A STUDY ON THE PREVALENCE AND THERAPEUTIC MANAGEMENT OF COLIBACILLOSIS IN CALVES

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THESIS

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MASTER OF VETERINARY SCIENCES
(Veterinary Medicine)

2011

Dedicated

TO MY
Beloved PARENTS
&
SISTER

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
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Shuhama Campus Srinagar– 190 006
-::o::-

Certificate – I

This is to certify that the thesis entitled, **"A Study on the Prevalence and Therapeutic Management of Colibacillosis in Calves"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Sciences (Veterinary Medicine)**, to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Dr. Riyaz Ahmed Bhat (Regd. No. 2009-V-131-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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We, the members of the Advisory Committee of **Dr. Riyaz Ahmed Bhat (Regd. No. 2009-V-131-M**, a candidate for the degree of **Master of Veterinary Sciences (Veterinary Medicine)** have gone through the manuscript of the thesis entitled, **"A Study on the Prevalence and Therapeutic Management of Colibacillosis in Calves"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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ABSTRACT

Studies on prevalence of colibacillosis in calves under different organizational setup and seasons were conducted during August, 2010 to July, 2011. The overall prevalence recorded was 86.8%. The season wise prevalence was 86.96, 91.33, 90.32 and 63.33% during autumn, winter, spring and summer respectively being higher in winter (91.33%) and lower in summer (63.33%). Prevalence was higher in un-organized farms (88.89%) as compared to organized farms (85.96%). One hundred and twenty nine isolates of *E. coli* got serotyped, of which one hundred sixteen serotypes belonged to 22 different 'O' serogroups (O20, O22, O69, O11, O84, O147, O68, O107, O123, O153, O157, O1, O6, O17, O36, O51, O60, O92, O102, O140, O158 and O159), 8 isolates were untypable and 5 were rough. The highest percentage was found for O20 serotype (22.48%) followed by O22 (14.73%) and O69 (10.08%).

In-vitro drug sensitivity pattern indicated that most of the serotypes were highly sensitive to four antibacterials, viz., ciprofloxacin, gentamicin, neomycin and co-trimoxazole. Tetracycline and enrofloxacin were moderately sensitive. Penicillin was low sensitive, where as, the isolates were resistant to amoxicillin and ampicillin.

Haematobiochemical studies in infected animals revealed significant increase in packed cell volume, haemoglobin, total leucocyte count and total erythrocyte count, where as, the biochemical parameters viz., total protein, albumin, blood glucose, sodium and potassium were significantly decreased.

The therapeutic efficacy of four highly sensitive *in-vitro* drugs viz., ciprofloxacin, gentamicin, neomycin and co-trimoxazole was evaluated. Ciprofloxacin at the dose rate of 4mg/kg body weight was found 100 per cent effective at 144^{th} hour post treatment. Gentamicin at the dose rate of 4mg/kg body weight was found 83.33 per cent effective at 144^{th} hour post treatment where as neomycin @10mg/kg body weight and co-trimoxazole @ 15-30 mg/kg body weight were found 66.67 per cent effective in this study at 144^{th} hour post treatment. Treatment regimen used in four different groups of clinical cases of colibacillosis indicated ciprofloxacin being most effective drug followed by gentamicin, neomycin and co-trimoxazole which was comparable with *in-vitro* studies.

Key words : Colibacillosis, calves, prevalence, haematobiochemical, serotypes

Signature of Student

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Riyaz Ahmed Bhat

Place : Shuhama, Srinagar

Dated:

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CHAPTER – 1

INTRODUCTION

Livestock rearing is an integral part of Indian rural life and makes multifaceted contribution to the growth and development of the agriculture sector. Over last two decades, livestock sector has grown at an annual rate of 5.6 per cent, which is higher than growth in agriculture sector (3.3%) (Ali, 2007). Therefore, livestock industry is likely to emerge as an engine of agricultural growth in the coming decade. In India, over 70 per cent of rural house-holds own livestock and majority of them are small, marginal and landless farmers (Taneja and Birthal, 2004). Several empirical studies indicate that livestock rearing has a significant positive impact on equity in terms of income, employment and poverty reduction in rural areas (Thorton *et al.,* 2002) as distribution of livestock is more egalitarian compared to land (Taneja and Birthal, 2004).

The Indian sub-continent possesses the largest livestock population in the world. Cattle population in India is about 187.38 million which is about 15 per cent of the world cattle population (1.3 billion). Out of the 187.38 million cattle, 22.63 million are cross bred, which is 12.07 per cent of the total cattle population (17^{th} livestock census, 2003). Jammu and Kashmir has a total cattle population of 3.084 million as per live stock census of 2003 (Digest of Statistics, 2007-08).

The productivity of domesticated animals in India is poor due to varied complex factors. They are further compounded by clinical or sub-clinical diseases caused by bacteria, viruses and parasites. The bacterial diseases inflict losses through morbidity, mortality, decreased production and by way of costs incurred on treatment and control. Amongst the bacterial infections, colibacillosis is wide spread in the Indian sub-continent and has been frequently reported from plains and hilly tracts.

Colibacillosis is one of the important diseases in new born calves, caused by pathogenic serotypes of *Escherichia coli* (Carlton, 1992) and characterized by

prostration, profuse diarrhoea and septicemia. It is a major cause of economic loss in new born calves.

Escherichia coli belongs to a family *Enterobacteriaceae* and is a short Gram negative, non-spore forming and usually peritrichous and fimbriate bacillus. *Escherichia coli* was first isolated by Theobald Escherich in 1885 from faeces of infants. Laruelle in 1889 was the first to suggest the possible pathogenicity of this organism, while Jensen in 1893 reported it being the cause of white scour in calves. Over 700 antigenic types or serotypes of *Escherichia coli* have been recognized based on O, H and K antigens. Serotyping is important in distinguishing the small number of strains that actually cause disease.

Colibacillosis has been associated both with enteric or septicaemic disease in newborn farm animals (Radostits *et al*., 2000), enteric colibacillosis is characterized by varying degrees of diarrhoea, dehydration, acidosis, and death in a few days if not treated, whereas, coliform septicemia is characterized by severe illness and rapid death in several hours. Enteric colibacillosis is caused by enterotoxigenic strains of *Escherichia coli* that colonize and proliferate in the upper small intestine and produce enterotoxins, which cause an increase in net secretion of fluid and electrolytes from the systemic circulation. The adhesion of *Escherichia coli* to the intestinal epithelial cells is mediated by bacterial pili. *Septicemic colibacillosis* (coliform septicemia) occurs as a result of invasive strains of *Escherichia coli* invading the tissues and systemic circulation via, the intestinal lumen, nasopharyngeal mucosa and tonsillar crypts, or umbilical vessels. These strains are able to invade extraintestinal tissues, to resist the bactericidal effect of complement, to survive and multiply in body fluids, to escape phagocytosis and intracellular killing by phagocytes, and to induce tissue damage by the release of cytotoxins. Calves that are deficient in colostral immunoglobulins are highly susceptible to septicemic colibacillosis.

The prevalence of enterotoxigenic *Escherichia coli* in diarrhoeic calves varies widely geographically, between herds and depending on the age of the animals.

The prevalence can be as high as 50-60 per cent in diarrheic calves under 3 days of age and only 5-10 per cent in diarrhoeic calves 8 days of age but in some countries the prevalence is only 5-8 per cent in diarrhoeic calves under 3 days of age (Radostits *et al*., 2000). It is the major cause of calf scours causing mortality between 5-25 per cent (Basoglu, 1992) or even as high as 54.58 per cent (Khan and Khan, 1997). The importance of Colibacillosis in cattle depends on its prevalence and its effect on productivity and the value of the animal.

Although the administration of intravenous fluids and oral electrolyte solutions plays a central role in treatment, the efficacy of antimicrobial agents in treating calf diarrhoea is controversial (Constable, 2004). In treating Colibacillosis in neonatal calves, pefloxacin is most effective followed by amoxycilline and metranidazole-furazolidone combination (Kaur *et al*., 2002) however, Fernandes *et al.* (2009a) reported sulphadiazine-trimethoprim combination as drug of choice in neonatal calf diarrhoea.

The proposed study is, therefore, planned and aimed to detect and characterize the field isolates of *E. coli* from diarrhoeic calves by cultural, biochemical methods with the identification of key serotypes involved in disease progression in order to design effective therapeutics. The present study is thus conceived with the following objectives:

1) To study the prevalence of the colibacillosis in calves in organized and unorganized farms.
2) To study the haemato-biochemical changes in calves associated with colibacillosis.
3) To evaluate the *in-vitro* drug efficacy in clinical cases of colibacillosis.

CHAPTER – 2

REVIEW OF LITERATURE

2.1 Clinical studies

Aly *et al.* (1993) while studying clinical symptoms in neonatal buffalo calf diarrhoea recorded profuse watery diarrhoea, progressive dehydration, acidosis and finally death within a few days.

Constable *et al.* (1998) reported that clinical assessment of dehydration in neonatal calves is best assessed by utilizing extent of eyeball recession into the orbit and skin pliability in the lateral neck region. In calves with cachexia or chronic diarrhoea, skin pliability in the neck region is the most reliable indicator of hydration status.

Kumar *et al.* (2002) reported that crossbred calves suffering from enteritis revealed increased respiration and heart rate, profuse diarrhoea with loose consistency and mild dehydration is evidenced by sunken eyes and reduced skin elasticity in calves suffering with enteritis.

Ahmad (2007) recorded profuse watery to pasty, usually pale yellow and occasionally streaked with blood flecks and very foul smelling faeces in diarrhoeic buffalo calves.

Guzelbektes *et al.* (2007) reported that diarrhoeic calves with 4-8 per cent dehydration had a weak suckle reflex, dry mucous membranes, warm mouth, and partly good muscular tone, in addition to the other general clinical symptoms like enophthalmos; 2-4 mm, decreased cervical skin elasticity-tent duration 4-6 s, cool extremities and ability to stand but with difficulty. Calves with 10 per cent and above dehydration, were unable to stand, had no sucking reflex, and cold mouth, in addition to the other general clinical symptoms like enophthalmos; 6 mm and over, decreased cervical skin elasticity-tent duration; 7 s and above, cold extremities, white mucous membranes and recumbency.

Tikoo *et al.* (2009) reported calves with diarrhoea as having foul smelling, profuse watery pale yellow faeces with soiled tail and perineum, sunken eyes, dry mucus membrane, cold extremities and dehydration ranging from 8-12 per cent on the basis of skin tent time. Extreme depression as indicated by recumbency and loss of suckle reflex was also noticed in some diarrheic calves.

2.2 Sero-prevalence

Escherichia coli was first described by Theoder Escherich in 1885 while Jensen in 1893 reported it being the cause of white scour in calves. Over 700 antigenic types or serotypes of *Escherichia coli* have been recognized based on O, H, and K antigens. Serotyping is important in distinguishing the small number of strains that actually cause disease.

Kaura *et al.* (1991) isolated *E. coli* from diarrhoeic buffalo and cow calves and reported serotypes O4, O7, O9, O11, O17, O20, O24, O25, O40, O55, O74, O86, O101, O110, O123, O129, O131, O142, O149, O153 and O166 from buffalo calves and O1, O5, O7, O8, O9, O13, O17, O25, O71, O77, O86, O101, O117, O132, O153, and O169 from cow calves.

Oswald *et al*. (1991) studied forty three diarrhoeic bovine isolates of *E. coli*, out of which 29 strains were typable and belonged to 10 different O serogroups in which the three main serogroups were O78 (eight strains), O123 (four strains) and O4 (four strains).

Minakshi *et al.* (1992) isolated O4, O9, O25, O7, O8, O11, O26, O101, O109, O110, O129, O123 *E. coli* serotypes from diarrhoeic buffalo calves and O4, O17,. O18, O8, O19, O20, O22, O25, O55, O170, O101, O103, O113, O132, O153 serotypes from diarrhoeic cow calves.

Mainil (1999) reported serotypes O5:H-, O8:H8, O20:H9, O26:H11, O103:H2, O111:H-, O118:H16, O128:H- and O145:H- associated with diarrhoea in calves.

Hussain and Saikia (2000) analysed 93 faecal samples collected from 1 to 35 days old diarrhoeic calves wherein all the samples yielded *Escherichia coli* (101

strains),of which 88(87.12%) could be serotyped into 32 serogrpoups. Serogroup O8, O9, O21, O55 and O123 were found to be the most predominant.

Blanco *et al.* (2003) isolated VTEC from cattle and sheep. The most frequently detected serotypes in cattle were O20:H19, O22:H8, O26:H11, O77:H41, O105:H18, O113:H21, O157:H7, O171:H2 and untypable: H19 whereas serotypes O5: H-, O6:H10, O91: H, O117: H-, O128: H-, O128:H2, O146:H8, O146:H21, O156:H- and untypable :H21 were found more frequently in sheep.

Hussain *et al.* (2003) isolated 101 *E. coli* strains from diarrhoeic calves. These strains belonged to the serogroups O8, O21 and O116 (two strains each), O7, O15, O18, O22, O101, O111, O123 and O141 (one strain each) and one untypable strain.

Sharma *et al.* (2004) examined a total of 146 faecal samples from diarrhoeic calves and recovered thirty nine serotypes, three strains were untypable and three strains were rough. Serogroups predominantly obtained were O123 (12.12%), O78 (8.33%), O23 (7.5%), O2 (6.06%), O8 (5.30%), O25 (5.30%), O55 (3.03%), O60 (3.03%), O4 (3.03%), O33 (2.27%) and O157 (1.51%).

Wani *et al.* (2004) reported 144 strains of *E. coli* isolated from a total of 340 faecal samples from calves. Out of 144 strains 5(3.47% each) were untypable and rough and 134 strains were belonging to different serogroups viz., O2, O3, O6, O7, O8, O9, O10, O12, O15, O16, O20, O22, O25, O26, O33, O45, O46, O50, O54, O55, O60, O75, O77, O78, O85, O86, O88, O89, O91, O96, O100, O101, O102, O105, O107, O109, O111, O114, O115, O123, O125, O131, O132, O146, O148, O150, O153, O156, O157, O161, O162, O164 and O165.

Fernandes *et al.* (2009b) isolated *E. coli* in 48 out of 70 clinical cases of neonatal bovine entero-colibacillosis which belonged to nine different serotypes viz., O1, O4, O13, O20, O29, O60, O76, O78 and O109.

Vagh and Jani (2010) isolated *E. coli* from diarrhoeic cattle calves and buffalo

calves and recovered serotypes O56, O82, O8, O164, O66, O17, O5, O169, O76, O171, O103, O20 and O100 from cow calves and O56, O82, O97, O13, O89, O131, O5, O48, O169, O25, O3, O8, O4, O16, and O132 from buffalo calves.

2.3 Antibiotic sensitivity test

Neonatal calf diarrhoea is one of the stumbling blocks for the development of dairy industry. Despite vaccination programs and managemental measures, treatment with antibiotics may be required in some cases. Although antimicrobial susceptibility testing is recommended, information on drug resistance trends in a particular geographic area is helpful to veterinarians in drug selection for empirical therapy. The result of culture and antibiotic sensitivity studies may be valuable as background information for further therapy for effective treatment and control of disease. Otherwise indiscriminate use of antimicrobial drugs may lead to serious health hazards and development of drug resistance.

Patil *et al*. (1999) studied drug resistance pattern of *Escherichia coli* isolates from diarrhoeic calves and reported that all *Escherichia coli* (18) were multidrug resistant. The maximum resistance was observed against oxytetracycline (94.44%), followed by ampicillin (88.88%), triple sulpha (88.88%) and streptomysin (83.33%).Whereas least resistance was observed against ciprofloxacin (5.55%), nitrofurazone (5.55%) and gentamicin (11.11%).

Kobayashi *et al.* (2001) tested antimicrobial susceptibility among *E. coli* isolates using 14 antimicrobial agents and found that only eight isolates were resistant to some of the antimicrobial drugs tested. Six of these isolates were resistant to aminoglycosides (kanamycin and dihydrostreptomycin) and/or tetracyclines (chlortetracycline and oxytetracycline) and the other two showed resistance to colistin.

Chattopadhyay *et al.* (2001) studied the antibiotic sensitivity pattern of STEC strains from animal, human and food products and reported that they were uniformly sensitive to common antibiotics except tetracycline, cephalexin,

dicloxacillin, erythromycin and lincomycin.

Khan *et al.* (2002) examined antimicrobial resistance pattern of 63 shiga toxin producing *E. coli* isolates from 19 human stool samples, 40 cow faecal samples and 4 beef samples in Kolkata using 15 antimicrobials. Resistance was observed most commonly to ampicillin (25.4%), tetracycline (23.8%) and streptomycin (14.3%) and less frequently to cephalothin (1.1%), co-trimoxazole (9.5%), nalidixic acid (6.4%) and neomycin (3.2%). One third of these strains (35%) showed reduced susceptibility to different antimicrobial agents, but were not completely resistant to any of the antibiotics. About 14.3 per cent of the isolates were sensitive to all antimicrobials used. Fourteen *E. coli* strains showed multiple drug resistance and no common resistance pattern among the strains was recorded.

Chattopadhyay *et al*. (2003) tested 13 STEC strains for antimicrobial susceptibility amoxicillin, amikacin, cephalexin, cefotaxime, chloramphenicol, ciprofloxacin, co-trimoxazole, furaxone, gentamicin, nalidixic acid and norfloxacin and tetracycline. Antimicrobial susceptibility pattern showed that 68.2, 61.5, 46 and 30.76 per cent were resistant to co-trimoxazole, furaxone, tetracycline/nalidixic acid and cephalexin respectively. Most of the strains were sensitive to norfloxacin, gentamicin and chloramphenicol.

Hariharan *et al.* (2004) reported that among eight antimicrobial drugs (apramycin, ceftiofur, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, trimethoprim-sulfa) tested *in vitro*, least resistance (≤ 8%) was seen against ceftiofur followed by gentamicin and florofenicol among the ETEC isolates from diarrheic calves tested.

Sharma *et al.* (2004) carried out antibiotic sensitivity test in *E. coli* strains from diarrhoeic calves and found that *E. coli* strains were most sensitive to oflaxacin (97.28%), kanamycin (93.24%) followed by gentamicin (85.62%), co-trimoxazole (71%), nalidixic acid (57.15%), streptomycin (52.50%), doxycycline (50%), oxytetracycline (30.78%) and trimethoprim (26.27%).

2.4 Haemato-biochemical study

Radhakishan *et al*. (1991) recorded a significant elevation in PCV (43.7%) in diarrheic neonatal buffalo calves as against (35%) the normal calves. The DLC showed increased values for neutrophils and lymphocytes which indicated the presence of intestinal infection. The total proteins were decreased from 8.4 per cent in normal calves to 5.74 per cent in diarrheic calves and ascribed it to deficient anabolic nitrogen retention during diarrheic period.

Dubey *et al.* (1992) reported a decline in albumin levels in calves suffering from colibacillosis and ascribed it to loss through inflamed gut epithelium.

Deshpande *et al.* (1993) reported a significant decrease in plasma sodium and glucose level in scouring calves while as plasma potassium, chloride, blood urea nitrogen (BUN) and total protein were significantly increased.

Sahal *et al.* (1993) reported hyperkalemia in calves with colibacillosis.

Olutosin *et al.* (2001) reported that there were no significant differences in mean values for plasma sodium, potassium and chloride concentrations between healthy and diarrheic calves.

Kumar *et al.* (2002) reported a non significant increase in Hb concentration, packed cell volume &total erythrocyte count but significantly lower value of plasma sodium in crossbred calves with clinical colibacillosis.

Kaur *et al.* (2006) reported that colibacillosis results in a significant hemoconcentration and PCV estimation was the most sensitive indicator for assessing the dehydration. They also reported that there was no significant change in total plasma protein and globulin levels of diarrhoeic calves as compared to healthy control calves. Significant increase in plasma albumin however, was observed. Besides plasma sodium and potassium levels were high in diarrheic calves as compared to healthy calves.

Seifi *et al.* (2006) reported a significant difference in the hematological profile of

diarrheic calves from that of normal ones. The concentration of potassium was also significantly higher in diarrheic calves that died than in diarrheic calves that survived. Diarrheic calves with potassium levels above 5.63mEq/l were four times more likely to die.

Fernandes *et al.* (2009c) while studying the hematological profile recorded increased Hb concentration, PCV, MCH and TLC concurrent with decreased MCV in diarrhoeic calves.

Roy *et al.* (2009) reported that the mean PCV values of diarrhoeic calves were increased significantly in comparison to healthy calves.

Tikoo and Soodan (2009) recorded increased values of haemoglobin, total protein and BUN in diarrhoeic neonatal calves.

2.5 Therapeutic studies

Lofstedt *et al.* (1999) reported that routine use of oral and injectable antibiotics cannot be recommended in calves without systemic illness. In calves with diarrhoea and severe systemic involvement antimicrobial therapy must be pondered carefully as intercurrent disease is not uncommon and the risk of bacteremia or septicaemia is increased. High risk calves in this respect are under 5 days of age, have failure of passive transfer and are recumbent with no sucking reflex.

Kaur *et al.* (2002) reported that pefloxacin is highly effective as compared to amoxicillin in treating colibacillosis in neonatal calves. Metronidazole-Furazolidone combination was found to be moderately effective in the treatment of colibacillosis.

Chaleva *et al.* (2003) reported ampicillin trihydrate administered orally at a dose of 60 mg/kg body weight twice daily for seven successive days was successful in treating diarrheic calves.

Grove-White (2004) considered the use of antimicrobials to treat calf diarrhoea to

be controversial and not indicated.

Constable (2004) reported parenteral administration of broad spectrum β-lactam antimicrobials-ceftiofer (2.2 mg/kg IM) and amoxycilline or ampiclline (10 mg/kg IM) or potentiated sulphonamides (25 mg/kg IV) is effective in treating calves with diarrhoea and systemic illness.

Ortman and Svensson (2004) reported that antimicrobials should be avoided to diarrheic calves that have a normal appetite, activity level, rectal temperature, and hydration status and the absence of concurrent infections such as pneumonia or omphalophlebitis.

Morley *et al*. (2005) reported that veterinarians should use and prescribe antimicrobials conservatively to minimize potential adverse effects on animal or human health.

Fernandes *et al.* (2009a) reported that symptoms abated earliest in diarrhoeic calves treated with sulphadiazine trimethoprim followed by amoxicillin trihydrate, where as calves treated with ciprofloxacin-tinodazole recurrence of diarrhoea was observed in 2 calves.

CHAPTER – 3

MATERIALS AND METHODS

3.1 Study on prevalence

For conducting studies on the prevalence of colibacillosis in calves, 250 rectal swabs were collected from diarrhoeic calves below 3 months of age irrespective of sex and age for the isolation of *Escherichia coli* reared under different managemental conditions. The sample sites included:

a) Organized farms which included
   - Cattle Research Station (CRS) Manasbal
   - Military Dairy Farm, Qamarwari, Srinagar

b) Unorganized areas/ selected areas of district Ganderbal and Srinagar which include local villages.

c) Clinical complex, FVSc & AH.

The study was conducted during all the four seasons viz spring/ summer/ autumn/winter during the period from August 2010 to July 2011. Rectal swabs were collected aseptically and were processed within 2-4 hours for the isolation and identification of *Escherichia coli* using standard cultural, microbiological and biochemical tests. The isolates were subjected to drug sensitivity tests using standard procedures (Bauer *et al.,* 1966).

3.1.1 Isolation procedure

The procedure described by Cruickshank *et al.* (1975) was adopted for the isolation of *Escherichia coli*. The suspected material collected under sterile measures was inoculated in single strength MacConkey's broth/Nutrient broth with bile salts and incubated at 37^{o}C for 24 hours. A loopful of inoculam from each tube showing gas production was streaked on MacConkey's agar media plates and incubated at 37^{o}C for 24 hours. An isolated, Smooth, glossy and

translucent, centrally raised rose-pink colony was picked up and transferred on Eosin Methylene-blue (EMB) agar media and incubated in similar manner. The characteristic smooth, raised and mucoid colony with typical "metallic sheen" was considered to be *Escherichia coli* and were finished out. One or two such colonies were stained by Gram's Method and simultaneously transferred to nutrient agar slants for further study.

3.1.2 Identification of isolates

The isolates were identified on the basis of morphological, cultural and biochemical characteristics.

3.1.3 Morphological and cultural characteristics

Morphology of the isolates was studied by examining the smear under oil immersion lens after staining by Gram's Method. Young cultures were looked for the typical shapes, sizes, arrangements. Cultures were also grown in nutrient broth and few were tested for motility by hanging drop method.

3.1.4 Biochemical tests

E. coli isolates were preliminary identified by biochemical tests viz., Indole, Methylred, Voges proskaeur and citrate utilization. The isolates were further characterized for their biochemical activity by the following tests viz., carbohydrate fermentation, urea hydrolysis, production of H_2S on TSI.

3.1.4.1 Indole test

The ability of isolates to decompose amino-acid Tryptophan to indole was tested by adding 0.5 ml of Kovac's reagent to 2 ml of freshly prepared broth culture. After gentle shaking, appearance of a bright red or pink ring at the top of the fluid was taken as an indication of indole production.

3.1.4.2 Methyl red (MR) test

Production of sufficient acid during fermentation of glucose by the isolates was tested by addition of a few drops of methyl red indicator to 3 to 5 ml of culture

media grown in glucose phosphate peptone water. After thorough mixing, a bright red colour was considered as a positive reaction and that of yellow as negative.

3.1.4.3 Voges-Proskauer (V.P) test

Production of acetyl methyl carbinol or its reduction products 2, 3, butylenes glycol by the isolates in glucose phosphate peptone water was tested by adding 0.6 ml of 5 per cent ethanolic solution of alfa-naphthol and 0.2 ml of 40 per cent potassium hydroxide solution to 1 ml of 3 to 5 day old broth culture. Development of pink colour was considered as positive reaction.

3.1.4.4 Simmon's citrate utilization test

The test was preformed as per the procedure of Cowan (1974). The ability of isolates to utilize citrate as the sole source of carbon was demonstrated by streaking the simmon's citrate agar slants with a fresh broth culture. Growth on the streaks after incubation at 37^{o}C for 72 hours with a change in colour of media was considered positive for the utilization of citrate.

3.1.4.5 Triple sugar iron (TSI) agar test

Triple sugar iron agar slants were prepared by the method of Cowan (1974). Agar slants were stabbed and streaked with a fresh culture a using a straight wire loop. The slants were then incubated at 37^{o}C for 72 hours. Change of color from yellow to red without blackening of the butt was considered as a positive reaction for presence of members of members of Enterobacteriaceae.

3.1.4.6 Urease test

The ability of isolates of to hydrolyze urea was tested using Christensen's medium. The slant were inoculated with fresh cultures and incubated at 37^{o}C for 5 days, during which these were observed for the development of a purple pink colour as the positive reaction.

3.1.5 Serotyping of *E. coli*

The suspected *E. coli* isolates were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (Himachal Pradesh) for serotyping.

3.2 *In-vitro* drug sensitivity of *E. coli* isolates

The bacterial isolates were subjected to *in vitro* antibiotic sensitivity test as per the method of Bauer *et al.* (1966) against a panel of 9 antibiotics (Table 1). Isolates were grown in nutrient broth at 37^{o}C for 16 hrs. Individual broth cultures were smeared on the Mueller-Hinton (MH) agar plates with the help of a sterile cotton swabs. Plates were allowed to dry for few minutes, antibiotic discs (Hi-Media) were placed on the agar surface within 15 minutes of inoculation of plates and were incubated overnight at 37^{o}C. The sensitivity or resistance of an isolate for a particular antibiotic was determined by measuring the diameter of the zone of inhibition of growth. The results were interpreted as sensitive or resistant based as per the guidelines provided by National Committee for Clinical Laboratory Standards (2004).

Table 1: Various antimicrobial agents and their respective concentration

S. No.	Antibiotic	Concentration (µg)
1	Ampicillin	10
2	Amoxycillin	10
3	Ciprofloxacin	5
4	Neomycin	30
5	Penicillin-G	10 units
6	Gentamycin	10
7	Enrofloxacin	10
8	Tetracycline	30
9	Co-tromoxazole	25

3.3 Experimental studies

3.3.1 Selection of calves

The clinical trial was designed on thirty calves showing the symptoms of diarrhoea and dehydration and were divided into five groups (six animals each). Four drug regimens were used in four groups which contained four different antibacterials, oral rehydration therapy and other supportive therapy (Table 2). Only those antibacterials were used which showed moderate to high sensitivity pattern, were readily available and economically feasible. The below mentioned group wise regimen was given to calves.

Systematic clinical examination was done starting from the 0 day before treatment and 24 hours, 48 hours and 144 hours post treatment.

Table 2 : Therapeutic trail design in calves with colibacillosis

Groups	Group No.	No. of Animals	Drug	Dose	Route
Healthy	1	6	-	-	-
Infected and untreated	2	6	-	-	-
Infected and treated	3	6	Co-trimoxazole	15-30 mg/kg BW (Bolus 1/5th orally)	Orally
Infected and treated	4	6	Neomycin	10mg/kg BW (Bolus 1/2nd orally)	Orally
Infected and treated	5	6	Gentamicin	4mg/kg BW (2 ml i/m BID daily)	Parenterally
Infected and treated	6	6	Ciprofloxacin	4mg/kg (250mg tab-1/3rd BID)	Orally

3.3.2 Clinical Symptoms

Detailed symptoms and clinical observations were recorded in all the animals of different groups. The clinical symptoms observed included :

3.3.2.1 Vital parameters

Temperature (^{o}F)

Respiration Rate (/min)

Pulse Rate (/min)

Heart Rate (/min)

3.3.2.2 Clinical examination

1) Number of animals affected.
2) Time of onset and frequency of diarrhoea was recorded.
3) Hydration status of the calf was determined on the basis of:

i) Cervical Skin fold test (sec).

The skin fold test was performed by tenting the skin of the lateral portion of the cervical region of neck and measuring the time (seconds) required for the skin fold to return to normal.

ii) Visible mucous membranes and extremities.

Oral mucosa was examined for temperature, moisture and color.

iii) Capillary refill time was evaluated for peripheral perfusion.

3.3.3 Haematological and biochemical studies

3.3.3.1 Collection of blood samples

For haematological studies, blood was collected from each animal of all groups in clean sterilized glass vials containing EDTA, as anticoagulant. The samples were collected on "0" day and then after 48 hours, 96 hours and 144 hours post treatment. For separation of serum 10 ml of blood was collected in sterile centrifuge tubes and kept in slanting position for about an hour at room temperature. The blood clot was broken and subsequently centrifuged at 2000 rpm for 30 minutes to obtain serum.

3.3.3.2 Haematological studies

Haematological studies included :

i) Determination of haemoglobin by Sahli's method

ii) Packed cell volume (PCV) by Wintrobe method.

iii) Total erythrocyte count (TEC) and total leucocyte count (TLC) with haemocytometer as per methods of Schalm *et al.* (1986).

iv) Differential leucocyte count (DLC) was carried out by standard laboratory method.

3.3.3.3 Biochemical studies

Serum was used for the estimation of glucose, total proteins, albumin, globulin, sodium and potassium as per the following methods :

i) Glucose was estimated by Tinder method (1969) using reagents supplied by Crest Biosystems, Goa, India.

ii) Total protein and albumin was determined by Doumas method (1975) using the kits supplied by Coral-Clinical System (Crest Biosystems) Goa, India.

iii) The serum electrolytes, Sodium and Potassium were estimated using the Flame photometric method (Oser, 1965)

3.4 Statistical analysis

The results were subjected to statistical analysis as per the method described by Snedecor and Cochran (1994).

CHAPTER – 4

EXPERIMENTAL FINDINGS

4.1 Epidemiological studies

Studies on the prevalence of colibacillosis in calves was conducted during different seasons and geographical areas of Kashmir valley. In all 250 diarrhoeic faecal samples were screened for the presence of *E. coli* of which 217 tested positive with an overall prevalence of 86.8 per cent. Prevalence was higher (88.89%) in un-organised farms compared to organised farms (85.96%). Variability in the prevalence of colibacillosis was also observed with different seasons with higher infection rates in winter (91.36%) followed by spring (90.32%) autumn (86.96%) and summer (63.33%) (Table-3).

Table-3 : Overall colibacillosis prevalence during different seasons in calves of Kashmir valley

	Spring	**Summer**	**Autumn**	**Winter**	**Total**
Total samples	93	30	46	81	250
Positive	84	19	40	74	217
Negative	9	11	6	7	33
%age	90.32	63.33	86.96	91.36	86.8

4.1.1 Organization setup

Out of a total of 250 faecal samples collected, 217 were found positive with an overall prevalence percentage of 86.8. In organized farms- of the 178 samples collected, 153 were positive for *E. coli* with prevalence percentage of 86.96%, whereas, in the unorganized areas, out of 72 faecal samples collected, 64 were positive with a prevalence percentage of 88.89% (Table 4).

Table-4 : Prevalence of Colibacillosis in Organized and Unorganized Farms of Kashmir Valley

	Organized	Un-organized	Total
N	178	72	250
n	153	64	217
%age	85.96	88.89	86.8

N = Total number of samples, n = Number of positive samples

4.1.2 Isolation and identification of *E. coli* isolates

For isolation of *E. coli*, MacConkey agar (MCA) and eosin methylene blue agar (EMB) were used as differential and selective plating media. Afore mentioned 250 faecal samples were initially screened on MCA. Pink coloured colonies on MCA plates (Plate-1) were considered to be lactose fermenting isolates. Two such pink colonies from each MCA plate were picked up and transferred to EMB agar plate. Colonies with greenish metallic sheen (Plate-2) on EMB agar plates were tentatively considered to be of *E. coli*. The *E. coli* isolates were stained by Gram's method to check for the purity and then characterized by biochemical tests viz., IMViC pattern (indole production, Methyl Red (MR) test, Voges-Proskauer (V.P.) test, citrate utilization on

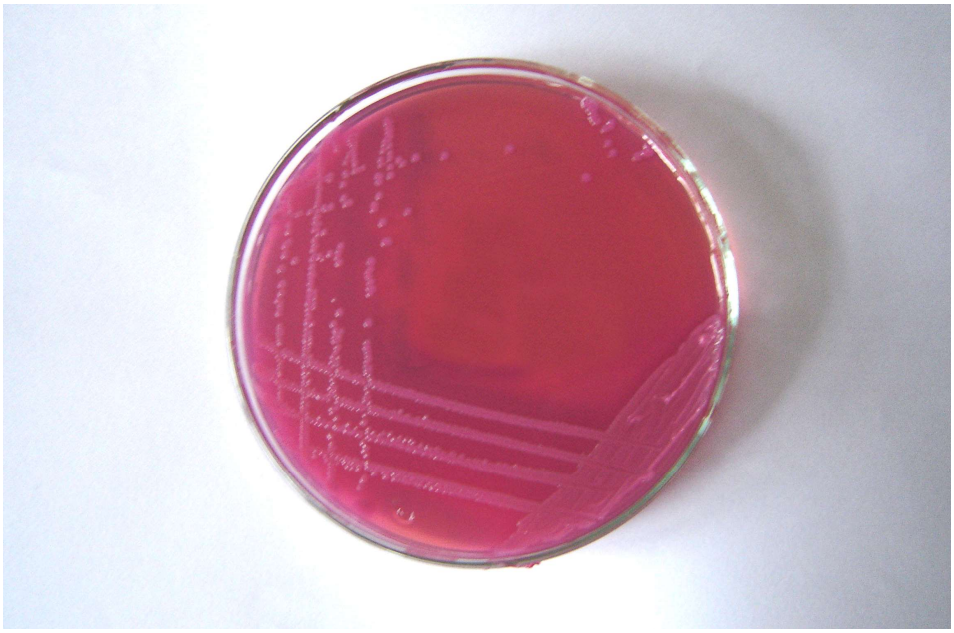

Plate-1 : **Lactose fermenting pink colonies of *E. coli* on MacConkey agar**

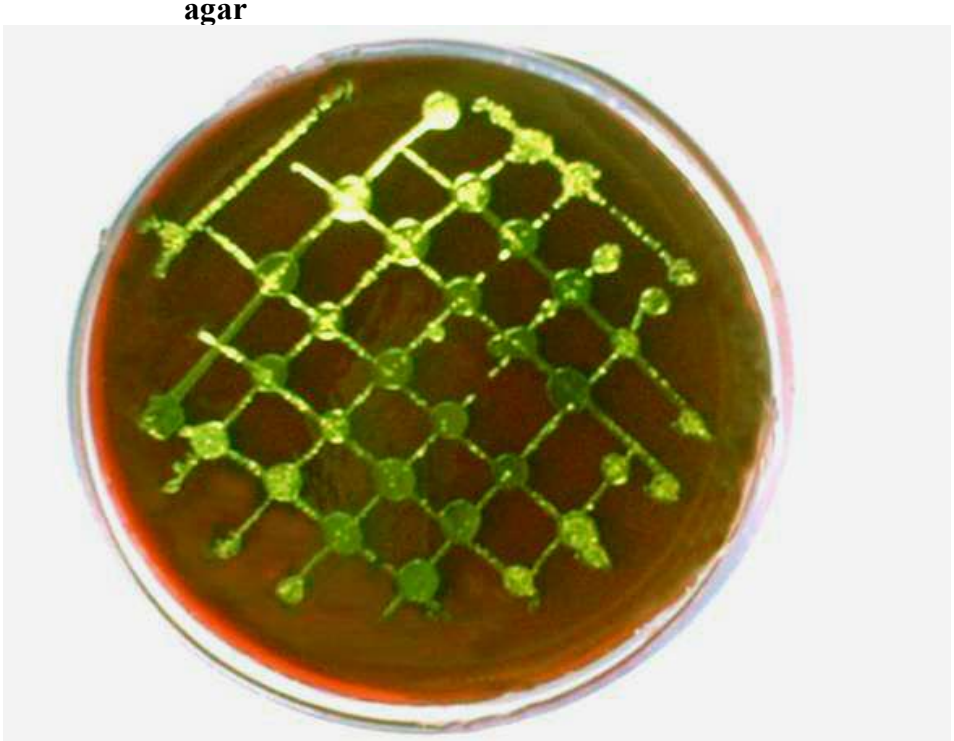

Plate-2 : ***E. coli* colonies with characteristic greenish metallic sheen on EMB agar**

Simmon's citrate medium), urea hydrolysis, production of H_2S on TSI agar, carbohydrate fermentation using seven sugars as per the method described by Edwards and Ewing (1972). On the basis of Gram's staining the isolates were found to be Gram negative bacilli. In all 217 isolates exhibited similar IMViC pattern of + + - - and were confirmed as *E. coli.*

4.1.3 Sero prevalence of *E. coli* isolates

One hundred twenty nine *E. coli* isolates were sent to National Salmonella and Escherichia Centre Kasauli (Himachal Pradesh) for serotyping. All the isolates of *E. coli* were typed for 'O' antigen. Out of 129 *E. coli* isolates 116 isolates belonged to 22 different 'O' serogroups while 8 isolates were untypable and 5 were rough isolates (Table 5). Among the 116 'O' serotypes the highest percentage of 22.48 was found for O20 serotype followed by O22 and O69 serotypes having percentage of 14.73 and 10.08 respectively. Serotypes O11 and O84 were having percentage of 6.98 each where as serotype O147 was having percentage 3.88. Percentage of 2.33 was seen in serotypes O68, O107, O123, O153 and O157. Serotypes O1, O6, O17, O36, O51 and O60 were having percentage of 1.55 each. The lowest percentage of 0.78 was found for O92, O102, O140, O158 and O159. Eight isolates were untypable with percentage 6.20 and 5 were rough isolates having percentage of 3.88.

Among the 129 isolates 55 were from organised farms and 74 were from un-organised areas (Table 6). Serotypes O11, O17, O51, O68, O102, O140 and O147 were found in organised farms where as serotypes O1, O6, O36, O60, O92, O153, O157, O158 and O159 were found in un-organised areas where as serotypes O20, O22, O69, O84, O107 and O123 were found in both organized farms as well as in un-organised areas.

Table-5 : ***Escherichia coli*** **serotypes isolated from calves suffering from colibacillosis**

S. No.	O Serotypes	frequency	%age
1	O20	29	22.48
2	O22	19	14.73
3	O69	13	10.08
4	O11	9	6.98
5	O84	9	6.98
6	O147	5	3.88
7	O68	3	2.33
8	O107	3	2.33
9	O123	3	2.33
10	O153	3	2.33
11	O157	3	2.33
12	O1	2	1.55
13	O6	2	1.55
14	O17	2	1.55
15	O36	2	1.55
16	O51	2	1.55
17	O60	2	1.55
18	O92	1	0.78
19	O102	1	0.78
20	O140	1	0.78
21	O158	1	0.78
22	O159	1	0.78
23	UT	8	6.20
24	Rough	5	3.88

Table-6 : ***Escherichia coli*** **serotypes isolated from calves from organised farms and un-organised areas**

S. No.	Organized farms		Un-organised areas	
	O Serotypes	**Frequency**	**O Serotypes**	**Frequency**
1	**O20**	**11**	**O20**	**18**
2	O11	9	**O69**	**10**
3	**O22**	**7**	**O22**	**12**
4	O147	5	**O84**	**7**
5	O68	3	O153	3
6	**O69**	**3**	O157	3
7	O17	2	O1	2
8	O51	2	O6	2
9	**O84**	**2**	O36	2
10	**O107**	2	O60	2
11	**O123**	2	O92	1
12	O102	1	**O107**	1
13	O140	1	**O123**	1
14	UT	3	O158	1
15	Rough	2	O159	1
			UT	5
			Rough	3

4.2 Antibiotic Sensitivity test

Antibiotic sensitivity patterns of the *E. coli* isolates were determined by disc diffusion method (Bauer *et al.,* 1966). The *E. coli* isolates were tested against nine commonly used antibiotics viz., Ampicillin (A), Ciprofloxacin (Cf), Co-trimoxazole (Co), Enrofloxacin (Ex), Amoxicillin (Am), Neomycin (N), Penicillin (P), Gentamicin (G) and Tetracycline (T).

Among the 95 isolates of *E. coli* tested against various antibiotics ciprofloxacin was found highest sensitive (71.58 %) where as amoxicillin, ampicillin and penicillin were having least sensitivity. The gentamicin, neomycin, co-trimoxazole, enrofloxacin and tetracycline were having 66.32, 54.74, 53.68, 45.26 and 32.63 per cent of sensitivity respectively (Table 7).

Enrofloxacin and tetracycline were having moderate sensitivity of 44.21 and 37.89 per cent respectively. Amoxicillin (85.26%), ampicillin (80.00%) were highly resistant where as ciprofloxacin, gentamicin, co-trimoazole and neomycin were found least resistance with percentage 2.11, 4.21, 4.22 and 5.26 per cent respectively (Plate-3).

4.3 Clinical Findings

Clinical signs recorded were diarrhoea, soiling of perineum and tail, depression, varying degree of dehydration, tachycardia, increased respiration rate, pale mucous membrane, prolonged capillary refill time, rough body coat, dry muzzle or mouth and profound weakness. The faeces was semisolid to watery with offensive odor, yellowish white in colour and sometimes blood stained (Plate-4).

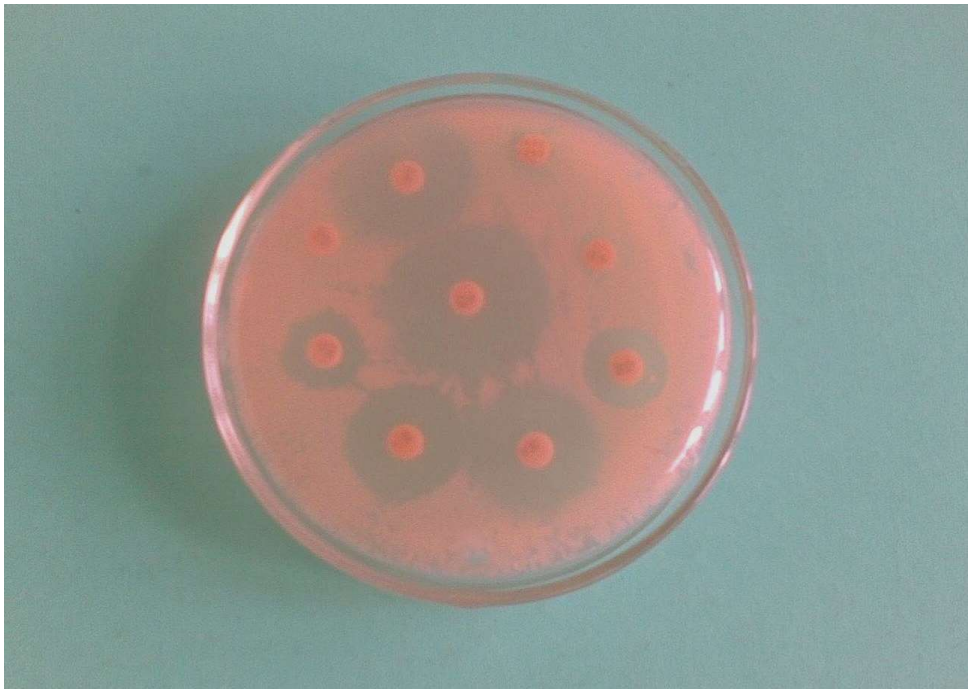

Plate-3 : ***In vitro*** **antimicrobial drug sensitivity pattern of** ***E. coli*** **isolates**

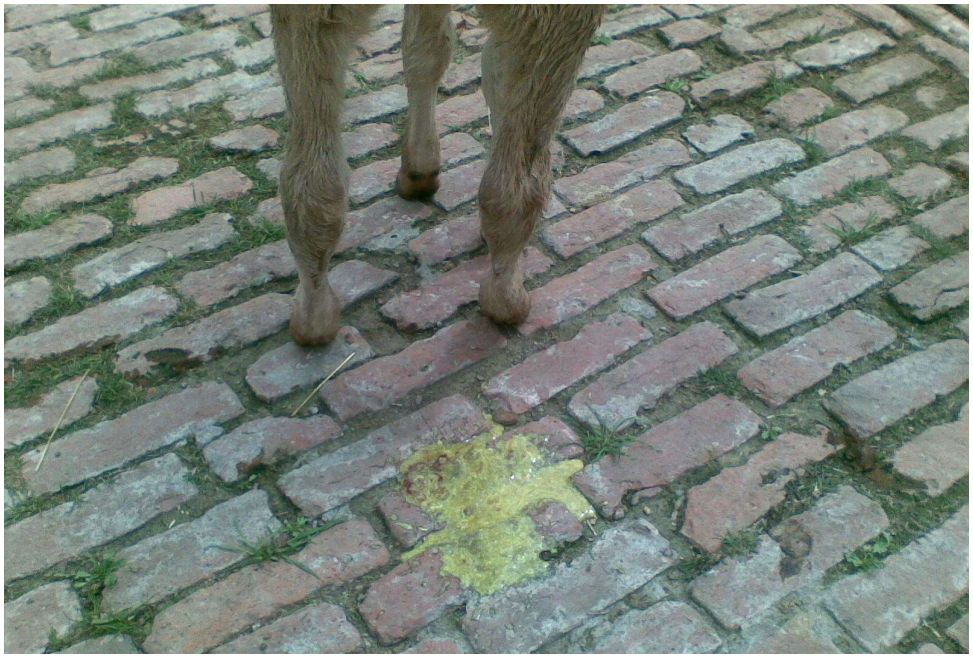

Plate-4 : Yellowish watery diarrhoea

Table-7 : Drug sensitivity pattern of *E. coli* isolates

S. No.	Antibiotics	Highly Sensitive N=95		Moderate Sensitive N=95		Low Sensitive N=95		Resistant N=95	
		No.	%age	No.	%age	No.	%age	No.	%age
1.	Ampicillin	-	-	-	-	19	20.00	76	80.00
2.	Amoxicillin	-	-	-	-	14	14.74	81	85.26
3.	Neomycin	52	54.74	35	36.84	3	3.16	5	5.26
4.	Gentamicin	63	66.32	24	25.26	4	4.21	4	4.21
5.	Ciprofloxacin	68	71.58	24	25.26	1	1.05	2	2.11
6.	Penicillin	-	-	-	-	67	70.53	28	29.47
7.	Co-trimoxazole (trimethoprim/ sulphamethoxazole)	51	53.68	37	38.95	3	3.16	4	4.21
8.	Enrofloxacin	43	45.26	42	44.21	4	4.21	6	6.32
9.	Tetracycline	31	32.63	36	37.89	18	18.95	10	10.53

4.3.1 Clinical Evaluation of Dehydration

4.3.1.1 Skin fold test

The hydration status of the diarrhoeic calves as revealed by skin fold test is represented in Table-8. In infected groups dehydration percentage ranged from 6-8 per cent, as compared to healthy control group in which no dehydration was seen at '0' hours.

There was gradual decrease in degree of dehydration in all infected treatment groups as compared to that of day 0 dehydration values. In co-trimoxazole treated group at 144^{th} hr post treatment mild dehydration was recorded but in case of neomycin and gentamicin there was no dehydration. In ciprofloxacin treated group the no detectable dehydration was recorded from 96^{th} hr after treatment.

4.3.1.2 Capillary refill time (seconds) (Mean)

The hydration status of the diarrhoeic calves as revealed by capillary refill time is represented in Table-9. In infected groups capillary refill time (CRT) ranged from 3.21-3.68 seconds, as compared to healthy control group in which capillary refill time was 1.51 at '0' hours.

There was gradual decrease in capillary refill time in all infected treatment groups as compared to 0 hr. In co-trimoxazole treated group at 144^{th} hr post treatment 2.22 sec of CRT was recorded but in case of neomycin and gentamicin CRT recorded was less than 2 sec. In ciprofloxacin treated group CRT recorded was less than 2 sec from 96^{th} hr after treatment.

Table-8 : Skin fold test (seconds) (Mean)

Groups	Group No.	0 hr		48 hr		96 hr		144 hr	
		sec	% dehydration	sec	% dehydration	sec	% dehydration	sec	% dehydration
Healthy	1	1.9	0	1.8	0	2.0	0	1.7	0
Infected and untreated	2	5.4	6	5.1	6	4.8	6	4.1	4
Co-trimoxazole	3	5.3	6	4.8	6	4.1	4	3.2	2
Neomycin	4	5.4	6	4.3	4	3.2	2	1.9	0
Gentamicin	5	5.9	8	4.4	4	2.3	2	1.3	0
Ciprofloxacin	6	5.1	6	3.2	2	2.3	0	1.2	0

Table-9 : Capillary refill time (seconds) (Mean)

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	1.51	1.48	1.50	1.47
Infected and untreated	2	3.62	3.44	3.12	2.92
Co-trimoxazole	3	3.39	2.91	2.13	2.22
Neomycin	4	3.43	2.97	2.34	1.99
Gentamicin	5	3.68	2.85	1.98	1.67
Ciprofloxacin	6	3.21	2.87	1.66	1.46

4.3.2 Temperature

The results of temperature (^{o}F) recorded at different stages in the healthy and infected animals are represented in Table-10 and Fig. 1. In infected groups the (Mean±SE) value of temperature (^{o}F) ranged between 101.33±0.43 to 102.30±0.21 (^{o}F) at 0 hr which were comparable with healthy control group (101.67±0.20 ^{o}F).

In co-trimoxazole and neomycin treated groups there was non-significant increase in temperature at 48th hr and after then there was progressive non-significant decrease in temperature which reached to 101.42±0.07 and 101.47±0.15^{o}F respectively at 144th hr post treatment which were non-significantly lower than healthy control (101.80±0.15 ^{o}F) and infected untreated groups (101.52±0.16^{o}F).

In gentamicin treated group there was significant decrease in temperature at 96th hr after treatment (101.80±0.12^{o}F) as compared to 0 hr (102.18±0.17^{o}F) and was comparable with healthy control group (101.72±0.18^{o}F) and infected untreated groups (102.00±0.15^{o}F).

In neomycin treated group there was non-significant increase in temperature at 48th hr post treatment and after then there was progressive non-significant decrease in temperature and its value reached to 101.62±0.07 ^{o}F at 144th hr of treatment which was significantly ($P<0.05$) lower than that recorded at 0 hr (102.30±0.21^{o}F) and was comparable with healthy control (101.80±0.15^{o}F) and infected untreated groups (101.52±0.16^{o}F).

4.3.3 Pulse Rate

The results of pulse rate (pulsation per min) (ppm) recorded at different stages in the healthy and infected animals are represented in Table-11 and Fig. 2. In infected groups the (Mean±SE) value of pulse rate (ppm) ranged between 74.50±2.11 to 78.00±1.34 ppm at 0 hr which were significantly ($P<0.05$) higher than healty control group 68.67±0.84 ppm.

Table-10 : Mean±SE of Temperature (^{o}F) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	101.67 ± 0.20^{Aa}	101.63 ± 0.09^{Aa}	101.72 ± 0.18^{Aa}	101.80 ± 0.15^{Aa}
Infected untreated	2	101.33 ± 0.43^{Aa}	101.78 ± 0.20^{ABa}	102.00 ± 0.15^{Aa}	101.52 ± 0.16^{Aa}
Co-trimoxazole	3	101.80 ± 0.44^{Aa}	102.00 ± 0.15^{ABCa}	101.77 ± 0.07^{Aa}	101.42 ± 0.07^{Aa}
Neomycin	4	101.70 ± 0.35^{Aa}	101.88 ± 0.16^{ABCa}	101.67 ± 0.11^{Aa}	101.47 ± 0.15^{Aa}
Gentamicin	5	102.18 ± 0.17^{Abc}	102.22 ± 0.14^{BCc}	101.80 ± 0.12^{Aab}	101.53 ± 0.08^{Aa}
Ciprofloxacin	6	102.30 ± 0.21^{Aa}	102.35 ± 0.13^{Ca}	101.93 ± 0.09^{Aab}	101.62 ± 0.07^{Ab}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

Table-11 : Mean±SE of Pulse Rate (ppm) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	68.67 ± 0.84^{Aa}	69.33 ± 1.09^{Aa}	69.17 ± 0.48^{Aa}	69.33 ± 1.38^{ABa}
Infected untreated	2	74.50 ± 2.11^{Ba}	73.67 ± 2.62^{ABa}	71.88 ± 1.97^{ABa}	71.22 ± 1.70^{Aa}
Co-trimoxazole	3	73.00 ± 2.97^{Ba}	72.67 ± 1.38^{ABa}	69.00 ± 1.03^{Aa}	68.83 ± 1.25^{Ba}
Neomycin	4	78.00 ± 1.34^{Ca}	78.00 ± 0.93^{Ca}	74.00 ± 0.82^{Bab}	70.12 ± 0.93^{ABb}
Gentamicin	5	78.00 ± 0.93^{Ca}	76.83 ± 1.85^{Ba}	72.17 ± 1.99^{ABb}	68.50 ± 1.23^{Bb}
Ciprofloxacin	6	77.00 ± 1.44^{Ca}	76.00 ± 1.71^{Bab}	71.83 ± 1.62^{ABbc}	68.33 ± 1.05^{Bc}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

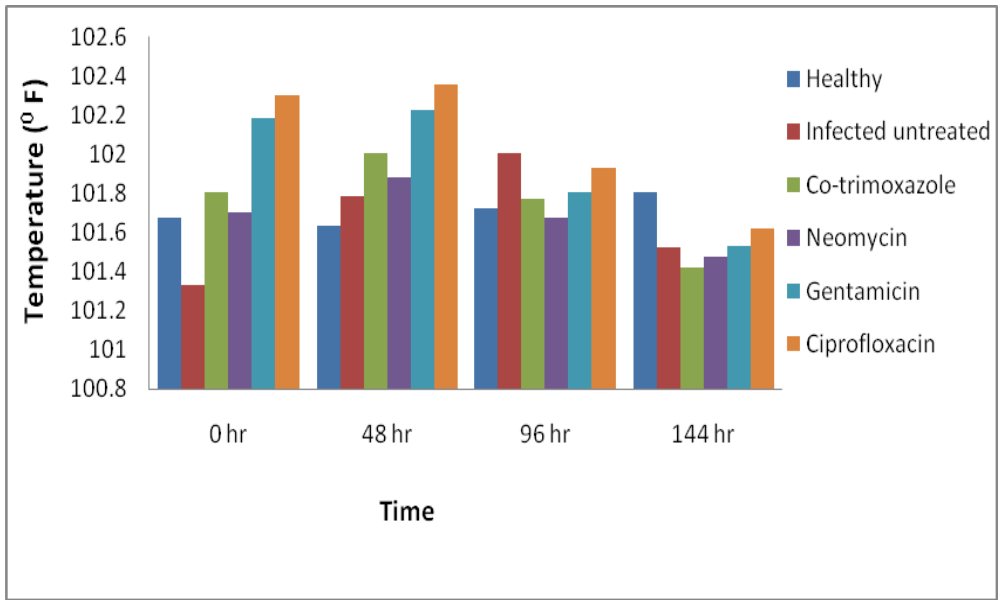

Fig. 1 : Temperature (°F) in Calves with Clinical Colibacillosis before and after treatment

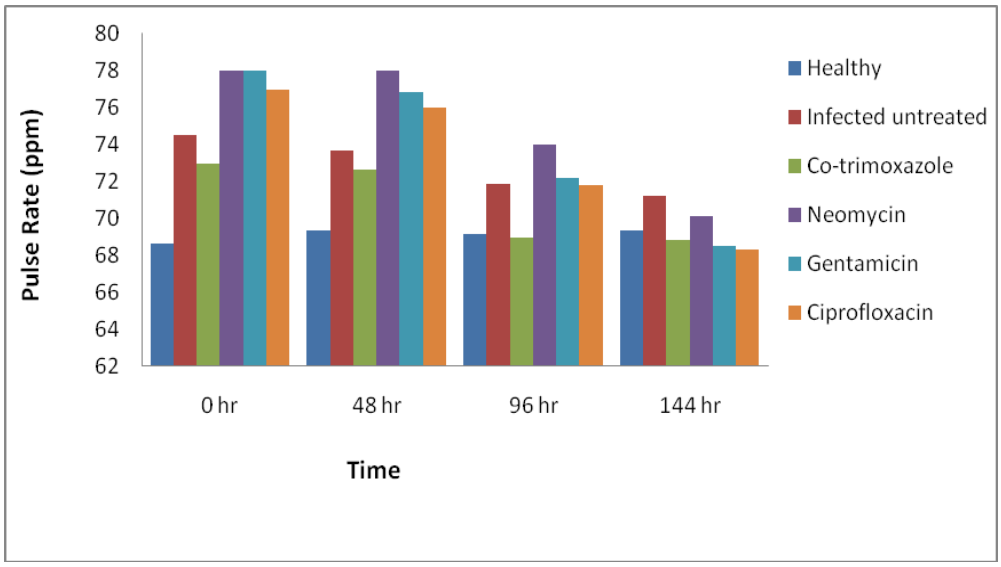

Fig. 2 : Pulse Rate (ppm) in Calves with Clinical Colibacillosis before and after treatment

In co-trimoxazole treated group there was progressive non-significant decrease in pulse rate its value reached to 68.83±1.25 ppm at 144th hr post treatment which was comparable with healthy control group (69.33±1.38 ppm) and significantly (P<0.05) lower than infected untreated control group (71.22±1.70 ppm).

In neomycin treated group there was significant decrease in pulse rate at 144th hr post treatment (70.12±0.93 ppm) as compared to 0 hr (78.00±1.34 ppm) and was comparable with healthy control group (69.33±1.38 ppm) and non-significantly lower than infected untreated control group (71.22±1.70 ppm).

In gentamicin and ciprofloxacin treated groups there was significant decrease in pulse rate at 96th hr after treatment (72.17±1.99 and 71.83±1.62 ppm respectively) as compared to 0 hr and were comparable with healthy control group (69.17±0.48 ppm) and non-significantly lower than infected untreated control group (71.88±1.97 ppm).

4.3.4 Respiration

The results of respiration breaths per min (bpm) recorded at different stages in the healthy and infected animals are represented in Table-12 and Fig. 3. In infected groups the (Mean±SE) value of respiration (bpm) ranged between 34.17±1.62 to 36.33±1.59 bpm at 0 hr which were significantly (P<0.05) higher than healthy control group (30.00±1.39 bpm).

In co-trimoxazole treated group there was progressive decrease in respiration rate and reached to 29.33±1.31 bpm at 144th hr after treatment and was significantly (P<0.05) lower compared to that recorded at 0 hr and was also non-significantly lower than infected untreated control group (31.33±1.36 ppm) but was comparable with healthy control group (29.50±0.56 bpm).

In neomycin, gentamicin and ciprofloxacin treated groups respiration rate recorded at 96th hr post treatment (33.83±1.64, 31.00±0.52 and 30.33±1.12 bpm respectively) were significantly (P<0.05) lower as compared to that recorded at 0 hr and lower than infected untreated control (34.00±1.24 bpm) and were comparable with healthy control group (28.83±0.79 bpm).

Table-12 : Mean±SE of Respiration Rate (bpm) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	30.00 ± 1.39^{Aa}	28.33 ± 0.84^{Aa}	28.83 ± 0.79^{Aa}	29.50 ± 0.56^{Aa}
Infected untreated	2	34.66 ± 0.58^{Ba}	33.17 ± 1.08^{Ba}	34.00 ± 1.24^{Ba}	31.33 ± 1.36^{Aa}
Co-trimoxazole	3	35.33 ± 1.36^{Ba}	34.33 ± 1.33^{Ba}	31.83 ± 1.56^{ABab}	29.33 ± 1.31^{Ab}
Neomycin	4	36.33 ± 1.59^{Ba}	35.67 ± 1.54^{Ba}	33.83 ± 1.64^{Bb}	30.00 ± 1.00^{Ab}
Gentamicin	5	34.17 ± 1.62^{Ba}	33.67 ± 0.72^{Bab}	31.00 ± 0.52^{ABbc}	28.50 ± 0.56^{Ac}
Ciprofloxacin	6	34.50 ± 1.09^{Ba}	32.83 ± 0.98^{Bab}	30.33 ± 1.12^{ABbc}	28.33 ± 1.09^{Ac}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

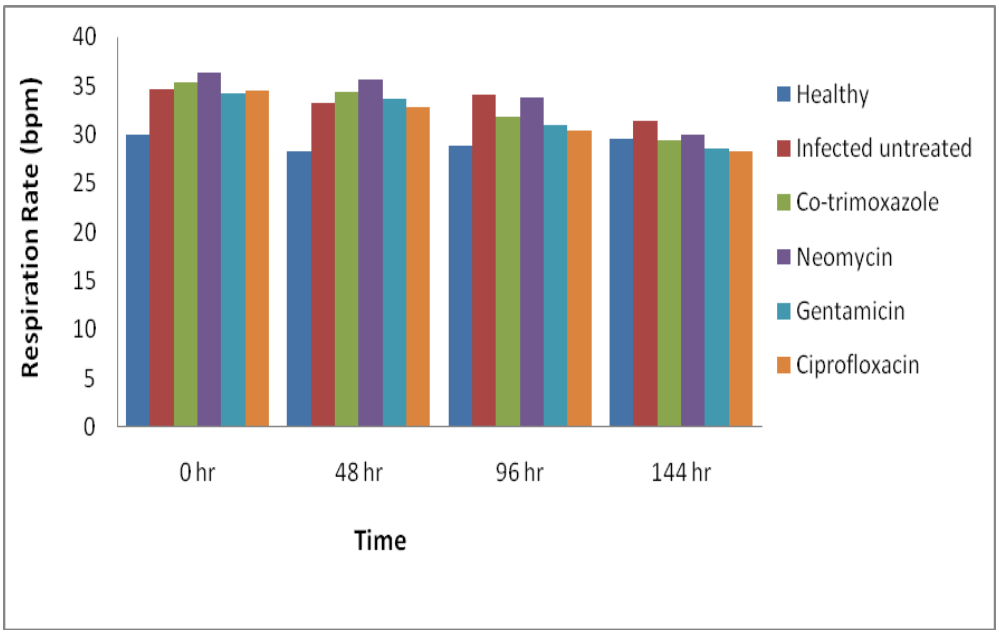

Fig. 3 : Respiration Rate (bpm) in Calves with Clinical Colibacillosis before and after treatment

4.3.5 Heart Rate

The results of heart rate (beats per min) (bpm) recorded at different stages in the healthy and infected animals are represented in Table-13 and Fig. 4. In infected groups the (Mean±SE) value of heart rate (bpm) ranged between 72.33±2.88 to 76.50±1.46 bpm at 0 hr which were significantly ($P<0.05$) higher than healthy control group (67.33±0.92 bpm).

In co-trimoxazole and neomycin treated groups the heart rate recorded at 144th hr post treatment (67.67±1.33 and 69.00±1.07 bpm respectively) were non-significantly lower than that recorded at 0 hr and were also lower than infected untreated group (71.01±1.67 bpm) but comparable with healthy control (67.50±1.43 bpm).

In gentamicin and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in heart rate at 96th hr of treatment (71.17±1.89 and 70.33±1.52 bpm respectively) as compared to that recorded at 0 hr and at 144th hr of treatment became significantly ($P<0.05$) lower than infected untreated group (71.01±1.67 bpm) but comparable with healthy control (67.50±1.43 bpm).

4.4 Haematological findings

4.4.1 Packed cell volume

The results of packed cell volume (PCV) percentage estimated at different stages in the healthy and infected animals are represented in Table-14 and Fig. 5. In infected groups the (Mean±SE) value of PCV percentage ranged between 40.27±1.55 to 44.22±0.86 percent at 0hr and were significantly ($P<0.05$) higher as compared to healthy control group (37.72±0.55 %).

In co-trimoxazole group there was significant ($P<0.05$) decrease in PCV % at 144th hr post treatment (38.26±1.40%) as compared to 0 hr (43.38±1.53%) and was comparable with healthy control group (38.48±0.27%) and infected untreated group (39.07±1.42%).

Table-13 : Mean±SE of Heart Rate (bpm) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	67.33 ± 0.92^{Aa}	68.33 ± 1.28^{Aa}	69.00 ± 0.52^{ABa}	67.50 ± 1.43^{Ba}
Infected untreated	2	73.33 ± 1.76^{Ba}	73.17 ± 2.06^{ABa}	70.22 ± 1.76^{ABa}	71.01 ± 1.67^{Aa}
Co-trimoxazole	3	72.33 ± 2.88^{Ba}	71.17 ± 1.99^{ACa}	67.17 ± 1.66^{Aa}	67.67 ± 1.33^{Ba}
Neomycin	4	76.50 ± 1.46^{Ca}	76.67 ± 1.26^{Ba}	71.83 ± 0.87^{Ba}	69.00 ± 1.07^{ABa}
Gentamicin	5	76.17 ± 0.98^{Ca}	75.83 ± 1.80^{Ba}	71.17 ± 1.89^{Bb}	67.21 ± 0.87^{Bb}
Ciprofloxacin	6	75.33 ± 1.65^{Ca}	75.50 ± 1.43^{Ba}	70.33 ± 1.52^{ABb}	66.50 ± 1.06^{Bb}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

Table-14 : Mean±SE of Packed Cell Volume Percentage (PCV%) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	37.72 ± 0.55^{Aa}	37.59 ± 0.54^{Aa}	37.91 ± 0.51^{Aa}	38.48 ± 0.27^{ABa}
Infected untreated	2	40.27 ± 1.55^{Ba}	42.40 ± 1.76^{Ba}	40.47 ± 1.51^{Aa}	39.07 ± 1.42^{ABa}
Co-trimoxazole	3	43.38 ± 1.53^{Ca}	40.63 ± 1.37^{ABab}	39.44 ± 1.72^{Aab}	38.26 ± 1.40^{ABb}
Neomycin	4	41.29 ± 2.08^{Ba}	39.19 ± 1.64^{Aa}	37.30 ± 1.55^{Ab}	36.47 ± 1.62^{Bb}
Gentamicin	5	41.36 ± 0.69^{Ba}	40.09 ± 0.62^{ABa}	37.78 ± 0.71^{Ab}	36.41 ± 0.88^{Bb}
Ciprofloxacin	6	44.22 ± 0.86^{Ca}	42.89 ± 0.82^{Bab}	41.25 ± 0.86^{Ab}	40.31 ± 1.05^{Ab}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

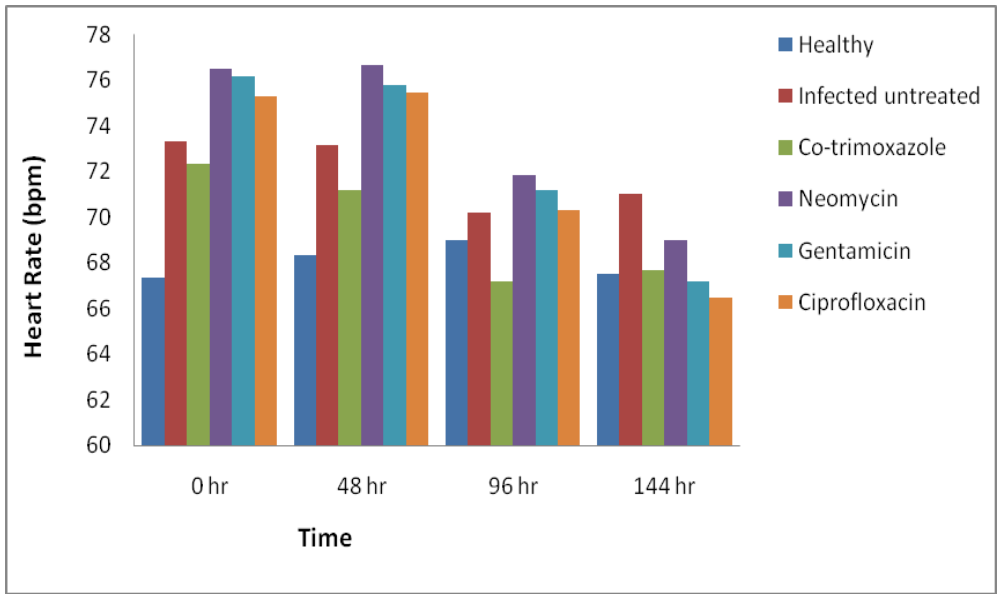

Fig. 4 : Heart Rate (bpm) in Calves with Clinical Colibacillosis before and after treatment

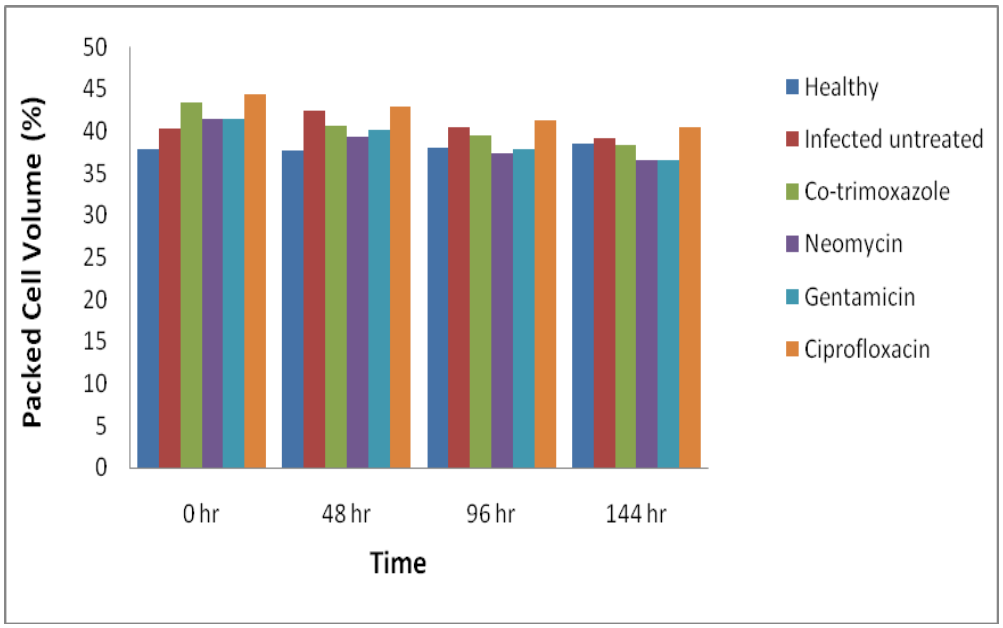

Fig. 5 : Packed Cell Volume Percentage (PCV%) in Calves with Clinical Colibacillosis before and after treatment

In neomycin, gentamicin and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in PCV% at 96^{th} hr post treatment and values recorded were 37.30±1.55, 40.09±0.62 and 42.89±0.82%, respectively as compared to 0 hr and were comparable with healthy control group (37.91±0.51%) and infected untreated group (40.47±1.51%).

4.4.2 Haemoglobin

The results of haemoglobin (g/dl) estimated in calves suffering from Colibacillosis and healthy control group are represented in Table-15 and Fig. 6. In infected groups the (Mean±SE) value of haemoglobin (g/dl) ranged between 12.99±0.92 to 13.71±0.46 g/dl at 0 hr and were significantly ($P<0.05$) higher as compared to healthy control group (11.57±0.24 g/dl).

In co-trimoxazole, neomycin and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in haemoglobin (g/dl) at 96^{th} hr post treatment and values recorded were 11.08±0.76, 11.30±0.30 and 11.44±0.33 g/dl respectively as compared to haemoglobin (g/dl) at 0 hr and were comparable to healthy control group (11.34±0.12 g/dl) and significantly ($P<0.05$) lower than infected untreated group (13.40±0.56 g/dl).

In gentamicin treated group there was significant ($P<0.05$) decrease in haemoglobin (g/dl) at 96^{th} hr and value recorded was 12.00±0.48 as compared to haemoglobin (g/dl) at 0 hr (13.71±0.46 g/dl) and was non-significantly higher than healthy control group (11.34±0.12 g/dl) and non-significantly ($P<0.05$) lower than infected untreated group (13.40±0.56 g/dl).

4.4.3 Total Leukocyte Count

The results of total leukocyte count (TLC $\times 10^3/\mu l$) estimated at different stages in the healthy and infected animals are represented in Table-16 and Fig. 7. In infected groups the (Mean±SE) value of total leukocyte count ranged between 7.99±0.31 to 10.67±0.34 $\times 10^3/\mu l$ at 0 hr and were significantly ($P<0.05$) higher than healthy control group (7.00±0.27 $\times 10^3/\mu l$).

Table-15 : Mean±SE of Haemoglobin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	11.57±0.24^{Aa}	11.98±0.26^{ABa}	11.34±0.12^{Ba}	11.41±0.09^{Ba}
Infected untreated	2	12.98±0.20^{Ba}	13.31±0.36^{Ba}	13.40±0.56^{Aa}	12.71±0.50^{Aa}
Co-trimoxazole	3	13.38±0.92^{Ba}	12.60±0.85^{ABab}	11.08±0.76^{Bb}	10.79±0.77^{Cb}
Neomycin	4	12.99±0.42^{Ba}	11.94±0.36^{ABab}	11.30±0.30^{Bbc}	10.69±0.26^{Cc}
Gentamicin	5	13.71±0.46^{Ba}	12.93±0.42^{ABab}	12.00±0.48^{ABbc}	11.11±0.45^{Bc}
Ciprofloxacin	6	13.00±0.33^{Ba}	12.23±0.29^{ABab}	11.44±0.33^{Bbc}	10.00±0.37^{Cc}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly (P<0.05

Table-16 : Mean±SE of Total Leukocyte Count (TLC × 10^3/µl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	7.00±0.27 ^{Aa}	7.27±0.26 ^{Aa}	7.35±0.35 ^{Aa}	7.20±0.31 ^{Aa}
Infected untreated	2	7.99±0.31 ^{Ba}	7.51±0.27 ^{ABa}	6.99±0.22 ^{Aa}	6.93±0.29 ^{Aa}
Co-trimoxazole	3	10.67±0.34 ^{Ca}	9.56±0.36 ^{Cb}	8.87±0.42 ^{Bb}	8.68±0.36 ^{Bb}
Neomycin	4	8.78±0.47 ^{Ba}	8.31±0.41 ^{Bab}	7.66±0.33 ^{Aab}	7.16±0.34 ^{Ab}
Gentamicin	5	8.38±0.16 ^{Ba}	7.87±0.28 ^{ABab}	7.34±0.34 ^{Abc}	6.74±0.27 ^{Ac}
Ciprofloxacin	6	8.78±0.16 ^{Ba}	8.45±0.16 ^{Ba}	7.80±0.21 ^{Ab}	7.11±0.21 ^{Ac}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

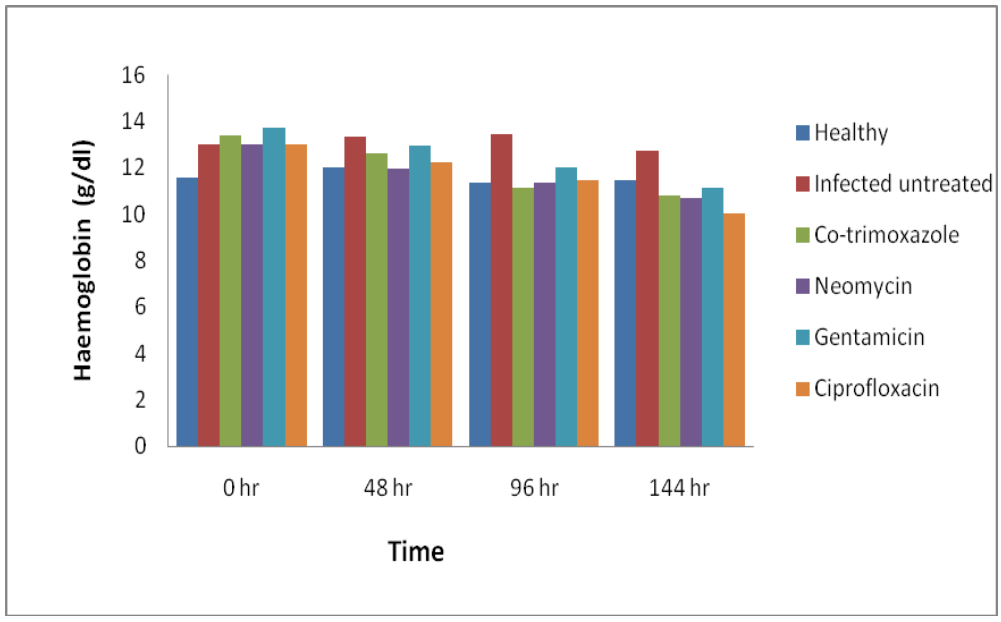

Fig. 6 : Haemoglobin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

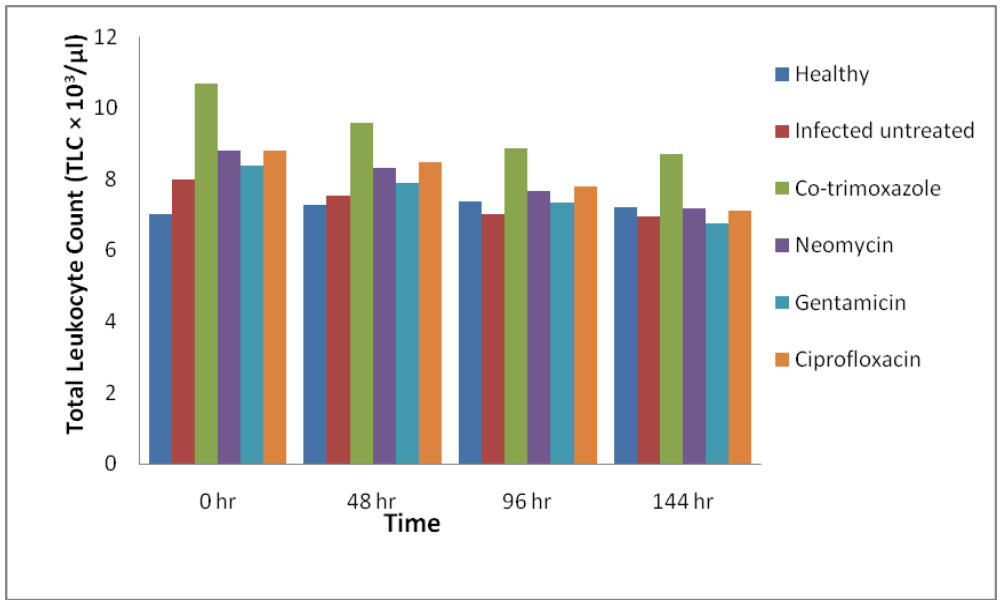

Fig. 7 : Total Leukocyte Count (TLC × 10^3/µl) in Calves with Clinical Colibacillosis before and after treatment

In co-trimoxazole treated group at 48th hr post treatment there was significant ($P<0.05$) decrease in TLC (9.56±0.36× 10^3/µl) as compared to TLC at 0 hr (10.67±0.34× 10^3/µl) and was significantly ($P<0.05$) higher than healthy control group (7.27±0.26 × 10^3/µl) and infected untreated group (7.51±0.27 × 10^3/µl).

In neomycin treated group there was progressive non-significant decrease in TLC and by the 144th hr post treatment the value was 7.16±0.54 × 10^3/µl which varied non-significantly with healthy control group (7.20±0.31 × 10^3/µl) and infected untreated group (6.93±0.28 × 10^3/µl).

In gentamicin and ciprofloxacin treated groups at 96th hr the values of TLC were 7.34±0.34 and 7.80±0.21 × 10^3/µl respectively, and were significantly ($P<0.05$) different from that of 0 hr of pretreatment but varied non-significantly from that of healthy control group (7.35±0.35 × 10^3/µl) and infected untreated group (6.99±0.22 × 10^3/µl).

4.4.4 Total Erythrocyte Count

The results of total erythrocyte count (TEC × 10^6/µl) at different stages of experimental trail are represented in Table-17 and Fig. 8. In infected groups the (Mean±SE) value of total erythrocyte count ranged between 8.02±0.31 to 8.74±0.44 × 10^6/µl at 0 hr and were higher as compared to healthy control group (7.39±0.14 × 10^6/µl).

In co-trimoxazole and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in TEC at 144th hr post treatment (7.32±0.26 and 7.34±0.24 × 10^6/µl) as compared to 0 hr and were comparable with healthy control group (7.56±0.32 × 10^6/µl) and were non-significantly lower than infected untreated group (8.11±0.70 × 10^6/µl).

In neomycin treated group there was progressive non-significant decrease in TEC and by the 144th hr post treatment the value was 6.97±0.34 × 10^6/µl which was non-significantly lower than healthy control group (7.56±0.32 × 10^6/µl) and infected untreated group (8.11±0.70 × 10^6/µl).

Table-17 : Mean±SE of Total Erythrocyte Count (TEC × 10^6/µl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	7.39±0.14 ^{Aa}	7.35±0.22 ^{Aa}	7.60±0.20 ^{Aa}	7.56±0.32 ^{ABa}
Infected untreated	2	8.52±0.40 ^{Ba}	8.71±0.38 ^{Ba}	8.50±0.29 ^{Ba}	8.11±0.70 ^{Ba}
Co-trimoxazole	3	8.53±0.43 ^{Ba}	8.14±0.44 ^{ABab}	7.75±0.28 ^{ABab}	7.32±0.26 ^{ABb}
Neomycin	4	8.02±0.31 ^{ABa}	7.90±0.35 ^{ABa}	7.45±0.33 ^{Aa}	6.97±0.34 ^{Aa}
Gentamicin	5	8.42±0.34 ^{ABa}	7.87±0.29 ^{ABab}	7.55±0.23 ^{Ab}	7.30±0.21 ^{ABb}
Ciprofloxacin	6	8.74±0.44 ^{Ba}	8.32±0.42 ^{ABab}	7.83±0.35 ^{ABab}	7.34±0.24 ^{ABb}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

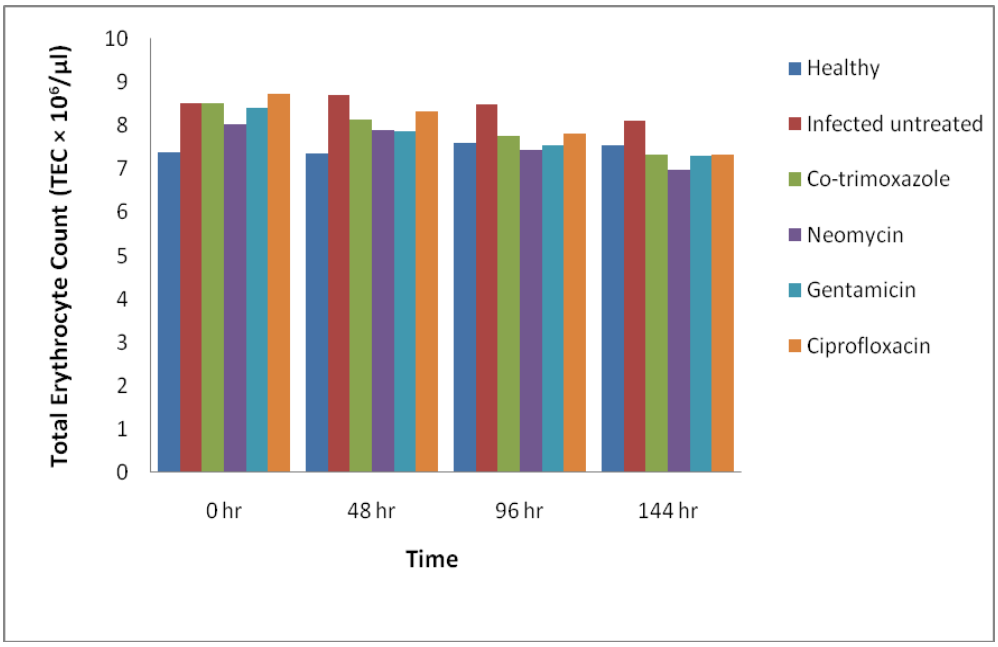

Fig. 8 : Total Erythrocyte Count (TEC × 10^6/µl) in Calves with Clinical Colibacillosis before and after treatment

In gentamicin treated group there was significant (P<0.05) decrease in TEC at 96th hr post treatment (7.55±0.23× 10^6/µl) as compared to 0 hr (8.42±0.34× 10^6/µl) and was comparable with healthy control group (7.60±0.20 × 10^6/µl) and significantly (P<0.05) lower than infected untreated group (8.50±0.29× 10^6/µl).

4.4.5 Neutrophil

The results of neutrophil percentage estimated at different stages in the healthy and infected animals are represented in Table-18 and Fig. 9. In infected groups the (Mean±SE) value of neutrophil percentage ranged between 32.17±1.22 to 35.50±0.76 percent at 0hr which varied from that of the healthy control group (33.00±1.29%).

In co-trimoxazole treated group there was progressive decrease in neutrophil percentage and by the 144th hr it reached to 30.50±0.67% and varied non-significantly with healthy control group (32.00±0.93%) and infected untreated group (33.00±1.39%).

In neomycin and ciprofloxacin treated groups at 96th hr post treatment there was significant decrease in neutrophil percentage (32.50±0.67and 32.00±0.52% respectively) as compared to the percentage at 0 hr and were non-significantly higher than healthy control group (31.67±0.56%) and non-significantly lower than infected untreated group (34.00±1.44%).

In gentamicin treated group at 144th hr post treatment there was significant decrease in neutrophil percentage (32.33±0.76 %) as compared to 0 hr (35.17±0.91%) and was comparable with healthy control group (32.00±0.93%) and non-significantly (P<0.05) lower than infected untreated group (33.00±1.39%).

4.4.6 Lymphocyte

The results of lymphocyte percentage estimated at different of experimental trail stages in the healthy and infected animals are represented in Table-19 and Fig. 10. In infected groups the (Mean±SE) value of neutrophil

Table-18 : Mean±SE of Neutrophil (%) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	33.00±1.29 ^{ABa}	30.83±0.95 ^{Aa}	31.67±0.56 ^{Aa}	32.00±0.93 ^{Aa}
Infected untreated	2	32.17±1.22 ^{Aa}	33.50±1.15 ^{Ba}	34.00±1.44 ^{Aa}	33.00±1.39 ^{Aa}
Co-trimoxazole	3	32.17±0.95 ^{Aa}	32.67±0.42 ^{ABa}	32.17±0.98 ^{Aa}	30.50±0.67 ^{Aa}
Neomycin	4	35.50±0.76 ^{Ba}	34.17±0.70 ^{Bab}	32.50±0.67 ^{Abc}	31.50±0.56 ^{Ac}
Gentamicin	5	35.17±0.91 ^{ABa}	33.67±0.80 ^{Bab}	33.17±0.87 ^{Aab}	32.33±0.76 ^{Ab}
Ciprofloxacin	6	35.33±0.56 ^{Ba}	33.83±0.60 ^{Ba}	32.00±0.52 ^{Ab}	30.67±0.67 ^{Ab}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

Table-19 : Mean±SE of Lymphocyte (%) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	58.83 ± 0.65^{Aa}	58.83 ± 0.40^{Aa}	58.67 ± 0.49^{Aa}	57.83 ± 0.54^{Aa}
Infected untreated	2	57.83 ± 1.08^{ABa}	57.00 ± 0.97^{ABCa}	57.00 ± 1.00^{Aa}	57.83 ± 1.08^{Aa}
Co-trimoxazole	3	57.17 ± 1.01^{ABa}	56.33 ± 0.49^{BCa}	56.83 ± 0.79^{Aa}	58.00 ± 0.52^{Aa}
Neomycin	4	55.33 ± 0.72^{Ba}	56.67 ± 0.76^{BCab}	58.17 ± 0.54^{Ab}	58.33 ± 0.62^{ABb}
Gentamicin	5	55.83 ± 0.60^{Ba}	58.00 ± 0.37^{ABb}	58.67 ± 0.21^{Ab}	60.17 ± 0.31^{Bc}
Ciprofloxacin	6	55.00 ± 0.93^{Ca}	55.33 ± 0.56^{Cab}	57.17 ± 0.48^{Abc}	58.00 ± 0.63^{Ac}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

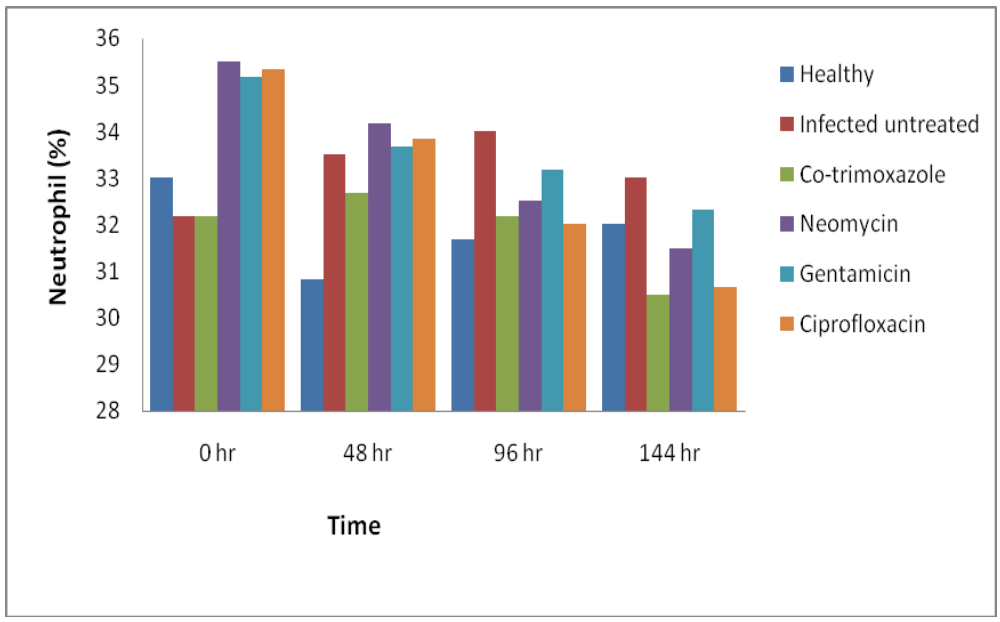

Fig. 9 : Neutrophil (%) in Calves with Clinical Colibacillosis before and after treatment

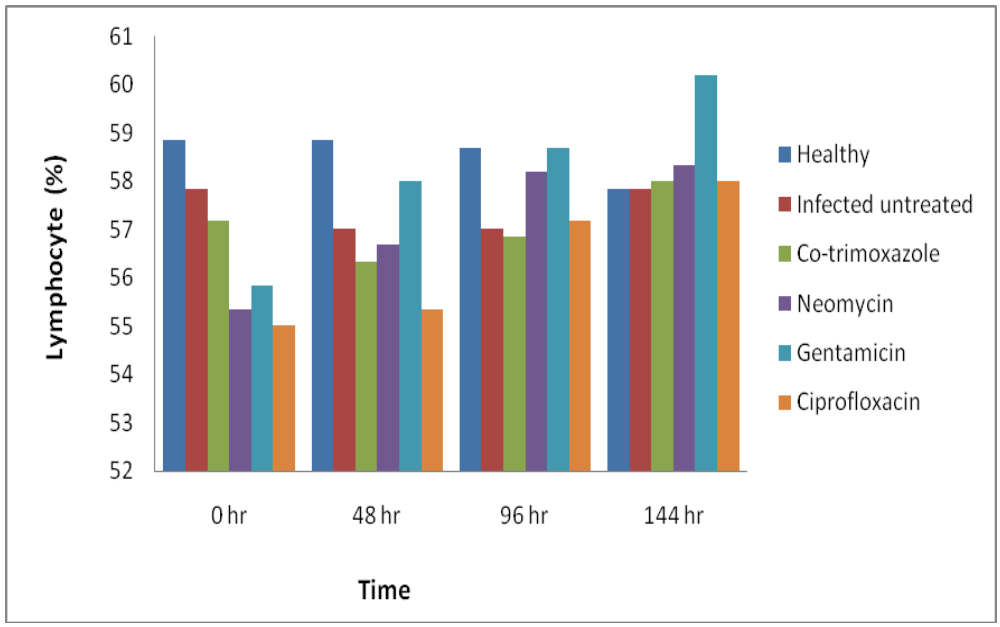

Fig. 10 : Lymphocyte (%) in Calves with Clinical Colibacillosis before and after treatment

percentage ranged between 55.00±0.93 to 57.83±1.08 percent at 0 hr which varied from that of the healthy control group (58.83±0.65%).

In co-trimoxazole treated group lymphocyte percentage varied non-significantly and reached to 58.00±0.52% at 144th hr post treatment which was non-significantly higher than healthy control group (57.83±0.54%) and infected untreated control group (57.83±1.08%).

In neomycin and ciprofloxacin groups treated groups there was significant ($P<0.05$) increase in lymphocyte% at 96th hr post treatment (58.17±0.54 and 57.17±0.48% respectively) compared to 0 hr and were comparable to healthy control group (58.67±0.49%) and infected untreated control group (57.00±1.00%).

In gentamicin groups treated group there was significant increase in lymphocyte% at 48th hr post treatment (58.00±0.37%) compared to 0 hr (55.83±0.60%) which were comparable to healthy control group (58.83±0.40%) and infected untreated control group (57.00±0.97%).

4.5 Biochemical Findings

4.5.1 Total Protein

The results of total protein (g/dl) estimated at different stages of experimental trail in the healthy and infected animals are represented in Table-20 and Fig. 11. In infected groups the (Mean±SE) value of total protein (g/dl) ranged between 5.50±0.30 to 5.94±0.18 g/dl at 0 hr which were significantly ($P<0.05$) lower than healthy control group (6.71±0.19 g/dl).

In co-trimoxazole and neomycin treated groups there was non-significant increase in total protein values at 144th hr post treatment (6.29±0.22and 6.49±0.22 respectively) as compared to 0 hr and were comparable with healthy control group (6.44±0.20 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (5.55±0.18 g/dl).

Table-20 : Mean±SE of total protein (gm/dl) in Calves with clinical colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	6.71±0.19 ^{Ba}	6.25±0.14 ^{Aa}	6.35±0.23 ^{Aa}	6.44±0.20 ^{Ba}
Infected untreated	2	5.94±0.18 ^{Aa}	5.63±0.18 ^{Aa}	5.59±0.19 ^{Ba}	5.55±0.18 ^{Aa}
Co-trimoxazole	3	5.91±0.29 ^{Aa}	6.12±0.19 ^{Aa}	6.15±0.17 ^{Aa}	6.29±0.22 ^{Bab}
Neomycin	4	5.88±0.40 ^{Aa}	6.01±0.28 ^{Aa}	6.35±0.23 ^{Aab}	6.49±0.22 ^{Bab}
Gentamicin	5	5.63±0.18 ^{Aa}	5.92±0.20 ^{Aa}	6.21±0.19 ^{ABab}	6.52±0.19 ^{Bb}
Ciprofloxacin	6	5.50±0.30 ^{Aa}	5.99±0.19 ^{Aa}	6.16±0.18 ^{ABab}	6.34±0.18 ^{Bb}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

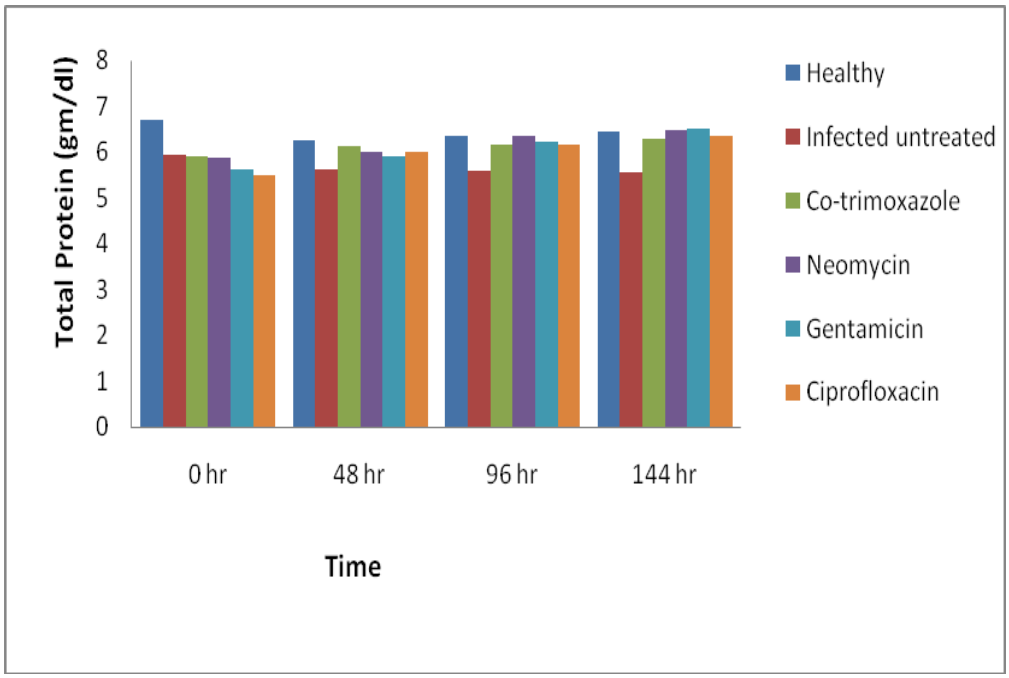

Fig. 11 : Total Protein (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

In gentamicin and ciprofloxacin treated groups there was significant (P<0.05) increase in total protein values at 144th hr post treatment (6.52±0.19 and 6.34±0.18 respectively) as compared to 0 hr and were comparable with healthy control group (6.44±0.20 g/dl) and significantly (P<0.05) higher than infected untreated control group (5.55±0.18 g/dl).

4.5.2 Serum Albumin

The results of serum albumin (g/dl) estimated at different stages in the healthy and infected animals are represented in Table-21 and Fig. 12. In infected groups the (Mean±SE) value of serum albumin (g/dl) ranged between 1.93±0.22 to 2.32±0.17 g/dl at 0 hr which were significantly (P<0.05) lower as compared to the healthy control group (2.93±0.13 g/dl).

In co-trimoxazole and neomycin treated groups the albumin levels at 144th hr post treatment (2.76±0.09 and 2.80±0.07 g/dl respectively) were significantly (P<0.05) higher as compared to values recorded at hr of treatment and were comparable with healthy control group (2.91±0.18 g/dl) and non-significantly higher than infected untreated control group (2.40±0.14 g/dl).

In gentamicin and ciprofloxacin treated groups the albumin levels at 96th hr post treatment were 2.77±0.19 and 2.51±0.13 g/dl respectively and were significantly (P<0.05) higher as compared to values recorded at 0 hr and at 144th hr of treatment became comparable with healthy control group (2.91±0.18 g/dl) but were significantly (P<0.05) higher than infected untreated control group (2.40±0.14 g/dl).

4.5.3 Serum Globulin

The results of total globulin (g/dl) estimated at different stages in the healthy and infected animals are represented in Table-22 and Fig. 13. In infected groups the (Mean±SE) value of globulin (g/dl) ranged between 3.43±0.12 to 3.68±0.15 g/dl at 0 hr which were comparable with healthy control group (3.78±0.90 g/dl).

Table-21 : Mean±SE of Serum Albumin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	2.93±0.13 ^{Aa}	2.62±0.13 ^{Aa}	2.72±0.17 ^{Aa}	2.91±0.18 ^{Ba}
Infected untreated	2	2.26±0.30 ^{Ba}	2.32±0.26 ^{Aa}	2.39±0.19 ^{Aa}	2.40±0.14 ^{Aa}
Co-trimoxazole	3	2.27±0.20 ^{Ba}	2.53±0.14 ^{Aab}	2.66±0.07 ^{Aab}	2.76±0.09 ^{ABb}
Neomycin	4	2.32±0.17 ^{Ba}	2.44±0.15 ^{Aa}	2.60±0.13 ^{Aab}	2.80±0.07 ^{ABb}
Gentamicin	5	2.20±0.21 ^{Ba}	2.43±0.14 ^{Aab}	2.77±0.19 ^{Abc}	2.97±0.12 ^{Bc}
Ciprofloxacin	6	1.93±0.22 ^{Ba}	2.33±0.14 ^{Aab}	2.51±0.13 ^{Ab}	3.07±0.19 ^{Bc}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

Table-22 : Mean±SE of Serum Globulin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	3.78±0.90 ^{Aa}	3.63±0.11 ^{Aa}	3.63±0.16 ^{Aa}	3.53±0.20 ^{ABa}
Infected untreated	2	3.68±0.15 ^{Aa}	3.31±0.16 ^{Aab}	3.20±0.13 ^{Ab}	3.15±0.12 ^{Ab}
Co-trimoxazole	3	3.64±0.14 ^{Aa}	3.40±0.18 ^{Aa}	3.50±0.19 ^{Aa}	3.53±0.21 ^{Aa}
Neomycin	4	3.56±0.29 ^{Aa}	3.57±0.20 ^{Aa}	3.75±0.26 ^{Aa}	3.70±0.25 ^{Ba}
Gentamicin	5	3.43±0.12 ^{Aa}	3.49±0.15 ^{Aa}	3.43±0.04 ^{Aa}	3.56±0.09 ^{ABa}
Ciprofloxacin	6	3.57±0.26 ^{Aa}	3.66±0.23 ^{Aab}	3.65±0.18 ^{Aab}	3.28±0.07 ^{ABb}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

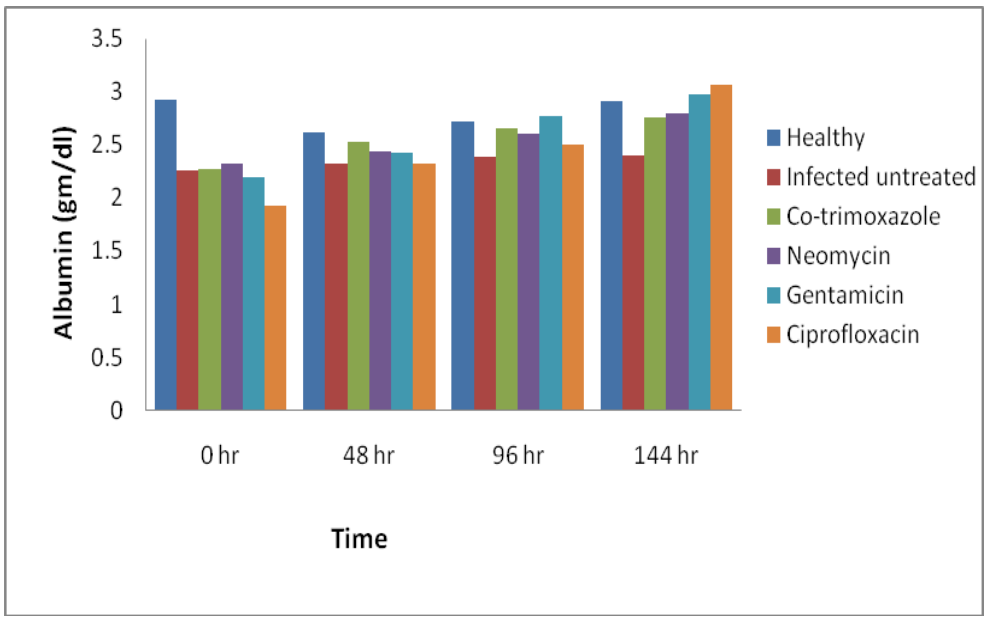

Fig. 12 : Serum Albumin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

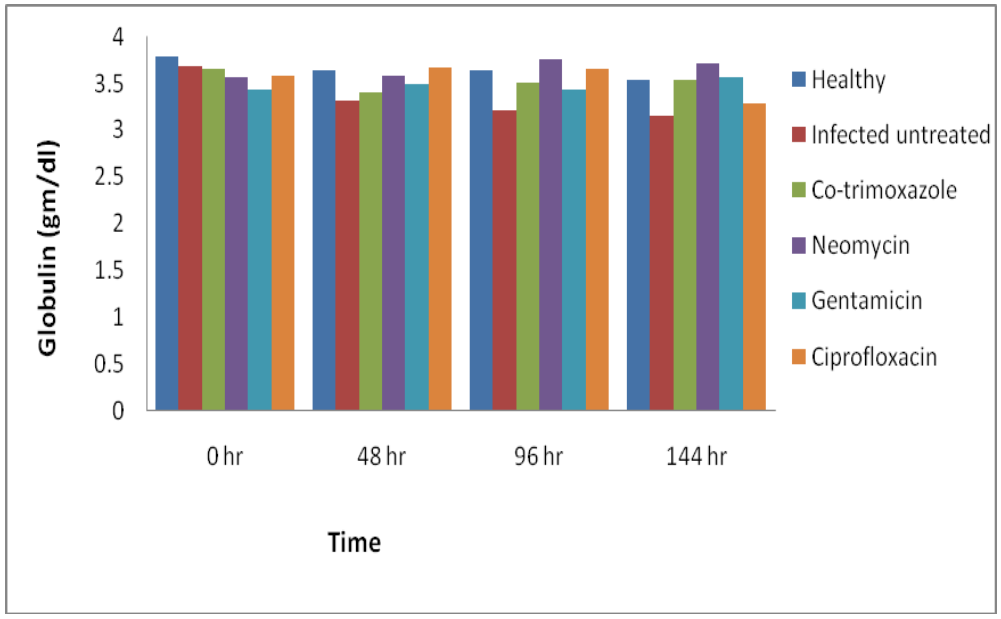

Fig. 13 : Serum Globulin (gm/dl) in Calves with Clinical Colibacillosis before and after treatment

In co-trimoxazole and gentamicin treated groups the globulin levels varied non-significantly and by 144th hr reached to 3.53±0.21 and 3.56±0.09 g/dl respectively and were comparable with healthy control group (3.53±0.20 g/dl) and infected untreated control group (3.15±0.12 g/dl).

In neomycin treated group there was progressive non-significant increase in globulin levels which reached 3.70±0.25 g/dl at 144th hr post treatment and was comparable with healthy control group (3.53±0.20 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (3.15±0.12 g/dl).

In ciprofloxacin treated group the albumin levels varied non-significant and at 144th hr of treatment value recorded was 3.28±0.07 g/dl and was significantly ($P<0.05$) lower than the value recorded at 0 hr (3.57±0.26 g/dl). The value recorded at 144th hr of treatment varied non-significantly with healthy control group (3.53±0.20 g/dl) and infected untreated control group (3.15±0.12 g/dl).

4.5.4 Blood Glucose

The results of blood glucose (mg/dl) estimated at different stages in the healthy and infected animals are represented in Table-23 and Fig. 14. In infected groups the (Mean±SE) value of blood glucose (mg/dl) ranged between 57.56±6.93 to 69.18±1.93 mg/dl at 0 hr which were significantly ($P<0.05$) lower than healthy control group (77.58±2.01 mg/dl).

In co-trimoxazole and neomycin treated groups at 144th hr post treatment blood glucose value i.e., 75.71±3.66 and 72.20±2.17 mg/dl respectively were significantly ($P<0.05$) higher than that recorded at 0 hr and were also significantly ($P<0.05$) higher than infected untreated group (62.69±2.14 mg/dl) and comparable with healthy control group (77.20±1.83 mg/dl).

In gentamicin and ciprofloxacin treated groups blood glucose values recorded at 96th hr post treatment (73.21±2.04 and 72.68±1.84 mg/dl respectively) were significantly ($P<0.05$) higher than that recorded at 0 hr and were also

Table-23 : Mean±SE of Blood Glucose (mg/dl) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	77.58±2.01 ^{Aa}	76.30±2.44 ^{Aa}	75.89±1.50 ^{Aa}	77.20±1.83 ^{Aa}
Infected untreated	2	59.17±2.57 ^{Ba}	59.56±2.57 ^{Ba}	59.68±2.60 ^{Ba}	62.69±2.14 ^{Ba}
Co-trimoxazole	3	57.56±6.93 ^{Ba}	63.06±5.72 ^{Bab}	69.39±4.44 ^{Aab}	75.71±3.66 ^{Ab}
Neomycin	4	69.18±1.93 ^{Ba}	68.72±1.79 ^{ABa}	72.01±2.17 ^{Aab}	76.98±2.27 ^{Ab}
Gentamicin	5	61.93±4.00 ^{Ba}	66.23±2.57 ^{Bab}	73.21±2.04 ^{Abc}	78.20±2.26 ^{Ac}
Ciprofloxacin	6	65.10±1.87 ^{Ba}	68.10±2.02 ^{ABab}	72.68±1.84 ^{Abc}	77.14±1.86 ^{Ac}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

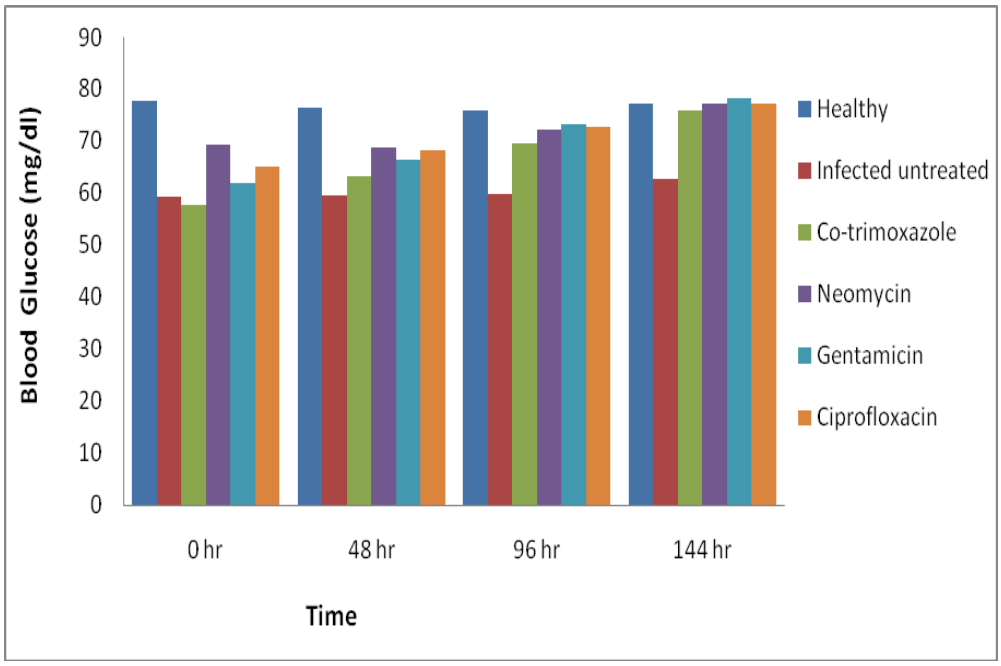

Fig. 14 : Glucose (mg/dl) in Calves with Clinical Colibacillosis before and after treatment

significantly (P<0.05) higher than infected untreated group (59.68±2.60 mg/dl) and comparable with healthy control group (75.89±1.50 mg/dl).

4.5.5 Serum Sodium

The results of serum sodium (mEq/L) estimated at different stages in the healthy and infected animals are represented in Table-24 and Fig. 15. In infected groups the (Mean±SE) value of serum sodium (mEq/L) ranged between 126.00±1.52 to 133.61±1.26 mEq/L at 0 hr which were significantly (P<0.05) lower than healthy control group (139.98±0.94mEq/L).

In co-trimoxazole treated group there was progressive significant increase in sodium values till 144th hr when its values was 136.29±2.24 mEq/L which was comparable with healthy control group (140.47±1.24 mEq/L) and significantly (P<0.05) higher than infected untreated group (132.02±2.21 mEq/L).

In neomycin and gentamicin treated groups the serum sodium recorded at 144th hr post treatment (139.72±1.58 and135.15±1.87 mEq/L respectively) were significantly (P<0.05) higher than that recorded at 0 hr of and were also significantly (P<0.05) higher than infected untreated group (132.02±2.21 mEq/L) and comparable with healthy control group (140.47±1.24 mEq/L).

In ciprofloxacin treated group at 48th hr after treatment the serum sodium recorded was 131.20±1.13 mEq/L which was significantly (P<0.05) higher than that recorded at 0 hr but was significantly (P<0.05) lower than healthy control group (140.91±1.2 mEq/L) and was comparable to infected untreated group (132.01±1.72 mEq/L).

4.5.6 Serum Potassium

The results of serum potassium (mEq/L) estimated at different stages of experimental trail in the healthy and infected animals are represented in Table-25 and Fig. 16. In infected groups the (Mean±SE) value of serum potassium (mEq/L) ranged between 3.96±0.07 to 4.10±0.20 mEq/L at 0 hr and were comparatively lower than healty control group (4.32±0.09 mEq/L).

Table-24 : Mean±SE of Serum Sodium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	139.98±0.94 ^{Aa}	140.91±1.23 ^{Aa}	140.91±1.45 ^{Aa}	140.47±1.24 ^{Aa}
Infected untreated	2	133.61±1.26 ^{Ba}	132.01±1.72 ^{Ba}	131.65±2.22 ^{Ba}	132.02±2.21 ^{Ba}
Co-trimoxazole	3	132.59±1.82 ^{Ba}	132.00±2.40 ^{Ba}	133.57±2.36 ^{BCa}	136.29±2.24 ^{ABa}
Neomycin	4	133.07±2.28 ^{Ba}	135.20±2.39 ^{Bab}	138.12±1.87 ^{ACab}	139.72±1.58 ^{Ab}
Gentamicin	5	129.46±2.26 ^{BCa}	131.47±2.17 ^{Ba}	134.58±2.09 ^{BCab}	137.15±1.87 ^{Ab}
Ciprofloxacin	6	126.00±1.52 ^{Ca}	131.20±1.13 ^{Bb}	134.55±0.95 ^{BCc}	139.70±0.74 ^{Ad}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

Table-25 : Mean±SE of Serum Potassium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

Groups	Group No.	0 hr	48 hr	96 hr	144 hr
Healthy	1	4.32 ± 0.09^{Aa}	4.27 ± 0.12^{Aa}	4.25 ± 0.14^{ABa}	4.31 ± 0.17^{ABa}
Infected untreated	2	4.09 ± 0.09^{ABa}	3.99 ± 0.09^{Aa}	3.95 ± 0.09^{Aa}	3.96 ± 0.14^{Ba}
Co-trimoxazole	3	4.10 ± 0.20^{ABa}	4.18 ± 0.14^{Aa}	4.42 ± 0.08^{Bab}	4.45 ± 0.07^{Ab}
Neomycin	4	3.95 ± 0.05^{Ba}	4.00 ± 0.10^{Aa}	4.10 ± 0.19^{ABab}	4.29 ± 0.07^{ABb}
Gentamicin	5	4.04 ± 0.02^{ABa}	4.13 ± 0.11^{Aa}	4.26 ± 0.11^{ABab}	4.51 ± 0.13^{Ab}
Ciprofloxacin	6	3.96 ± 0.07^{Ba}	4.06 ± 0.08^{Aa}	4.21 ± 0.13^{ABab}	4.46 ± 0.14^{Ab}

Values with Similar superscript (Capital letters-Between groups and small letters-Within groups) do not differ significantly ($P<0.05$)

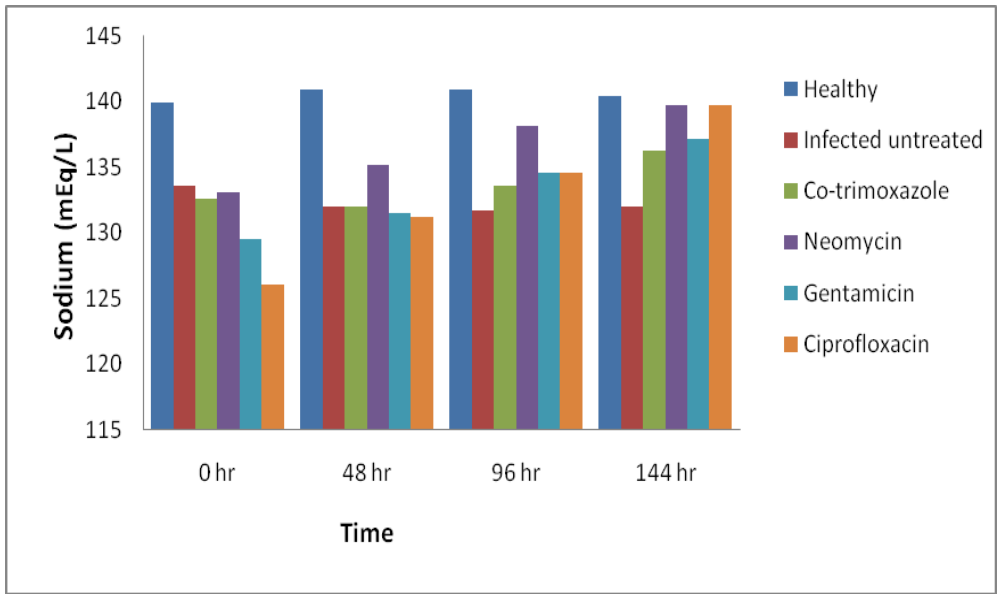

Fig. 15 : Serum Sodium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

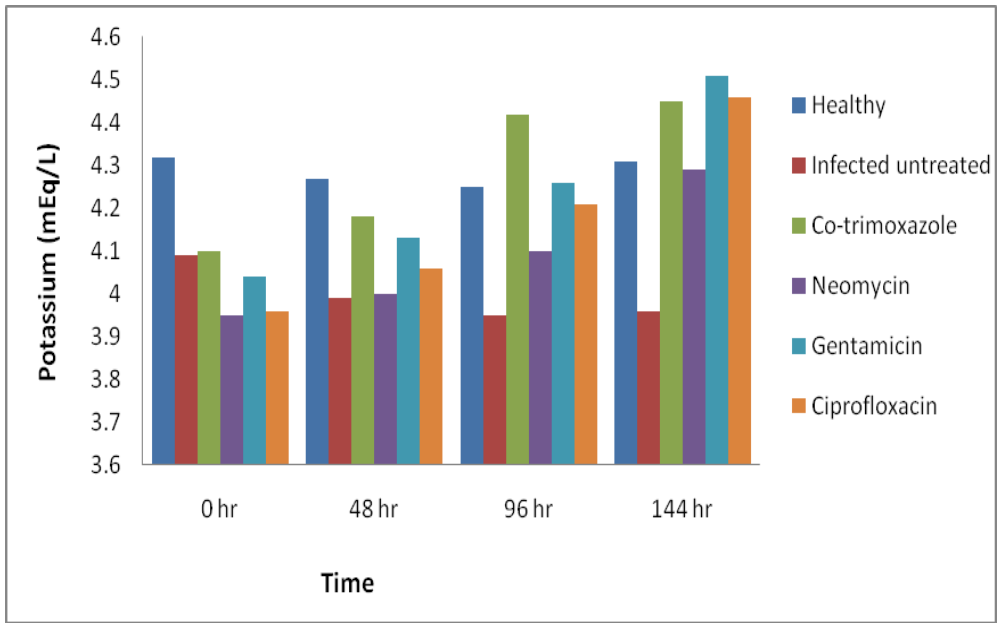

Fig. 16 : Serum Potassium (mEq/L) in Calves with Clinical Colibacillosis before and after treatment

In co-trimoxazole, gentamicin and ciprofloxacin treated groups potassium values 4.45±0.07, 4.51±0.13 and 4.46±0.14 mEq/L respectively at 144th hr post treatment and were significantly ($P<0.05$) higher than that recorded at 0 hr and were comparable with healthy control (4.31±0.17 mEq/L) and significantly ($P<0.05$) higher than infected untreated control group (3.96±0.14 mEq/L).

In neomycin treated group potassium value 4.29±0.07 mEq/L at 144th hr was significantly ($P<0.05$) higher than that recorded at 0 hr (3.95±0.05 mEq/L) and was comparable with healthy control (4.31±0.17 mEq/L) and non-significantly higher than infected untreated control group (3.96±0.14 mEq/L).

4.6 Therapeutic Trail

For evaluating the efficacy of different treatment regimens 30 animals were divided into 5 groups, each with six animals and one group comprising of apparently six healthy animals kept as healthy control. Therapeutic efficacy of different drugs was made through clinical evaluation of different parameters on daily basis (Table 26).

Following observations on clinical trials were made in different groups of animals.

The calves of group 2 were not given any treatment but recovered after the trial period and remained weak. Calves of the 3rd group were treated with oral Co-trimoxazole bolus. Out of six calves, four completely recovered with a delay in recovery of two cases. Fourth group of calves were given oral neomycin tablets with a delay in recovery of 2 cases, however, all calves recovered after 7th day. Calves of the group 5 were treated with gentamicin parenterally, a delay in recovery of 1 case which also recovered after 7th day. Six calves of group 6 were treated with ciprofloxacin oral tablets. All of six calves recovered completely within six days, hence, the drug proved to be 100% effective.

Table-26 : Effect of therapeutic regimens on clinical recover

Group No.	Therapeutic Regimen	Dosage of the antibiotics	No. of cases treated	Response to Treatment						
				0 hrs	48 hrs	96 hrs	144 hrs	Case Recovery	%age	No. of cases Delayed
1	Healthy control	-	-	-	-	-	-	-	-	-
2	Infected untreated Group	-	six	-	-	-	-	-	-	6
3	Co-trimoxazole (Aroprim Bolus)	15-30 mg/kg BW (Bolus 1/5th orally)	six	+	++	++	+++	four	66.67	2
4	Neomycin (Unimycin)	10mg/kg BW (Bolus 1/2nd orally)	six	+	++	++	+++	four	66.67	2
5	Gentamicin (Gentacip)	4mg/kg BW (2 ml i/m BID daily)	six	+	++	+++	++++	five	83.33	1
6	Ciprofloxacin (ciprobid)	4mg/kg (250mg tab-1/3rd BID)	six	+	++	+++	++++	**six**	**100**	-

+ = slightly improved, ++ = improved, +++ = recovery and ++++ = complete recovery

CHAPTER – 5

DISCUSSION

Colibacillosis is one of the important diseases in new born calves, caused by pathogenic serotypes of *Escherichia coli* (Carlton, 1992) and characterized by prostration, profuse diarrhea and septicemia. It is a major cause of economic loss in new born calves. It has been associated both with enteric or septicaemic disease in newborn farm animals (Radostits *et al*., 2000), enteric colibacillosis is characterized by varying degrees of diarrhea, dehydration, acidosis, and death in a few days if not treated, whereas, coliform septicemia is characterized by severe illness and rapid death in several hours. Calves that are deficient in colostral immunoglobulins are highly susceptible to septicemic colibacillosis.

The present investigation on prevalence of colibacillosis in calves was undertaken in different geographical areas during different seasons of Kashmir valley. The overall prevalence of colibacillosis recorded in the present study was 86.8% which is relatively higher than the earlier findings who recorded a prevalence 50 to 60% of enterotoxigenic *Escherichia coli* in diarrhoeic calves (Newman, 1973; Radostits *et al*., 2006) causing mortality between 5 to 25% (Basoglu, 1992) or even high as 54.58% (Khan and Khan, 1997). Prevalence of *E. coli* in calf diarrhoea varies widely from region to region, and 20 to 51% has been recorded from different parts of the world (Simeonov *et al.,* 1982; Sizov *et al.,* 1984; Nagy *et al.,* 1986; Debnath *et al.,* 1987; Barrandeguy *et al.*, 1988). The high prevalence recorded in the study could be due to overcrowding, decreased immunity, un-hygienic measures leading to stressful conditions which subsequently resulted in heavy infection in calves and also because present study was carried out in calves of less than 3 months of age. High risk for calves to develop colibacillosis during the first months of life has been reported (Frank and Kaneene, 1993; Bendali *et al*., 1999; Scott *et al.,* 2004)

In the present study prevalence of colibacillosis in calves both in organized and

un-organized farming systems was also studied. In the un-organized areas, prevalence was comparatively higher (88.89%) than organized sector (85.96%). It appears that the quality of zoohygienic conditions of animal husbandry and managemental practices influence the exposure of animals to colibacillosis. The animals in the un-organized farming systems were kept under poor managemental conditions with the flooring being cleaned less frequently, thus increasing the chances of cross infections through direct contact with the faeces of infected animals. On the contrary, the organized farms had separate calving pens and batten type flooring but still managmental practices were not enough to prevent the infection.

Seasonal variation in colibacillosis in calves was markedly observed in the present study. The prevalence recorded during spring, summer, autumn and winter was 90.32, 63.33, 86.96 and 91.36% respectively, being highest in winter (91.36%) and lowest (63.33%) in summer. Low prevalence in summer may be because of early release of calves to the pastures and reducing the period of close proximity and opportunity for potentially infective animal interactions in the calving sheds. High prevalence during winter season may be due to close confinement, and built up of contamination as a result of presence of increased number of diarrhoeic animals and low frequency of sheds cleaning. Gutzwiller (2002) also reported higher incidence of neonatal diarrhoea in calves which were born in winter than in summer.

5.1 Isolation and Identification of *E. coli*

E. coli is a facultative anaerobe that can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions; growth of these organisms has also been observed at 44°C. *E. coli* is Gram negative, catalase positive, oxidase negative, non spore forming, and rod shaped bacterium. The motility of the organism occurs due to peritrichous flagella, although some non-motile strains have also been recorded. When growing anaerobically there is an absolute requirement for fermentable carbohydrate. Glucose is fermented to

give acid and gas. These organisms are able to utilize carbon and nitrogen sources for all their metabolic and energy needs (Edwards and Ewing, 1972). *E. coli* in faecal samples are most often recovered on MacConkey or Eosin Methylene Blue (EMB) agar. Merchant and Packer (1967) considered EMB agar as a suitable medium for isolation of *E. coli* from faeces and foods because of ability to produce distinctive colonies having greenish metallic sheen not produced by other bacteria of *Enterobacteriaceae* family. For epidemiological or clinical purposes *E. coli* strains are often selected from MacConkey agar plates after presumptive visual identification of lactose fermenting pink colonies. However, this method should be used only with caution because only 90 per cent of *E. coli* are lactose positive, some diarrhoeagenic *E. coli* strains are typically lactose negative. The indole test positive in 99 per cent of *E. coli* strains is the single best test for differentiation from other members of *Enetrobacteriaceae* (Nataro and Kaper, 1998).

5.2 Serotypes of *E. coli* associated with colibacillosis in calves

E. coli of specific serogroup can be associated with reproducibility of certain clinical syndrome. The distribution of different serotypes of *E. coli* varies with geographical regions and their prevalence in man and animals in a particular area. A shift in 'O' serogroups along with their virulence factors has also been observed (Soderlind *et al.,* 1988). In the present study, 129 *E. coli* isolates with different 'O' serogroups were recorded in different locations viz., Organized farms; Unorganized areas/ selected areas which included local villages and Clinical Service Complex, FVSc & AH. Shuhama, Out of 129 *E. coli* isolates 116 isolates belonged to 22 different 'O' serogroups while 8 isolates were untypable and 5 were rough isolates. Among the 116 'O' serotypes the highest frequency of 29 with percentage 22.48 was found for O20 serotype followed by O22 and O69 serotypes having percentage of 14.73 and 10.08 respectively. Serotypes O11 and O84 were having percentage of 6.98 where as serotype O147 was having percentage of 3.88. Serotypes O68, O107, O123, O153 and O157 were having

percentage of 2.33 each where as serotypes O1, O6, O17, O36, O51 and O60 were having percentage of 1.55 each. The lowest frequency of one was found for O92, O102, O140, O158 and O159 with percentage of 0.78 each. Eight isolates were untypable with percentage 6.20 and 5 were rough isolates having percentage of 3.88.

The serogroup O157, O68, O36, O17 and O11 recorded in present study has also been reported earlier (Panda and Panda, 1987; Wani *et al.,* 2004; Tripathi and Soni, 1984; Minakshi *et al.,* 1992; Kaura *et al.,* 1991).

The serogroups O1, O20, O22, O153, O123, O60 and O102 recovered in this study has also been reported earlier in diarrhoeic and non- diarrhoeic calves (Joon and Kaura, 1993; Hussain *et al.,* 2003; Wani *et al.,* 2004).

The serogroup O69 recovered in this study has also been reported by Aniruddha *et al.* (2009) from diarrhoeic calves.

5.3 Antibiotic sensitivity test

Though antibiotics use has its advantages, the intensive and extensive use of antibiotics has led to the emergence of antimicrobial resistance. The indiscriminate and uncontrolled use of antimicrobial drugs exerts a selection pressure and encourages the proliferation of drug resistant strains of *E. coli* in animal population. When this is coupled with poor environmental sanitation and low personal hygiene, the situation may constitute a danger to public health.

In the present study *in-vitro* antibiotic resistance patterns of the *E. coli* isolates were determined by disc diffusion method of Bauer *et al.* (1966). The *E. coli* isolates were tested against commonly used antibiotics viz. Ampicillin, Ciprofloxacin, Co-trimoxazole, Enrofloxacin, Amoxicillin, Neomycin, Penicillin, Gentamicin and Tetracycline. *E. coli* isolates were found highly sensitive to ciprofloxacin (71.58%), gentamicin (66.32%), neomycin (54.74%) and co-trimoxazole (53.68%). Patil *et al.* (1999) also reported ciprofloxacin and gentamicin highly sensitive against *E. coli* isolates. The present findings for

sensitivity pattern of co-trimoxazole was similar to that of Saravanbava *et al.* (1990). Moderate sensitivity was observed towards enrofloxacin (44.21%) and tetracycline (37.89%), while penicillin (70.53%) showed intermediate sensitivity. Orden *et al.* (1999) similar sensitivity pattern of enrofloxacin which is comparable to the present findings. The present findings of neomycin and tetracycline are in accordance with the reports of Khan *et al.* (2002). The low sensitivity pattern shown by penicillin may be due to its action on peptidoglycan of cell wall, which is present in low amounts in case of gram negative bacteria. However isolates were resistant to ampicillin (80.00%) and amoxicillin (85.26%). Samad *et al.* (2003) also reported ampicillin and amoxicillin resistant against *E. coli* isolates.

5.4 Clinical findings

Clinical signs recorded were diarrhoea, soiling of perineum and tail, depression, varying degree of dehydration, tachycardia, increased respiration rate, pale mucous membrane, prolonged capillary refill time, rough body coat, dry muzzle or mouth and profound weakness. The faeces was semisolid to watery with offensive odor, yellowish white in colour and sometimes blood stained. These findings were similar to the earlier reports (Bellamy *et al.,* 1979; Radostits *et al.,* 2000). Yellowish white diarrhoea faeces might be due to high content of salt particularly bicarbonates (Ward, 1976) with heavy secreation of water in intestinal lumen (Szancer, 1980).

There was definite increase in the skin fold time in the diarrhoeic calves which was indicative of decreased skin elasticity due to extracellular fluid loss in these animals. The skin fold time of ranging from 5.1 to 5.9 seconds was recorded in infected calves which corresponded approximately to the dehydration of 6 to 8 per cent. The capillary refill time was also increased in infected calves which ranged from 3.21 to 3.68 seconds. Increased skin fold test as well as CRT are indicative of a compromised circulating volume and reduced peripheral perfusion in these animals which was towards normalization at 144^{th} hour post treatment.

Most of the calves naturally infected with *E. coli* showed signs of higher rectal temperature which ranged between 101.33±0.43 to 102.30±0.21 (^{o}F) at 0 hr. High temperature may be due to bacterial infection and septicemia and low temperature may be due to dehydration which leads to reduced peripheral perfusion. The temperature restoring towards normal value by day 6th. Samad *et al.* (2003) reported increase in temperature in colibacillotic calves where as Fernandes *et al.* (2009a) reported subnormal body temperature in calf diarrhoea.

A marked increase in respiration rate was recorded in *E. coli* infected calves. The dehydration may be accompanied by marked variation in the depth and rate of respiration leading to hyperpnoea and polypnoea. Increase in heart rate and pulse rate was recorded in the present study, which may be due to metabolic upsets (Radostits *et al.,* 2000 ; Kumar *et al*., 2002).

5.5 Haematological findings

5.5.1 Packed cell volume

In all the groups of calves with clinical colibacillosis the Packed Cell Volume (PCV) percentage ranged from 40.27±1.55 to 44.22±0.86 percent at 0 hr and were significantly ($P<0.05$) higher as compared to healthy control group (37.72±0.55%). Increase in packed cell volume in diarrhoeic calves might be due to haemoconcentration confirming the fluid loss from vascular compartment. This increase in PCV% is in agreement with the earliar observations (Radhakishan *et al.,* 1991; Kumar and Mandial, 2002; Kaur *et al.,* 2006).

In co-trimoxazole group there was significant ($P<0.05$) decrease in PCV % at 144th hr (38.26±1.40%) post treatment as compared at 0 hr (43.38±1.53%) and was non-significantly lower than healthy control group (38.48±0.27%) and infected untreated group (39.07±1.42%).

In neomycin, gentamicin and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in PCV% at 96th hr post treatment and values recorded were 37.30±1.55, 40.09±0.62 and 42.89±0.82%, respectively as compared to PCV % at

0 hr and were comparable with healthy control group (37.91±0.51%) and infected untreated group (40.47±1.51%).

5.5.2 Haemoglobin

In colibacillosis suffering groups of calves, the values of haemoglobin (g/dl) ranged from 12.99±0.92 to 13.71±0.46 g/dl at 0 hr and were significantly ($P<0.05$) higher as compared to healthy control group (11.57±0.24 g/dl). Increase in haemoglobin values in diarrhoeic calves might be due to fluid loss from vascular compartment (Naylor, 1987). Similar trend of higher values were also recorded by Sridhar *et al.* (1988) and Kumar *et al.* (2002).

In co-trimoxazole, neomycin and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in haemoglobin (g/dl) at 96^{th} hr post treatment and values recorded were 11.08±0.76, 11.30±0.30 and 11.44±0.33 g/dl respectively as compared to haemoglobin (g/dl) at 0 hr and were comparable to healthy control group (11.34±0.12 g/dl) and significantly ($P<0.05$) lower than infected untreated group (13.40±0.56 g/dl).

In gentamicin treated group there was significant ($P<0.05$) decrease in haemoglobin (g/dl) at 96^{th} hr post treatment and value recorded was 12.00±0.48 as compared to haemoglobin (g/dl) at 0 hr (13.71±0.46 g/dl) and was comparable with healthy control group (11.34±0.12 g/dl) and non-significantly lower than infected untreated group (13.40±0.56 g/dl).

5.5.3 Total Leukocyte Count

The total leukocyte count (TLC) in groups of calves suffering from colibacillosis ranged between 7.99±0.31 and 10.67±0.34 × 10^3/µl at 0 hr and significantly ($P<0.05$) higher than healthy control group (7.00±0.27 × 10^3/µl). The increase in total leucocyte count might have occurred due to the normal reaction of body defense mechanism against *E. coli* infection and also due to dehydration and haemoconcentration. The increase in TLC in the present study was in agreement with the earlier findings of Fernandes *et al.* (2009c).

In co-trimoxazole treated group at 48th hr post treatment there was significant ($P<0.05$) decrease in TLC (9.56±0.36× 10^3/µl) as compared to TLC at 0 hr (10.67±0.34× 10^3/µl) and was significantly ($P<0.05$) higher than healthy control group (7.27±0.26 × 10^3/µl) and infected untreated group (7.51±0.27 × 10^3/µl).

In neomycin treated group there was progressive non-significant decrease in TLC and by the 144th hr post treatment the value was 7.16±0.54 × 10^3/µl which varied non-significantly with healthy control group (7.20±0.31 × 10^3/µl) and infected untreated group (6.93±0.28 × 10^3/µl).

In gentamicin and ciprofloxacin treated groups at 96th hr post treatment the values of TLC were 7.34±0.34 and 7.80±0.21 × 10^3/µl respectively, and were significantly ($P<0.05$) different from that of 0 hr but varied non-significantaly from that of healthy control group (7.35±0.35 × 10^3/µl) and infected untreated group (6.99±0.22 × 10^3/µl).

5.5.4 Total erythrocyte count

In infected groups the value of total erythrocyte count ranged from 8.02±0.31 to 8.74±0.44 × 10^6/µl at 0 hr of and were higher as compared to healthy control group (7.39±0.14 × 10^6/µl). Increase in TEC values in diarrhoeic calves might be due to reduction in the plasma value and haemoconcentration due to loss of extra cellular fluid in diarrhoeic faeces on account *E. coli* induced intestinal epithelial damage (Fayet, 1971). The increase in TEC in the present study is in agreement with the earlier findings (Fernandes *et al.,* 2009c; Kumar and Mandial, 2002).

In co-trimoxazole and ciprofloxacin treated groups there was significant ($P<0.05$) decrease in TEC at 144th hr post treatment (7.32±0.26 and 7.34±0.24 × 10^6/µl) as compared to 0 hr and were non-significantly lower than healthy control group (7.56±0.32 × 10^6/µl) and infected untreated group (8.11±0.70 × 10^6/µl).

In neomycin treated group there was progressive non-significant decrease in TEC and by the 144th hr post treatment the value was 6.97±0.34 × 10^6/µl which was non-significantly lower than healthy control group (7.56±0.32 × 10^6/µl) and

infected untreated group (8.11±0.70 × 10^6/µl).

In gentamicin treated group there was significant (P<0.05) decrease in TEC at 96th hr post treatment (7.55±0.23× 10^6/µl) as compared to 0 hr (8.42±0.34× 10^6/µl) and was non-significantly lower than healthy control group (7.60±0.20 × 10^6/µl) and significantly (P<0.05) lower than infected untreated group (8.50±0.29× 10^6/µl).

5.5.5 Neutrophil

In infected groups the value of neutrophil percentage ranged from 32.17±1.22 to 35.50±0.76 percent at 0 hr which varied from that of the healthy control group (33.00±1.29%). Increased values of neutrophils and lymphocytes has been reported earlier (Sridhar *et al.,* 1988; Radhakishan *et al.,* 1991) indicating the presence of intestinal infection where as Donovan *et al.* (1998) reported neutropenia in neonates with diarrhoea.

In co-trimoxazole treated group there was progressive decrease in neutrophil percentage and by the 144th hr post treatment it reached to 30.50±0.67% and varied non-significantly with healthy control group (32.00±0.93%) and infected untreated group (33.00±1.39%).

In neomycin and ciprofloxacin treated groups at 96th hr post treatment there was significant decrease in neutrophil percentage (32.50±0.67and 32.00±0.52% respectively) as compared to the percentage at 0 hr and were comparable with healthy control group (31.67±0.56%) and non-significantly lower than infected untreated group (34.00±1.44%).

In gentamicin treated group at 144th hr of treatment there was significant (P<0.05) decrease in neutrophil percentage (32.33±0.76 %) as compared to the percentage at 0 hr and was comparable with healthy control group (32.00±0.93%) and non-significantly lower than infected untreated group (33.00±1.39%).

5.5.6 Lymphocyte

In infected groups the value of neutrophil percentage ranged from 55.00±0.93 to 57.83±1.08 percent at 0 hr which were comparable as compared to healthy control group (58.83±0.65%). Marginal change in lymphocyte % might be due to early institution of therapy and less severity of the disease.

In co-trimoxazole treated group lymphocyte percentage varied non-significantly and reached to 58.00±0.52% at 144th hr after treatment which was non-significantly higher than healthy control group (57.83±0.54%) and infected untreated control group (57.83±1.08%).

In neomycin and ciprofloxacin groups treated groups there was significant ($P<0.05$) increase in lymphocyte% at 96th hr after treatment (58.17±0.54 and 57.17±0.48% respectively) compared to 0 hr and were comparable to healthy control group (58.67±0.49%) and infected untreated control group (57.00±1.00%).

In gentamicin groups treated group there was significant increase in lymphocyte% at 48th hr post treatment (58.00±0.37% respectively) compared to 0 hr (55.83±0.60%) which were comparable to healthy control group (58.83±0.40%) and infected untreated control group (57.00±0.97%).

5.6 Biochemical findings

5.6.1 Total protein

In infected groups of calves suffering from colibacillosis the value of total protein (g/dl) ranged from 5.50±0.30 to 5.94±0.18 g/dl at 0 hr which were significantly ($P<0.05$) lower than healthy control group (6.71±0.19 g/dl). This lowered protein values may be due to some loss of protein as a result of diarrhoea. Calves affected with diarrhoea may also have maldigestion and malabsorption and may have protein loss from erosive or ulcerative colonic lesions causing protein losing enteropathy. Anemia due to fecal blood loss might further contribute to protein loss. These findings are in agreement with the earlier findings (Dhaliwal, 1993; Radhakishan *et al.,* 1991; Aly *et al.,* 1996). However, Dubey *et al.* (1992) and

Deshpande *et al.* (1993) reported increase in total plasma protein in diarrheic calves which might be due to dehydration and haemoconcentration (Radostitis *et al.,* 1994) and also due to release of intracellular proteins from damaged tissues (Barber *et al.,* 1975). Non-significant change in total plasma protein has also been reported by Kaur *et al.* (2006) and as such shows limited utility as an indicator of hydration status of diarrheic neonatal calves (Roussel and Kasari, 1990).

In co-trimoxazole and neomycin treated groups there was non-significant increase in total protein values at 144th hr post treatment (6.29±0.22and 6.49±0.22 g/dl respectively) as compared to 0 hr and were comparable with healthy control group (6.44±0.20 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (5.55±0.18 g/dl).

In gentamicin and ciprofloxacin treated groups there was significant increase in total protein values at 144th hr post treatment (6.52±0.19 and 6.34±0.18 respectively) as compared to 0 hr and were comparable with healthy control group (6.44±0.20 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (5.55±0.18 g/dl).

5.6.2 Serum albumin

In infected groups the value of serum albumin (g/dl) ranged from 1.93±0.22 to 2.32±0.17 g/dl at 0 hr which were significantly ($P<0.05$) lower as compared to the healthy control group (2.93±0.13 g/dl). These findings corroborate with the earlier reports of Dubey *et al.* (1992) in diarrhoeic calves and ascribed it its loss through inflamed gut epithelium. However, Thortan *et al.* (1972) and Kaur *et al.* (2006) reported significant increase in plasma albumin level in diarrhoeic calves and ascribed to dehydration during diarrhoea.

In co-trimoxazole and neomycin treated groups the albumin levels at 144th hr after treatment (2.76±0.09 and 2.80±0.07 g/dl respectively) were significantly ($P<0.05$) higher as compared to values recorded at 0 hr and were comparable with healthy

control group (2.91±0.18 g/dl) and non-significantly higher than infected untreated control group (2.40±0.14 g/dl).

In gentamicin and ciprofloxacin treated groups the albumin levels at 96th hr post treatment were 2.77±0.19 and 2.51±0.13 g/dl respectively and were significantly ($P<0.05$) higher as compared to values recorded at 0 hr and at 144th hr of treatment became comparable with healthy control group (2.91±0.18 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (2.40±0.14 g/dl).

5.6.3 Serum globulin

In infected groups the value of globulin (g/dl) ranged between 3.43±0.12 and 3.68±0.15 g/dl at 0 hr which were comparable with healthy control group (3.78±0.90 g/dl). Slight decrease may be due to loss through inflamed gut epithelium. Unchanged globulin levels might be due to early institution of therapy and less severity of the disease Kaur *et al.* (2006).

In co-trimoxazole and gentamicin treated groups the globulin levels varied non-significantly and by 144th hr post treatment reached to 3.53±0.21 and 3.56±0.09 g/dl respectively and were comparable with healthy control group (3.53±0.20 g/dl) and infected untreated control group (3.15±0.12 g/dl).

In neomycin treated group there was progressive non-significant increase in globulin levels which reached 3.70±0.25 g/dl at 144th hr post treatment and was comparable with healthy control group (3.53±0.20 g/dl) and significantly ($P<0.05$) higher than infected untreated control group (3.15±0.12 g/dl).

In ciprofloxacin treated group the albumin levels varied non-significant and at 144th hr after treatment value recorded was 3.28±0.07 g/dl and was significantly ($P<0.05$) lower than the value recorded at 0 hr (3.57±0.26 g/dl). The value recorded at 144th hr after treatment varied non-significantly with healthy control group (3.53±0.20 g/dl) and infected untreated control group (3.15±0.12 g/dl).

5.6.4 Blood glucose

In infected groups of calves suffering from colibacillosis the value of blood glucose (mg/dl) ranged between 57.56±6.93 and 69.18±1.93 mg/dl at 0 hr which were significantly (P<0.05) lower than healthy control group (77.58±2.01 mg/dl). During the present study hypoglycemia was an important biochemical finding in diarrheic calves. The decreased glucose levels in diarrhoeic calves as compared to healthy calves has been also reported earlier (Bijwal *et al.,* 1987; Deshpande *et al.,* 1993; Walker *et al.,* 1998; Seifi *et al.,* 2006). The factors responsible for development of hypoglycemia seem to be anorexia and decreased intestinal absorption of glucose.

In co-trimoxazole and neomycin treated groups at 144th hr post treatment blood glucose value i.e., 75.71±3.66 and 72.20±2.17 mg/dl respectively were significantly (P<0.05) higher than that recorded at 0 hr and were also significantly (P<0.05) higher than infected untreated group (62.69±2.14 mg/dl) and non-significantly lower than healthy control group (77.20±1.83 mg/dl).

In gentamicin and ciprofloxacin treated groups blood glucose values recorded at 96th hr after treatment (73.21±2.04 and 72.68±1.84 mg/dl respectively) were significantly (P<0.05) higher than that recorded at 0 hr and were also significantly (P<0.05) higher than infected untreated group (59.68±2.60 mg/dl) and non-significantly lower than healthy control group (75.89±1.50 mg/dl).

5.6.5 Serum sodium

In all the groups of calves with clinical colibacillosis the value of serum sodium (mEq/L) ranged between 126.00±1.52 and 133.61±1.26 mEq/L at 0 hr which were significantly (P<0.05) lower than healthy control group (139.98±0.94mEq/L). The decreased sodium values may be due to increased loss sodium through gastrointestinal tract in diarrhoea (Tennat *et al.,* 1972). A significant decrease of serum sodium in diarrheic calves has also been reported (Deshpande *et al.,* 1993 and Bijwal *et al.,* 1987). Kumar and Mandial (2002) also reported hyponatraemia

and ascribed it to an excessive secreation of sodium along with water into the intestinal lumen. However, Kaur *et al.* (2006) reported hypernatraemia in colibacillotic calves and ascribed it to increased absorption of sodium on account of high glucose content of rehydration solution which was used for treatment purpose.

In co-trimoxazole treated group there was progressive significant increase in sodium values till 144th hr when its values reached to 136.29±2.24 mEq/L which was comparable with healthy control group (140.47±1.24 mEq/L) and significantly (P<0.05) higher than infected untreated group (132.02±2.21 mEq/L).

In neomycin and gentamicin treated groups the serum sodium recorded at 144th hr post treatment (139.72±1.58 and135.15±1.87 mEq/L respectively) were significantly (P<0.05) higher than that recorded at 0 hr of pretreatment and were also significantly (P<0.05) higher than infected untreated group (132.02±2.21 mEq/L) and comparable with healthy control group (140.47±1.24 mEq/L).

In ciprofloxacin treated group at 48th hr post treatment the serum sodium recorded was 131.20±1.13 mEq/L which was significantly (P<0.05) higher than that recorded at 0 hr but was significantly (P<0.05) lower than healthy control group (140.91±1.2 mEq/L) and was comparable to infected untreated group (132.01±1.72 mEq/L).

5.6.6 Serum potassium

In infected groups the value of serum potassium (mEq/L) ranged from 3.96±0.07 to 4.10±0.20 mEq/L at 0 hr and were comparatively lower than healty control group (4.32±0.09 mEq/L). The decreased potassium values may be due to increased loss potassium through gastrointestinal tract in diarrhoea. Hussain *et al.* (2001) and Chaleva and Encheva (2003) also reported hypokalemia in diarrhoeic calves. However, Fischer (1965) and Hasso *et al.* (1985) recorded hyperkalemia in diarrhoeic calves and ascribed it to increased K^+ retention by the kidney and also due to cellular damage.

In co-trimoxazole, gentamicin and ciprofloxacin treated groups potassium values 4.45±0.07, 4.51±0.13 and 4.46±0.14 mEq/L respectively at 144th hr were significantly (P<0.05) higher than that recorded at 0 hr and were comparable with healthy control (4.31±0.17 mEq/L) and significantly (P<0.05) higher than infected untreated control group (3.96±0.14 mEq/L).

In neomycin treated group potassium value 4.29±0.07 mEq/L at 144th hr was significantly (P<0.05) higher than that recorded at 0 hr (3.95±0.05 mEq/L) and was comparable with healthy control (4.31±0.17 mEq/L) and non-significantly higher than infected untreated control group (3.96±0.14 mEq/L).

5.7 Therapeutic trials in clinical cases of colibacillosis in calves

In the present study, the treatment of the clinical cases of colibacillosis in calves was studied on four trail groups comprising of six calves each. In these trail groups, therapeutic efficacy of four different antibacterials viz., Ciprofloxacin, Gentamicin, Neomycin and Co-trimoxazole was evaluated (which were selected on the basis of higher *in-vitro* sensitivity against *E. coli* isolates) against clinical cases of colibacillosis in calves. The efficacy of the drugs was assessed on the basis of clinical recovery and time taken for the disappearance of the clinical symptoms. Group 6 was given ciprofloxacin @4mg/kg body weight orally (250 mg tab-1/3rd bid) for five days and it proved to be most effective drugs wherein, drug helped in restoration of the disturbed haematobiochemical changes and abating clinical symptoms. The drugs helped in normalizing the haematobiochemical changes like hemoglobin, packed cell volume, total leucocyte count, blood glucose, serum sodium, total protein and albumin. The drug showed good results and proved to be 100 per cent effective for treating enteric colibacillosis. This might be due to the fact that these fluroquinolones group of antibiotics has got less resistance as they have recently been introduced and less used in Veterinary practice (Patil *et al.,* 1999; Choudhary and Das, 2003). The Fernandes *et al.* (2009a) also reported ciprofloxacin, effective against G-ve organisms in calf diarrhoea.

Group 5 was treated with another conventional gut acting antibacterial gentamicin @ 4mg/kg body weight (2 ml i/m bid), which showed 83.33 per cent efficacy in this study. Five of the calves clinically responded well with the fecal consistency returning to normal within six days. There was however, a significant increase in the blood glucose, serum sodium, serum potassium, total protein and albumin fraction with restoration of hematological values post treatment in the gentamicin treated group within six days which suggest that drug was effective for treating colibacillosis. This finding is in agreement with the findings of Jones *et al.* (1977) who reported that gentamicin improved the stool consistency in the calves with experimentally induced *E. coli* diarrhoea.

The infected calves of group 4 treated with neomycin @10mg/kg body weight orally ($1/2^{nd}$ bolus) proved 66.67 per cent efficacious in this study. There was marked improvement in clinical signs in four cases by day six with delay in recovery in two cases even after 144 hour post treatment. This drug helps in restoration of disturbed haematobiochemical parameters towards normalcy by day six. This drug given orally is currently labeled in United States for the treatment of calf diarrhoea (Constable, 2004).

The infected calves of group 3 were given co-trimoxazole at the dose rate of 15-30 mg/kg body weight orally ($1/5^{th}$ bolus), showed 66.67 per cent efficacy in this study. There was marked improvement in clinical signs in four cases by day six with delay in recovery in two cases even after 144 hour post treatment. This drug helps in restoration of disturbed haematobiochemical parameters towards normalcy by day six. Oral was also tried by Dubey and Rao (1991). Though endowed with a wide range of anti-bacterial activity, sulphonamides are not very effective against *E. coli*. Trimethoprim is, however, highly effective against both G-ve and G+ve bacteria. Sulphonamide-trimethoprim combination is claimed to possess drug potentiating effect (Dupont *et al.,* 1982; Hill and Pearson, 1988).

In the present study in severe cases electrolyte solution (oral rehydration salt solution) and dextrose saline 5 per cent was given intravenously. Blood *et al.* (1989) emphasized on the use of balanced oral or parentral electrolyte solution.

On the basis of therapeutic trials of different antibacterials given to the colibacillotic calves in this study, it is recommended that ciprofloxacin, gentamicin, neomycin and co-trimoxazole should be recommended for the field use.

CHAPTER – 6

SUMMARY AND CONCLUSION

Of the 250 diarrhoeic fecal samples, 217 tested positive for *Escherichia coli* with an overall prevalence of 86.8 per cent. The prevalence in organized and unorganized farms was 85.96 and 88.89 per cent, respectively. Season wise prevalence recorded in present study was 86.96, 91.36, 90.32 and 63.33 per cent during autumn, winter, spring and summer respectively.

One hundred and twenty nine isolates of *E. coli* were identified, characterized and got serotyped. One hundred sixteen serotypes belonged to 22 different 'O' serogroups (O20, O22, O69, O11, O84, O147, O68, O107, O123, O153, O157, O1, O6, O17, O36, O51, O60, O92, O102, O140, O158 and O159), 8 isolates were untypable and 5 were rough.

Among the different serogroups the highest percentage of 22.48 was found for O20 serotype followed by O22 and O69 serotypes having percentage of 14.73 and 10.08 respectively. Serotypes O20, O22, O69, O84, O107 and O123 were found in both organized farms as well as in unorganized areas.

In-vitro drug sensitivity pattern indicated that most of the serotypes were highly sensitive to four antibacterials, viz., ciprofloxacin, gentamicin, neomycin and co- trimoxazole. Tetracycline and enrofloxacin were moderately sensitive. Penicillin was low sensitive, where as, the isolates were resistant to amoxicillin and ampicillin.

Clinical signs recorded were, scant fecal volume to profuse watery diarrhoea with offensive odor, yellowish white in colour and sometimes blood stained, soiling of perineum and tail, depression, varying degree of dehydration, tachycardia, pale mucous membrane, prolonged capillary refill time, rough body coat, dry muzzle or mouth and profound weakness. Increased capillary refill time was reliable and a significant indicator of peripheral perfusion among diarrhoeic calves.

In infected animals haematobiochemical studies revealed significant increase in packed cell volume, haemoglobin, total leucocyte count and total erythrocyte count, where as, total protein, albumin, blood glucose and serum sodium were significantly decreased in diarrhoeic calves.

The therapeutic efficacy of four highly sensitive *in-vitro* drugs was evaluated, viz., ciprofloxacin, gentamicin, neomycin and co-trimoxazole. Ciprofloxacin at the dose rate of 4mg/kg body weight was found 100 per cent effective at 144th hour post treatment. Gentamicin at the dose rate of 4mg/kg body weight was found 83.33 per cent effective at 144th hour post treatment where as neomycin @10mg/kg body weight and co-trimoxazole @ 15-30 mg/kg body weight were found 66.67 per cent effective in this study at 144th hour post treatment. Treatment regimen used in four different groups of clinical cases of colibacillosis indicated ciprofloxacin being most effective drug followed by gentamicin, neomycin and co-trimoxazole which was comparable with *in-vitro* studies.

CONCLUSION

On perusal of the results obtained in the investigation, the following conclusions were drawn:

1. The prevalence of colibacillosis in calves was 86.8 per cent.
2. The prevalence was higher in un-organized (88.89 %) areas compared to organized areas (85.96 %).
3. Highest prevalence was recorded in winter (91.36%) and least in summer (63.33%).
4. One hundred sixteen serotypes belonged to 22 different 'O' serogroups (O20, O22, O69, O11, O84, O147, O68, O107, O123, O153, O157, O1, O6, O17, O36, O51, O60, O92, O102, O140, O158 and O159), 8 isolates were untypable and 5 were rough.
5. *In-vitro* drug sensitivity pattern indicated that most identified isolates were

highly sensitive to four antibacterials, viz., ciprofloxacin, gentamicin, neomycin and co-trimoxazole.

6. The clinical signs associated with colibacillosis ranged from profuse watery to whitish/ yellowish diarrhoea, soiling of perineum and tail, depression, varying degree of dehydration, tachycardia, pale mucous membrane, prolonged capillary refill time, rough body coat, dry muzzle or mouth and profound weakness.

7. A prolonged skin fold and capillary refill time were the other significant clinical findings in calves with diarrhoea.

8. There was significant increase in packed cell volume, haemoglobin, total leucocyte count and total erythrocyte count. Biochemical parameters were decreased like, total protein, albumin, blood glucose and serum sodium.

9. As per present study the clinical cases of colibacillosis in calves were effectively treated by ciprofloxacin followed by gentamicin, neomycin and co-trimoxazole which is comparable with *in-vitro* study and is recommended for the field use.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. S.K. Gupta, Professor & Head, Division of Clinical Veterinary Medicine, SKUAST-J, Jammu during Viva-Voce examination held on December 8, 2011 have been incorporated in the manuscript entitled **"A Study on the Prevalence and Therapeutic Management of Colibacillosis in Calves"** submitted by **Mr. Riyaz Ahmed Bhat (Regd. No. 2009-V-131-M)**.

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