.36
Group III Mean ± SE	27.33±1.33	18.66±0.66
Group IV Mean ± SE	29.66±1.20	17.66±0.95

ANOVA OF ALT ACTIVITY

	d.f.	S.S.	M.S.S.	F
Between groups	3	430.03	143.34	11.49**
Between days	1	588.21	588.21	47.15**
Error	43	536.46	12.48	
Total	47			

** Significant at 1% level

Table 13: ALP ACTIVITY (KA units)

Characteristics	Day 0	After treatment Day 14
Group I Mean ± SE	6.03±0.15	6.04±0.12
Group II Mean ± SE	7.33±0.10	6.73±0.14
Group III Mean ± SE	7.38±0.09	6.59±0.09
Group IV Mean ± SE	7.46±0.14	6.38±0.03

ANOVA OF ALP ACTIVITY

	d.f.	S.S.	M.S.S.	F
Between groups	3	8.18	2.73	22.37**
Between days	1	4.52	4.52	37.05**
Error	43	5.25	0.122	
Total	47			

** Significant at 1% level

in group I animals on day 0 and 14 were 6.03±0.15 and 6.04±0.12 KA units respectively.

In group II animals the mean values of ALP on day 0 and 14 were 7.33±0.10 and 6.73±0.14 KA units. The ALP activity was high on day 0 and decreased by day 14.

The mean values of ALP activity in group III animals were 7.38±0.09 and 6.59±0.09 KA units on day 0 and 14 respectively. The ALP activity was high on day 0 and decreased by 14 day.

In animals of group IV the ALP activity was high on 0 day and decreased by 14th day. The mean values of ALP level on day 0 and 14 were 7.46±0.14 and 6.38±0.03 KA units respectively. There is significant difference between groups on the ALP activity ($P<0.01$).

4.4.5 Serum immunoglobulins

The levels of serum immunoglobulins in healthy and affected calves are presented in table 14. Out of 18 affected calves (77.77%) studied, 14 had immunoglobulins less than 5 mg/ml of serum and 4 (22.23%) calves showed 5 to 15 mg/ml of serum. Whereas most of the healthy calves (66.66%) had the immunoglobulins between 5 to 15 mg/ml of serum and only one animal (16.66%) each showed immunoglobulins less than 5 mg and more than 15 mg/ml of serum, respectively.

Table 14: Serum immunoglobulin levels in healthy and affected calves

S.No.	Immunoglobulin concentration (mg/ml of serum)	Healthy calves		Ascarid infected calves	
		Examined	Percentage	Examined	Percentage
1.	Less than 5 mg	1	16.66	14	77.77
2.	Between 5 to 15 mg	4	66.66	4	22.23
3.	More than 15 mg	1	16.66	-	-

4.5 THERAPY

4.5.1 Results of EPG in buffalo calves treated with Piperazine

Results of EPG in ascarid infected calves treated with Piperazine are presented in table 15. The mean EPG count before treatment was 41900±8056 and on day 2, 3 and 7 post treatment it was 10666.66±4132.44, 850±131.02, 75±65.5 and 50±50 respectively. Only one animal out of 6 animals showed an egg count of 300 on day 14, thereafter the faecal samples were negative on days 21 and 28.

4.5.2 Results of EPG in buffalo calves treated with Ivermectin

The results of EPG in calves treated with Ivermectin are presented in table 16. The mean EPG count before treatment was 43933.33±12093.43 and after treatment on days 2, 3 and 7 was 11800±5714.01, 1900±1232.88 and 33.33±33.33 respectively. All the animals in this group were free of parasitic ova on day 14.

4.5.3 Results of EPG in buffalo calves treated with Levamisole

Results of EPG in ascarid infected calves treated with Levamisole are presented in table 17. The mean EPG count before treatment was 43683.33±12067. On day 2 post treatment one animal out of 6 was free of infection whereas on day 3, three animals out of 6 were positive for

Table 15: Results of EPG in buffalo calves treated with Piperazine

Animal No.		After treatment					
	Day 0	2	3	7	14	21	28
1	55400	29800	900	Nil	Nil	Nil	Nil
2	28600	5600	1200	400	300	Nil	Nil
3	42700	6300	800	Nil	Nil	Nil	Nil
4	36300	5800	400	Nil	Nil	Nil	Nil
5	16400	2500	600	50	Nil	Nil	Nil
6	72000	14000	1200	Nil	Nil	Nil	Nil
Mean ± S.E.	41900 ± 8056	10666.66 ± 4132.44	850 ± 131.02	75 ± 65.5	50 ± 50	-	-

Table 16: Results of EPG in buffalo calves treated with Ivermectin

Animal No.	After treatment						
	Day 0	2	3	7	14	21	28
1	66600	15500	900	Nil	Nil	Nil	Nil
2	20100	6000	200	Nil	Nil	Nil	Nil
3	91500	38700	8000	200	Nil	Nil	Nil
4	18700	5000	300	Nil	Nil	Nil	Nil
5	42900	3200	1400	Nil	Nil	Nil	Nil
6	23800	2400	600	Nil	Nil	Nil	Nil
Mean ± S.E.	43933.33 ± 12093.43	11800 ± 5714.01	1900 ± 1232.88	33.33 ± 33.33	-	-	-

Table 17: Results of EPG in buffalo calves treated with Levamisole

Animal No.	Day 0	After treatment					
		2	3	7	14	21	28
1	58800	13000	1100	Nil	Nil	Nil	Nil
2	86300	1500	Nil	Nil	Nil	Nil	Nil
3	16600	Nil	Nil	Nil	Nil	Nil	Nil
4	62600	12500	300	Nil	Nil	Nil	Nil
5	17500	900	Nil	Nil	Nil	Nil	Nil
6	20300	11700	1800	Nil	Nil	Nil	Nil
Mean ± S.E.	43683.33 ± 12067	6600 ± 2606.65	533.33 ± 307.31	-	-	-	-

ascarid eggs. All the animals were free of eggs in faeces on day 7 after treatment and continued to remain free from infection till day 28.

4.5.4 Comparative efficacies of Piperazine, Ivermectin and Levamisole

The comparative efficacies of Piperazine, Ivermectin and Levamisole are projected in table 18. In Piperazine treated group, 4 animals (66.66%) were free from parasitic ova as determined by faecal examination on day 7, whereas on day 14 only one animal was positive for ascarid ova, out of 6 showing an efficacy of 83.33 per cent. However, the drug showed cent per cent efficacy on day 21 and 28.

In Ivermectin treated group, none of the animals examined were free of infection on day 3 whereas on day 7 only one out of 6 animals was positive for ascarid ova showing an efficacy of 83.33 per cent. However, the drug showed cent per cent efficacy on day 21 and 28, post treatment.

The results indicated that Levamisole showed 16.66 per cent and 50 per cent efficacy on day 2 and 3 respectively and cent per cent efficacy on day 7 post treatment as against the other two drugs Piperazine and Ivermectin which showed cent per cent efficacy on day 21 and day 14 post treatment, respectively.

Table 18: Comparative efficacies of Piperazine, Ivermectin and Levamisole against ascariasis as determined by faecal egg counts

Drugs used	No.of animals treated	Animals found free of ascarid ova in faeces											
		Day 2		Day 3		Day 7		Day 14		Day 21		Day 28	
		Number	Efficacy %	Number	Efficacy %	Number	Efficacy %	Number	Efficacy %	Number	Efficacy %	Number	Efficacy %
Piperazine	6	Nil	-	Nil	-	4	66.66	5	83.33	6	100	6	100
Ivermectin	6	Nil	-	Nil	-	5	83.33	6	100	6	100	6	100
Levamisole	6	1	16.66	3	50	6	100	6	100	6	100	6	100

DISCUSSION

CHAPTER V

DISCUSSION

In order to find out the causes of calf mortality the old records of the organised dairy farms were screened for the previous five years. The calves with natural infection of ascariasis were also studied. In addition to incidence an investigation was also undertaken to study the haematological and biochemical parameters. Therapeutic trial using new generation ascaricidal drugs were also carried out.

The retrospective studies on causes of mortality in buffalo calves revealed pneumonia (30%), followed by enteritis (18%), septicaemia (16%), cirrhosis of liver (6%), nephritis (6%) and coccidiosis (2%) whereas no cause was attributed to 14 per cent of mortality. Singh and Singh (1972), Bali *et al*. (1979) and Patil *et al*.(1991) reported similar mortality pattern with respect to pneumonia and enteritis, whereas higher mortality due to pneumonia and enteritis was reported by Srivastava and Sharma (1980) and Lakshmi Prasad (1991). On the contrary Khera (1981) reported higher mortality rates with respect to digestive and respiratory diseases and lower mortality rates due to septicaemia. The present findings are on similar lines with Lakshmi Prasad (1991) with respect to liver disorders. Khera (1981) and Lakshmi Prasad (1991) reported lower mortality rates with respect to septicaemia

and Bali et al. (1979) reported higher mortality rates (15%) with relation to coccidiosis.

In the present study 93 calves out of 309 were positive for ascariasis giving an overall incidence of 30.09 per cent. Almost similar findings have been reported earlier by Nagappapai (1955), Bhatnagar et al. (1980) and Gupta et al. (1985) who recorded 37.9 per cent, 27.5 per cent and 29.1 per cent incidence respectively in buffalo calves. Contrary to the present findings, Baruah et al. (1981), Swain et al. (1987), Gupta (1986) and Sahoo et al. (1991) reported higher incidence of ascariasis infection in calves, whereas the lower incidence was reported by Hariprasad (1985). The variation in the incidence rates might be due to the difference in the climatic conditions and managemental practices.

In this study incidence (42.55%) in calves upto the age of 30 days was highly significant ($P<0.01$). These findings were in agreement with the reports of Bhatnagar et al. (1980) and Hariprasad (1985), whereas Barbosa and Correa (1989) reported 58 per cent incidence in the first week of life, 87.5 per cent in the second week, 96 per cent in the third and 100 per cent in the fourth week. The lower incidence of 33 per cent was observed in the age group of 30 to 60 days. Corresponding findings of Bhatnagar et al. (1980), Hariprasad (1985) were almost

similar in the age group of 2 to 3 months whereas Baruah et al. (1981) observed higher incidence in the age group of 46 to 53 day old calves.

In the age group of 60 to 90 days 27.27 per cent incidence was observed. Baruah et al. (1981) reported slightly higher (35.7%) incidence in 81 to 90 days old calves. Sahoo et al. (1991) reported higher incidence in the age group of 2 to 3 months. In the present study the incidence of Toxocara vitulorum in the group of calves above 90 days age was only 4.08 per cent. These findings are similar to those of Bhatnagar et al.(1980), Hariprasad (1985) and Gupta (1986).

The lower incidence in the higher age group might be due to the age resistance of the host (Vaidyanathan, 1949). Swain et al. (1987) stated that the incidence rate sharply declined in calves above three months of age and he opined that it might be due to the natural expulsion of the adult worms.

The incidence of ascariasis in relation to the sex of the calf revealed 29.16 per cent in males and 30.68 per cent in female calves indicating an insignificant influence of sex on incidence of ascariasis. The present finding is in confirmity with those reported earlier by Bhatnagar et al. (1980), Gupta and Chhabra (1990), where the former concluded that the sex of the host has no

on the incidence of infection. However, Tawfik) and Hariprasad (1985) reported slightly higher ice in male than in female calves.

The incidence of ascariasis relating to breed was 30 per cent in graded murrah buffalo calves, 30.43 per cent in murrah buffalo calves and 29.76 per cent in the non descript variety of calves. The difference in incidence was insignificant. The data could not be compared for want of earlier reports.

The clinical findings observed in the present study included poor body coat, unthrifty condition, alopecia over the ears and neck region, loose faeces with foul smell, some times diarrhoea followed by constipation, dehydration, sunken eye balls, pale conjunctiva. Body temperature was normal in many cases and slightly less than normal in few cases. In advanced cases recumbency and coma was noticed with weak and feeble pulse. Similar clinical findings were also observed earlier by Nagappapai (1955) and Christopher (1984).

In addition Nagappapai (1955) also observed high coloured urine, pyrexia, colic in some cases. Christopher (1984) recorded the pathological lesions as cirrhosis, pneumonia in ascarid infected calves, which indicated the involvement of liver and lung. Abeydeera and Roberts (1991) in their experimental studies noted the lesions in

liver varying from cellular infiltrations in early stages to eosinophilic granulomata with degenerating larvae in later stages of infection.

The haematological findings in the calves affected with ascariasis revealed lower numbers of erythrocytes in comparison to that of healthy controls. These findings reconfirm the earlier reports of El-Abdin (1975), Lau and Singh (1985) and Hariprasad (1985). The reduction in erythrocytic count might be due to the suppression of erythropoietic activity of bone marrow to some parasitic toxins as reported earlier by Udall (1954). Contrary to present findings, Baruah et al. (1979) reported an increased erythrocyte count in Neoascaris vitulorum infected calves, whereas no change in the erythrocyte count was observed by Panebianco (1955).

The haematological studies relating to total leucocyte count revealed leucocytosis in ascarid infected calves. These findings corresponded well with the earlier reports of Lau and Singh (1985). The differential leucocyte count in the affected calves showed absolute increase in values of neutrophils. These findings corroborated with those of El-Abdin et al. (1975) whereas Lau and Singh (1985) reported no change in neutrophilic count. The differential leucocyte count of eosinophils revealed increased number of eosinophils in the infected calves. The increase in eosinophil count might be due to

the presence of high quantities of histamine in ascarid worms (Pautrizel et al., 1949). Mann (1969) contended that certain of the catalytic enzymes present in the lysosomal granules of eosinophil degrade histamine and thus the eosinophil represents some of the control mechanism for histamine release. Correspondingly similar findings were observed by Panebianco (1955), El-Abdin et al. (1975) and Lau and Singh (1985). As far as lymphocytes were concerned there was slight decrease in the number of lymphocytes in the infected calves. These findings corroborate with the findings of El-Abdin et al. (1975) but on contrary Lau and Singh (1985) reported lymphocytosis in the affected calves. Regarding the mean absolute values of monocyte count, much variation was not observed in all the groups indicating no monocytic response during the parasitic infection.

Haemoglobin concentration in all the infected calves showed lowered values in comparison to that of healthy calves indicating anaemia. The similar findings were observed by Hariprasad (1985), Lau and Singh (1985) and Pandey and Mishra (1985). However, Baruah et al. (1979) reported an increased haemoglobin content in the ascarid infected calves than in the control calves. The present study revealed a low haematocrit in the ascarid infected calves than in the healthy calves. These findings corroborate with the observations made by Pandey

and Mishra (1985), Lau and Singh (1985) and Hariprasad (1985).

The investigations into the biochemical profiles of the affected calves in the present study indicated lowered serum glucose and increased values of aspartate amino transferase, alanine amino transferase and alkaline phosphatase.

The lowered serum glucose values in the ascarid infected calves are in agreement with those of Baruah et al. (1979) and Hariprasad (1985) who recorded low blood glucose values in the ascarid infected calves. The reduction in the serum glucose might be due to the fact that Toxocara vitulorum adult worms thrive on carbohydrates available in the gastrointestinal tract of calves depleting the host the required glucose (Von Brand, 1966) and also might be a result of liver damage caused by visceral larva migrans (Srivastava, 1963) and resultant hepatic insufficiency (Das and Singh, 1955). However, Pandey and Mishra (1985) reported no change in the blood glucose values in their studies.

There was an increase in the levels of aspartate amino transferase, alanine amino transferase and alkaline phosphatase in Toxocara vitulorum infected calves when compared to the healthy control calves. The results were almost identical with those reported by El-Abdin et al.

(1975). The present findings were however suggestive of severe hepatic insufficiency which might be due to either liberation of toxins (Das and Singh, 1955) or damage caused by the visceral larva migrans (Srivastava, 1963).

The studies on serum immunoglobulins revealed majority of calves (77.77%) had less than 5 mg immunoglobulins when compared to those (22.23%) with 5-15 mg immunoglobulins and none with above 15 mg immunoglobulins. The assessment of the immunoglobulin status of infected calves suggested that majority of the calves had either little or no absorption of immunoglobulins and as such no protection (Radostits et al., 1994).

In piperazine treated group the mean EPG counts declined on day 2 and 3 compared to pretreatment values. Thereafter on day 7, four animals were free of ascarid ova indicating 66.66 per cent efficacy whereas on day 14 five animals were free of infection indicating 83.33 per cent efficacy and by day 21 all the animals were free of infection indicating 100 per cent efficacy of the drug. The present findings were in agreement with that of Akhtar et al. (1982), Lee (1955) whereas Hossain et al. (1980) observed 96 per cent efficacy of the drug seven days after treatment.

The mean EPG counts in the ivermectin treated group before treatment were high, whereas the EPG counts

after treatment showed considerable decline on day 2, 3 and 7. The results indicated 83.33 per cent efficacy on day 7 and 100 per cent efficacy by day 21 post treatment. Gill et al. (1989) observed ivermectin as an effective drug eliminating worms within one week after treatment. Shastri (1989) observed 90.83 to 100 per cent reduction in faecal egg counts after administration of ivermectin in buffalo calves at 200 µg/kg body weight whereas Uysal (1989) noticed 99.5 per cent efficacy of the drug after 2 weeks of using ivermectin.

The other drug used in the present therapeutic trial was Levamisole. The EPG counts in Levamisole treated group of animals were high before treatment and declined after treatment. On day 2 one animal was free of infection giving 16.66 per cent efficacy of the drug and 3 animals were free of infection on day 3 showing 50 per cent efficacy. Thereafter on day 7 all the animals were free of infection showing 100 per cent efficacy of the drug.

The results of present therapeutic trials indicated that levamisole hydrochloride injection at the dose rate of 7.5 mg/kg body weight administered subcutaneously eliminated the ascarid worms and the faeces of all calves was negative for ascarid ova on 7th day itself whereas the haematological and biochemical parameters returned to normal after 14 and 21 days post

treatment. The present results corroborate well with those of Manuel and Rugay (1979) who reported a 100 per cent efficacy of levamisole against Neoascaris vitulorum at a dose rate of 6.12 mg/kg as injectable solution by day 7. Roberts (1989) studied the efficacy of levamisole (7.5 mg/kg body weight) and piperazine (200 mg/kg) and found that levamisole and piperazine were effective at 97 and 42 per cent respectively against immature worms and 83 per cent and 57 per cent respectively against mature worms whereas the other two drugs viz. ivermectin though effective was very costly and the piperazine though cheaper in cost, in some cases it may cause tympany and diarrhoea (Lee, 1955). Therefore, the drug levamisole is recommended as a routine dewormer in buffalo calves.

SUMMARY

CHAPTER VI

SUMMARY

An attempt was made to review the causes of buffalo calf mortality with particular reference to ascariasis based on old records of organised dairy farms maintained for the last five years. Studies were also made to find out incidence, epizootiology, blood changes, biochemical profiles along with therapeutic drug trial.

In the present study pneumonia was found to be major cause of mortality resulting in 30 per cent incidence followed by enteritis, (18%), septicaemia (16%), aflatoxicosis (8%), cirrhosis and nephritis (6%) and coccidiosis (2%).

Out of 309 faecal samples from buffalo calves examined 93 were positive for ascarid ova giving an incidence of 30.09 per cent.

In the present study, calves upto 30 days age showed 42.5 per cent incidence followed by 33 per cent in 30 to 60 days group, 27.27 per cent in calves of 60 to 90 days group whereas calves above 90 days showed 4.08 per cent incidence.

The sex had insignificant influence on calf mortality since males showed 29.16 per cent and females 30.66 per cent incidence of ascariasis.

As far as breed was concerned, graded murrah buffalo calves had 30.16 per cent, murrah calves 30.43 per cent and non descript calves revealed 29.76 per cent incidence.

The clinical findings in infected calves were poor body coat, unthrifty condition, alopecia, loose and foul faeces, dehydration, normal or subnormal body temperature and recumbency in advanced cases.

The blood studies in infected calves had low P.C.V., low haemoglobin content, reduced R.B.C's and increased total leucocytes along with increased neutrophils, eosinophils and decreased lymphocytes.

The biochemical estimations were as follows: low serum glucose level, increased A.S.T., increased A.L.T and increased A.L.P. The serum immunoglobulins in most of the infected calves were less than 5 mg suggesting majority of calves had either little or no absorption of immunoglobulin and as such no protection.

During therapeutic trial of three anthelmintics, the drug levamisole hydrochloride given parenterally proved more better when compared with oral piperazine and parenteral ivermectin.

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APPENDICES

APPENDIX - A

ESTIMATION OF SERUM GLUCOSE (O-TOLUIDINE METHOD)

PRINCIPLE

Glucose, in presence of hot acetic acid condenses with Ortho-Toluidine and gives a blue green coloured N-glucosylamine which is measured colorimetrically at 630 nm.

The following reagents were used :

Reagent 1: O-Toluidine Reagent

Reagent 2: Glucose Standard, 100 mg/dl

The following procedure was followed

	Test (T)	Standard (S)
Serum/Plasma	0.05 ml	-
Reagent 2: Glucose Standard, 100 mg/dL	-	0.05 ml
Reagent 1: O-Toluidine Reagent	5.0 ml	5.0 ml

The contents were mixed well and kept in a boiling water bath exactly for 10 minutes. Cooled under running tap water and measured the C.D. at 630 nm or Red filter against distilled water used as a blank to set zero.

CALCULATIONS

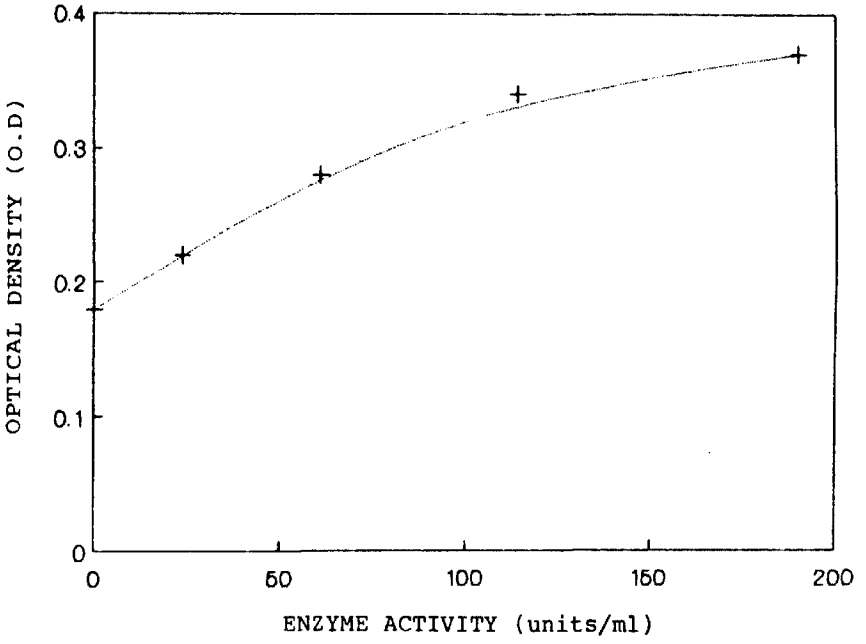
$$\text{Serum glucose in mg/100 ml} = \frac{\text{O.D. test}}{\text{O.D. std.}} \times 100$$

The following procedure was followed.

A. For colorimeter

Tube No.	1	2	3	4	5
Enzyme activity(units/ml)	0	23	61	114	190
Reagent 1: Buffered Aspartate-α-KG Substrate, pH 7.4 ml	0.5	0.45	0.4	0.35	0.3
Reagent 2: Working Pyruvate Standard 2 mM	-	0.05	0.1	0.15	0.2
Distilled water, ml	0.1	0.1	0.1	0.1	0.1
Reagent 2: DNPH Colour Reagent ml	0.5	0.5	0.5	0.5	0.5
The contents were mixed well and allowed to stand at Room Temperature for 20 minutes					
Solution 1, ml	5.0	5.0	5.0	5.0	5.0

The contents were mixed well by inversion, allowed to stand at room temperature for 10 minutes and measured the O.D. of all the five tubes against distilled water on a colorimeter with a green filter. Standard graph was plotted by taking enzyme activity on X-axis and optical density on Y-axis.



STANDARD CURVE : SERUM AST

Units/ml	O.D
0	0.18
24	0.22
61	0.28
114	0.34
190	0.37

For Colorimeter :

	Test (T)
Reagent 1: Buffered Aspartate-α-KG Substrate, pH 7.4	0.5 ml Incubated at 37°C for 5 minutes
Serum	0.1 ml Mixed well and incubated at 37°C for 60 minutes
Reagent 2: DNPH Color Reagent	0.5 ml Mixed well and allowed to stand at room temperature for 20 minutes
Solution I	5.0 ml

Mixed well and allowed to stand at room temperature for 10 minutes and read the O.D. against distilled water on a colorimeter using a green filter.

CALCULATIONS

Marked the O.D. of Test (T) on the Y-axis of the standard curve and extrapolate it to the corresponding enzyme activity on X-axis. The results obtained were expressed in RF units/ml.

APPENDIX - C

ESTIMATION OF ALANINE AMINO TRANSFERASE SGPT (ALT) (2,4-DNPH METHOD)

PRINCIPLE

SGPT (ALT) Catalyses the following reaction:

α-Keto glutarate + L.Alanine $\rightleftharpoons$ L.Glutamate + Pyruvate

Pyruvate so formed is coupled with 2,4 Dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown color in alkaline medium and this can be measured colorimetrically.

The following reagents were used :

Reagent 1: Buffered Alanine - α-KG Substrate, pH 7.4

Reagent 2: DNPH Color Reagent

Reagent 3: Sodium Hydroxide, 4 N

Reagent 4: Working Pyruvate Standard 2mM.

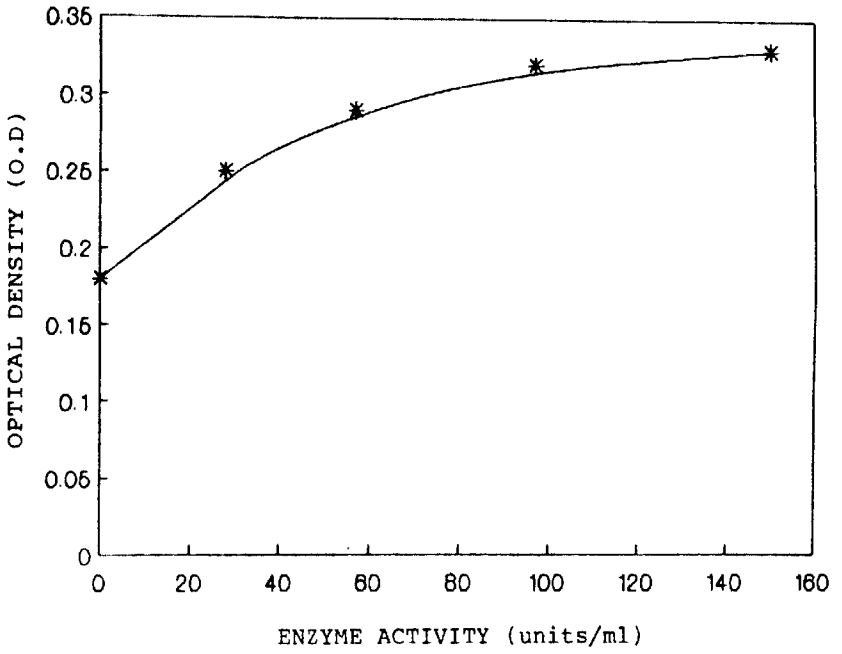
Working Solution I: Diluted 1 ml of reagent 3 to 10 ml with distilled water

The following procedure was followed.

A. For colorimeter

Tube No.	1	2	3	4	5
Enzyme activity(units/ml)	0	28	57	97	150
Reagent 1: Buffered Alanine α-KG Substrate, pH 7.4 ml	0.5	0.45	0.4	0.35	0.3
Reagent 4: Working Pyruvate Standard 2 mM	-	0.05	0.1	0.15	0.2
Distilled water, ml	0.1	0.1	0.1	0.1	0.1
Reagent 2: DNPH Colour Reagent ml	0.5	0.5	0.5	0.5	0.5
The contents were mixed well and allowed to stand at room temperature for 20 minutes					
Solution 1, ml	5.0	5.0	5.0	5.0	5.0

The contents were mixed well by inversion. Allowed to stand at room temperature for 10 minutes and measured the O.D. of all the five tubes against distilled water on a colorimeter with a green filter. Standard curve was plotted by taking enzyme activity on X-axis and optical density on Y-axis.



STANDARD CURVE : SERUM ALT

Units/ml	O.D
0	0.18
28	0.25
57	0.29
97	0.32
150	0.33

For Colorimeter :

		Test (T)
Reagent 1:	Buffered Substrate, pH 7.4	0.5 ml Incubated at 37°C for 5 minutes
Serum		0.1 ml Mixed well and incubated at 37°C for 30 minutes
Reagent 2:	DNPH Color Reagent	0.5 ml Mixed well and allowed to stand at room temperature for 20 minutes
Solution 1		5.0 ml

Mixed well and allowed to stand at room temperature for 10 minutes and read the O.D. against distilled water on a colorimeter using a green filter.

CALCULATIONS

Marked the O.D. of Test (T) on the Y-axis of the standard curve and extrapolate it to the corresponding enzyme activity on X-axis. The results obtained were expressed in RF units/ml.

APPENDIX - D

ESTIMATION OF ALKALINE PHOSPHATASE (ALP) (KIND & KING'S METHOD)

PRINCIPLE

Alkaline phosphatase from Serum converts Phenyl phosphate to inorganic phosphate and phenol at pH 10.0 phenol so formed reacts in alkaline medium with Aminoantipyrine in presence of oxidising agent potassium Ferricyanide and forms an orange-red colored complex, which can be measured colorimetrically. The colour intensity is proportional to the enzyme activity.

The Reaction can be represented as :

$$\text{Phenyl Phosphate} \xrightarrow[\text{pH 10.0}]{\text{Alk. Phosphatase}} \text{Phenol + pi}$$

$$\text{Phenol + 4-AA} \xrightarrow[OH^-]{\text{Pot. Ferricyanide}} \underset{(\lambda \text{ max} = 510\text{-}520 \text{ nm})}{\text{Orange-red coloured complex}}$$

The following reagents were used :

Reagent 1: Buffered Substrate, pH 10.0

Reagent 2: Sodium Hydroxide, 0.5 N

Reagent 3: Sodium Bicarbonate, 0.5 N

Reagent 4: 4-Aminoantipyrine, 0.6%

Reagent 5: Potassium Ferricyanide, 2.4%

Reagent 6: Stock Phenol Standard, 10 mg%.

Working solution I : Each vial of Reagent 1 was reconstituted with 3 ml of distilled water and mixed well.

Working solution II : Reagent 4 was dissolved in 50 ml distilled water.

Working solution III : Reagent 5 was dissolved in 50 ml distilled water.

Working standard : Diluted stock phenol standard 1:10 with distilled water.

Solution 1 and working standard are made fresh, just before use

The following procedure was followed :

For colorimeter

	Blank(B)	Standard(S)	Control(C)	Test(T)
Solution I:	-	-	1.0 ml	1.0 ml
Distilled water	2.1 ml	1.1 ml	1.0 ml	1.0 ml
	Mixed well and incubate at 37°C for 3 minutes			
Working standard	-	1.0 ml	-	-
Serum	-	-	-	0.1 ml
	Mixed well and incubated at 37°C for 15 minutes			
Reagent 2: Sodium Hydroxide 0.5 N	0.8 ml	0.8 ml	0.8 ml	0.8 ml
Serum	-	-	0.1 ml	-
Reagent 3: Sodium Bicarbonate 0.5 N	1.2 ml	1.2 ml	1.2 ml	1.2 ml
Solution II:	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Solution III	1.0 ml	1.0 ml	1.0 ml	1.0 ml

Mixed well after the addition of each reagent and measured the O.D. of Blank (B), Standard (S), Control (C) and Test (T) against distilled water using a green filter.

CALCULATIONS

Serum Alkaline Phosphatase activity in KA Units

$$= \frac{\text{O.D. test - O.D. control}}{\text{O.D. std. - O.D. blank}} \times 10$$

Mixed well after the addition of each reagent and measured the O.D. of Blank (B), Standard (S), Control (C) and Test (T) against distilled water using a green filter.

CALCULATIONS

Serum Alkaline Phosphatase activity in KA Units

$$= \frac{\text{O.D. test - O.D. control}}{\text{O.D. std. - O.D. blank}} \times 10$$

APPENDIX - E

ESTIMATION OF SERUM IMMUNOGLOBULINS BY SSPT* (Pfieffer and MC. Guire, 1977)

Reagents : Sodium sulfite (Na_2So_3) concentrations of 14, 16 and 18 per cent solutions were prepared by adding 14, 16 and 18 gms. of sodium sulphite each in a total volume of 100 ml distilled water.

Procedure : 0.1 ml of serum is added to 1.9 ml of each of the three concentrations of sodium sulphite solution respectively. The samples mixed thoroughly and allowed to stand undisturbed for one hour at room temperature to permit maximum precipitation.

Interpretation : If the precipitation was observed only in 18 per cent sodium sulphite solution, the Ig concentration was less than 5 mg/ml of serum. If the precipitation was seen both in 18 per cent and 16 per cent sodium sulphite solution the Ig concentration was between 5 to 15 mg/ml of serum. If the precipitation was observed in all 14, 16 and 18 per cent sodium sulphite solution, Ig concentration was more than 15 mg/ml of serum.

* Sodium sulphite precipation test

APPENDIX - F

DEPARTMENT OF MEDICINE
COLLEGE OF VETERINARY SCIENCE, RAJENDRANAGAR,
HYDERABAD - 500 030

SCORE SHEET

"BUFFALO CALF MORTALITY PARTICULARS FOR THE LAST 5 YEARS"

1. *Farm/Location* :
2. *Date* :
3. ***Number of calves*** :
4. No. of stock :
5. Particulars on colostrum feeding :
6. Date of birth :
7. Date of death :
8. No.of calves affected :
9. Season effected :
10. Particulars of the calf

    Age Sex: Male/Female; Breed _____
    Birth weight _____

11. Deworming routinely practised :
12. Symptoms observed :
13. Treatment given :
14. P.M. report :
15. Cause of death/ Final diagnosis :

APPENDIX - G

DEPARTMENT OF MEDICINE
COLLEGE OF VETERINARY SCIENCE, RAJENDRANAGAR,
HYDERABAD - 500 030

SCORE SHEET FOR ASCARIASIS IN BUFFALO CALVES

1. Place : 2. Date : 1993

3. Description of the calf :

   Age : Sex: Male/Female; Breed:

4. Colostrum given/not given

   Colostrum quantity given: Time of feeding after birth:

5. Faecal sample : Positive/Negative

Eggs per gram (E.P.G.)

Before therapy	After therapy					
0 day	2nd day	3rd day	7th day	14th day	21st day	28th day

6. Clinical signs :

7. Blood parameters

	Before therapy		After therapy 14th		After therapy 21st	
A. T.E.C. millions/c.m.m	_____		_____		_____	
B. T.L.C. Thousand/c.m.m	_____		_____		_____	
C. D.L.C %	N E B	L M	N E B	L M	N E B	L M
D. Hemoglobin g/dl	_____		_____		_____	
E. P.C.V.%	_____		_____		_____	
F. Serum glucose mg/dl	_____		_____			
G. SGOT (AST)	_____		_____			
H. SGPT (ALT)	_____		_____			
I. Alkaline phosphatase	_____		_____			
J. Immunoglobulins	_____		_____		_____	

8. Therapy: Group: 1. Healthy control
Group: 2. Piperazine
Group: 3. Ivermectin
Group: 4. Levamisole

9. Remarks

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