Epidemiological, Clinical and Haemato-biochemical Characterization of Calf Diarrhoea and Evaluation of Therapeutic Regimens

वत्स अतिसार का जनपादकीय, शयनिक एवं रक्त जैव-रासायनिक निरूपण एवं चिकित्सीय पथ्यों का मूल्यांकन

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THESIS

DOCTOR OF PHILOSOPHY (Veterinary Medicine)



2013

Department of Clinical Veterinary Medicine, Ethics and Jurisprudence College of Veterinary and Animal Science Rajasthan University of Veterinary and Animal Sciences, Bikaner 334001 (Rajasthan)

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THESIS

Submitted to the Rajasthan University of Veterinary and Animal Sciences, Bikaner In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Veterinary Medicine)

BY

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2013

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S.No.	Abbreviation	Full Form	

1	S.E.	Standard error
2	°F	Foreignheit
3	FCC	Faecal consistency score
4	Hb	Haemoglobin
5	g/dl	Gram per decilitre
6	PCV	Packed cell volume
7	%	Percentage
8	TEC	Total erythrocyte count
9	μl	Microlitre
10	MCV	Mean Corpuscular Volume
11	МСН	Mean Corpuscular Haemoglobin
12	MCHC	Mean Corpuscular Haemoglobin
13	TLC	Total leucocyte count
14	mmol/L	Milimole per litre
15	A/G	Albumin/ Globulin
16	mg/dl	Milligram per decilitre
17	mg/ml	Milligram per mililitre
18	i.v.	Intravenous
19	MI	Millilitre
20	kg	Kilogram
21	Gm	Gram
22	b wt	Body weight
23	@	At the dose rate of
24	i.m	Intramuscular
25	χ^2	Chi ²
26	СВ	Crossbred
27	ND	Non-descript
28	E. coli	Escherichia coli
29	spp.	Species
30	μg	Microgram
31	PAGE	Polyacrylamide gel electrophoresis
32	ANOVA	Analysis of variance

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1. Introduction

Animal husbandry is not merely a subsidiary to agriculture but major economic activity, particularly in the arid and semi arid areas of the country. The enterprise provides much needed insurance against frequently occurring droughts and scarcity conditions in the region. Next to crop production, animal husbandry is the most important activity closely interwoven with the agriculture as farming and with the medicine as the health of the animals is concerned. Livestock plays vital role in socio-economic structure in our country by providing employment, livelihood, nutrition and food security and stabilizing the household income. It serves as the backbone of agrarian economy contributing approximately 25% of the total share from agriculture to national GDP while in critical semi arid and arid region where conventional agriculture is always gamble, the contribution of livestock to national GDP is much higher. Farm animals contribute to the need of protein-rich foods including milk products and meat to human beings, besides providing work power, manure and leather.

Successful development of livestock depends upon proper health management. Diseases are the major cause of economic losses due to mortality, cost of treatment and inefficient production of livestock. Diseases are the main constraint in development of livestock in the country. Morbidity and mortality among the neonates have always proved a bottleneck and causes serious blow to the roots of dairy husbandry in India.

The future of any dairy production depends among other things, on the successful program of raising calves. Calf rearing is an important component of a dairy farm to sustain the production. Calves are the future replacement stocks. In the calf rearing, the key management objective is to ensure that calves survive and remain healthy (Jozica *et al.*, 2012).

Calf mortality accounts significantly to the total mortality in the dairy farm (Kumaresan *et al.*, 2009). Calf mortality causes the loss of genetic material for herd improvement and decrease the number of dairy heifers available for herd replacement and expansion. Radostits *et al.* (2009) reported an estimated reduction of 38 % of net profit due to 20% calf mortalities. Economic losses resulting from calf mortality and morbidity can be easily recognized, but the effect of morbidity on future health and performance, which may constitute a loss of even greater importance, is difficult to estimate. It is generally attributed to many diseases viz. enteritis, pneumonia, naval ill, parasitism etc. The

diseases of the gastrointestinal system account for the overwhelming majority of calf disease and death. Gastrointestinal diseases tend to be most common within the first month of life (Dargatz, 1992). Calf diarrhoea is the most common type of gastrointestinal disorder. It is accounted for the largest percentage of deaths in calves.

Calf diarrhoea is one of the most commonly encountered disease syndrome and is the significant cause of economic losses in dairy industry. Calf diarrhoea remains the leading cause of mortality in dairy calves and an important cause of morbidity and mortality in beef calves (Constable, 2004). It is a costly disease, with losses estimated to be over \$250million annually (Bicknell and Noon, 1993). Economic losses associated with the disease include decreased performance, high morbidity and mortality and the expenses of medication and labour to treat the sick calves. The effectiveness of treatment and control of herd epidemics of diarrhoea in calves is frustrating and causes heavy economical losses (Radostits *et al.*, 2003). Diarrhoea affects young calves at an age when they have immature immune status, lacks specific antibody, illustrate high metabolism with added stresses imposed by weaning and sometimes deprivation of immune colostrum feeding.

Calf diarrhoea is a multi-factorial disease complex characterized by increased frequency, fluidity or volume of faecal excretion in calves caused by excessive osmotic pressure in the intestine, intestinal damage caused by several organisms leading to malabsorption, toxins produced by organisms or excessive contractions of the intestine (Lorenz, 2006). The occurrence of diarrhoea in calves is a result of the complex interactions of three sets of factors: the calf and the dam, the calf's environment, including management, and infectious agents. It is a syndrome of great etiological complexity.

There are many infective as well as non-infective causes of diarrhoea in calves which lead to progressive dehydration, electrolyte

loss and metabolic acidosis (Mitchel et al., 1992). The infective causes include parasitic (ascariasis, coccidiosis etc.), bacterial (Escherichia coli, Salmonella spp. etc.) and viral pathogens (rotavirus). However, beside the parasitic causes, the most common cause is pathogenic enteric microorganisms which interact with the immunity, nutrition and environment of calves precipitating varied degree of diarrhoea (Webstar, 1982). Microorganisms like Escherichia coli, Salmonella spp., rotavirus, corona virus and Cryptosporidium spp. causes diarrhoea in neonates (Tzipori, 1985; Snodgrass et al., 1986; Bicknell and Noon, 1993, Stoltenow and Vincent, 2003) which are collectively responsible for 75-95% of infection in neonatal calves worldwide (Hansa et al., 2012). Escherichia coli are the most common causative agent of calf diarrhoea (Gyles, 1994) which accounts for its 50 per cent occurrence (Boyd, 1974). There is possibility of occurrence of cross infections in men, animals and birds (Baruah et al., 2011). Thus some strains have globally emerged as important zoonotic and diarrhoeal pathogens.

Newborn calves are born aggamaglobulinemic, without any measurable circulating IgG or IgM. The newborn calf derives passive immunity by absorbing immunoglobulins from colostrum provided within the first hour of life. In the calf, passively acquired immunity is of importance to the health of the calf for the extended period of time until they are capable of making their own antibodies (Morrill and Tyler, 2012). The risk of development of infectious diseases is greater in calves in which there has been failure of passive transfer of maternal immunoglobulins (Gay, 1983). Thus, colibacillosis occurs most commonly in neonates especially in those with low concentration of serum immunoglobulins (Ig).

E. coli infection in colostrum deprived calves results in acute and often fatal episode (Fey, 1972). Calves which do not receive enough colostrum or receive colostrum with a low concentration of Ig grow

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slower and are more prone to diseases or to death than calves which receive colostrum of good quality (Quigley *et al.*, 1995). Colostrum feeding protects the newborn calf against the infections by providing immunoglobulins to prevent or minimize invasion of microorganisms to intestinal mucosa. Thus, it is essential for neonates to consume colostrum within an hour of birth to absorb adequate immunoglobulins to gain immunity against a variety of neonatal diseases including diarrhoea (Boyd, 1989). In rural areas of the country, the colostrum feeding to newly borne calf is generally delayed for variable time. There is misconception that the first milking colostrum is not safe to be fed to the new borne calf. The delay in feeding colostrum to new borne calf may contribute to infections as a result of decreased immunity. It may therefore be necessary to find out the status of immunoglobulins concentration in the serum and correlate with the infection.

Rotaviruses are the major etiological agents of acute viral diarrhoea in young ones of a wide range of avian and mammalian species including cattle and human beings (Flewett and Woode, 1978, Estes and Kapikian, 2007). Rotaviruses have been isolated from the calves, piglets, lamb, chicken etc. in India (Wani *et al.*, 2004a). Rotaviruses are generally species specific but cross species transmission is also possible (Martella *et al.*, 2001). There may be a continual input of rotavirus strains or sequences in to the human population from the animal population albeit at a very low level (Cook *et al.*, 2004). The usual signs of rotavirus infection are anorexia, depression, diarrhoea and dehydration. Prevalence of rotaviral enteritis has been reported from only few states of the country.

Cryptosporidium is a ubiquitous coccidian parasite that causes diarrhoea in many mammalian species. It is the second most common pathogen from young calves with diarrhoea (Hall *et al.*, 1992). In 1993, Garcia and Lima reported 7 to 9% of Cryptosporidiosis in young calves less than 2 months old in Brazil. *Cryptosporidium* sparked great public

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health interest after the large human water borne outbreaks in Milwaukee in 1993 and it was rapidly recognized as one of the most serious water borne pathogens to date. There are only very few reports on the prevalence of Cryptosporidiosis in India.

Gastrointestinal parasitism is another serious cause of calf diarrhoea between 1 to 6 months of age (Bilal *et al.*, 2009). Among the parasitic diarrhoea in calves ascariasis, coccidiosis including cryptosporidiosis are of concern. Parasitic diarrhoea is not only responsible for fluid loss and dehydration but may also cause anaemia leading to weakness and poor growth in the affected animals. Regular deworming with appropriate anthelmintic is of great importance in young and growing calves and may reduce the calf mortality to a significant level (Singh and Pachauri, 2012).

Diarrhoea not only occurs due to pathogens alone but also influenced by effects and interactions between management and nutritional practices.

Identification of infectious agents which cause diarrhoea in calves in a herd is essential for implementing effective preventive and treatment measures and may show any potential zoonotic risks, as several organisms causing diarrhoea have the potential to cause severe disease in humans (Bazeley, 2003). Further, data on the occurrence of different causative agents of diarrhoea in calves are scanty in India.

Clinico-biochemical alterations in diarrhoea are complex in nature characterized by imbalance of fluid, electrolyte and acid base status (Radostits *et al.*, 2009) endangering the life of animal patients. The end result of diarrhoea is development of varying degrees of dehydration associated with severe electrolyte imbalance and acidosis. The worse the dehydration and electrolyte loss, the more severely affected will be the calf. Mildly affected calves will be somewhat weak and depressed. With increasing severity, calves will show more severe depression, may be unable to stand, will lose their nursing reflex and will drop to subnormal body temperature. In even more severe cases, dehydration will lead to a calf that is unable to rise and will become comatose. When fluid and electrolyte losses are severe enough, affected calves will die. Death in diarrhoea is attributed to severe dehydration and metabolic acidosis (Groutides and Michell, 1990). The effect of diarrhoea on fluid, electrolyte and acid base status depends on the type, duration and severity of diarrhoea as well as the host response. Diarrhoea leads to excess loss of intestinal fluid resulting in severe dehydration (Demigne *et al.*, 1980), electrolyte imbalances like hyponatraemia, hypochloraemia, hyperkalaemia (Constable *et al.*, 1996) and acid base imbalances like low blood pH, loss of bicarbonates and development of metabolic acidosis.

A good diagnostic workup is important for successful treatment and preventive measures. The clinico-biochemical profile of the calves suffering with diarrhoeal syndrome though documented by several workers needs further investigation to make clinico-biochemical characterization of diarrhoea in calves in a different environment, climate, and management conditions and to evaluate different therapeutic regimens.

Antibiotic resistance of *E.coli* is one of the major challenges being faced in the treatment of animal patients suffering with colibacillosis. *E. coli* can acquire high level of resistant to some of the antibiotics either by spontaneous genetic mutation or by transfer of drug resistant plasmid to the recipient cells. This generally occurs due to indiscriminate use of antibiotics as chemotherapeutic agent or feed additives (Cruickshank *et al.*, 1975) There is no doubt that antimicrobial therapy has become one of the pillar of medicine in last 60 years. Antibiotics have saved the lives and eased the suffering of millions of human and animal patients but currently pathogenic bacteria are becoming resistant to these antibiotics at an alarming rate. The

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emergence and dissemination of antimicrobial resistance in bacteria has been well documented as a serious problem worldwide (Cohen, 2000). The results of faecal antimicrobial susceptibility testing have traditionally been used to guide treatment decisions. It has clinical relevance only when applied to faecal isolates of *E. coli*. Antimicrobial sensitivity tests are needed to be carried out repeatedly in different populations and regions and thus also for enteric pathogens especially *E.coli*.

Antimicrobial treatment of diarrheic calves should be focused against E. coli in the small intestine and blood, the two sites of infection. Antimicrobial efficacy is best evaluated by the clinical response of a number of calves to treatment, with calves randomly assigned to treatment groups (Constable, 2004). The treatment of infectious diarrhoea in calves with specific antimicrobial therapy alone is apt to give disappointing results, if supportive therapies for correction of fluid, electrolyte and acid base imbalances as well as energy deficits are not administered. The most important therapeutic targets needed for life saving in diarrhoea are (a) correction of dehydration especially intracellular fluid volume and its reduction in associated hyponatraemia; and (b) correction of metabolic acidosis and its associated hyperkalaemia (Groutides and Michell, 1990). The supportive therapies in neonatal diarrhoea comprise of intravenous and / or oral fluid therapy, nutritional therapy and herbal therapy. The use of parenteral fluid therapy has recently been overtaken as "frontline" treatment by oral rehydration therapy. The oral rehydration therapy may be very useful in early cases especially of mild to moderate diarrhoea. It is also very convenient for animal patients, owners and clinicians in respect of administration (Brooks et al., 1997).

An ideal oral rehydration solution should facilitate normalization of extracellular fluid deficits, absorption of sodium and water from intestine, induce alkalizing effect and provide sufficient energy (Philips *et al.*, 1971). The standard ORS available commercially in the market have been developed for human use taking human physiology and metabolism in to consideration and thus may not fulfill all the requirements of diarrhoeic calf patients. Therefore there is need to formulate and evaluate therapeutically ORS for calves with the ability to supply energy in addition to correction of water, electrolyte and acid base imbalances.

India has a rich wealth of local and traditional knowledge of herbal medicine. Over 7500 species of plants are being used for treating common and complex ailments both in humans and animals (Tripathi and Mandape, 2002). Further, various medicinal plants have been used for years in daily life to treat diarrhoeal syndromes. It will not only help to fasten the recovery but economical also. WHO has also emphasized the need to integrate traditional indigenous health care system with modern facilities (Dalal, 1992). Shisham (Dalbergia sissoo) leaves, Bael (Aegal marmelos) unripe fruit, Anar (Punica granatum) bark etc. was reported to be used in diarrhoea and dysentery with very good results. These are reported to have astringent property and reduce irritation in the digestive tract (Kumar et al., 2000; Nadkarni, 2000). The rural people in India and Nepal used shisham leaves to treat animals suffering from non specific diarrhoea. The bark, roots, leaves, and mucilage of Shisham has medicinal value for treating infectious diarrhoea (Brijesh et al., 2006). The herbal or indigenous medicines are needed to be validated scientifically.

Looking towards the losses occurring in the national dairy industry and breed improvement programmes from calf morbidity and mortality due to diarrhoea and to evolve most effective clinico-therapeutic measures against it in local management conditions, the present study was undertaken with the following objectives.

 To identify various etiological agents associated with calf diarrhoea and find out the prevalence

- (ii) Clinical and haemato-biochemical characterization of calf diarrhoea
- (iii) Determination of antibiotic sensitivity pattern of *E. coli* isolated from faecal samples of diarrhoeic calves
- (iv) Evaluation of various therapeutic regimens in clinical cases of calf diarrhoea

2. Review of Literature

2.1 Epidemiological Studies

White *et al.* (1970) found that *E.coli* was the predominant bacterium in faecal samples collected from calves with diarrhoea and in the intestinal contents from dead calves.

Panziera *et a1*. (1971) reported the first finding of cryptosporidia in association with diarrhoeal disease in young cattle.

Glantz *et al.* (1972) isolated *E.coli* from faecal swabs of 11 calves suffering from diarrhoea and 6 calves which have died due to diarrhoea.

Boyd (1974) conducted a survey of neonatal diarrhoea in homebred calves on a farm and found that the pathogenic strains of the *E.coli* were isolated in large numbers from 50 % of the rectal swabs.

Srisuparbh (1978) identified the cause of neonatal diarrhoea as *E.coli* alone in 26.25 % cases, *E. coli* and rotavirus in 25 %, *E. coli*, rotavirus and corona virus in 11.25 %, *E. coli* and corona in 87.5 %, rotavirus alone in 10 % and *Salmonella* spp. alone in 3.75 % cases.

Fallon (1978) did not find any correlation between serum Ig levels in calves between 4-14 days of age and incidence of diarrhoea but there was a positive relationship between serum Ig and calf survival.

Anderson and Bulgin (1981) incriminated cryptosporidia as the cause of disease in 2 to 3 day old calves with signs of watery faeces, dehydration and bristled hair.

Logan *et al.* (1981) reported that first and second parity cows have significantly lower levels of immunoglobulins in their colostrum than higher parity cows. It has also been shown that cows that have a dry period of less than four weeks produce colostrum with low immunoglobulin levels.

Reference	Country	Incidence (%)	Age (weeks)	
McGuire et al. (1976)	USA	64.00	-	
Fink (1980)	Germany	30.80	1	
		56.40	2	
Jenny <i>et al.</i> (1981)	USA	19.10	4	
Freese and Gravert (1982)	Germany	50.00	Soon after birth	
Umoh (1982)	Nigeria	8.70	12	
Gusbi and Hird (1983)	Libya	12.50-26.00	4	
Gusbi and Hird (1983)	Libya	18.80	13	
Afzal <i>et al</i> . (1983)	Pakistan	39.80	Before 1 Year	
Braun and Tennant (1983)	USA	18.90	5	
Bhullar and Tiwana (1985)	India	34.00	12	
Peters (1986)	UK	3.96	5	
		14.30	Before 1 Year	
Zrelli <i>et al</i> . (1988)	Tunisia	18.80	-	
Varma <i>et al</i> . (1988)	India	12.50	4	

 Table 1
 Incidence of calf mortality at different weeks of age

Bendali <i>et al.</i> (1997)	France	3.80	4	
Silva et al. (2001)	Brazil	18.65	-	
USDA (2002)	U.S.A	10.50	12	
Temesgen (2004)	Ethiopia	18.00	up to 6 months	
Gupta <i>et al</i> . (2005)	India	38.59.	Before 1 Year	
Chase (2007)	U.S.A.	60.50	12	
Sreedhar <i>et al</i> . (2010)	India	22.00	Before 1 Year	

Reference	Mortality	Morbity	
Singh and Singh (1973)	61.04	50.00	
Williams et al. (1975)	16.49	-	
Fink (1980)	35.90	44.70	
Simensen (1982)	35.60	-	
Sharma <i>et al</i> . (1984)	26.43	-	
Sangwan and Anand (1985)	13.80	-	
Bhullar and Tiwana (1985)	63.00	-	
Peters (1986)	14.10	-	
Shimizu and Nagatomo (1987)	3.60	17.00	
Belows <i>et al.</i> (1987)	10.00	-	
Lau (1987)	23.00	-	
Zrelli <i>et al.</i> (1988)	24.50	-	
Varma <i>et al</i> . (1988)	36.60	-	
Basoglu <i>et al</i> . (1992)	5.00 -25.00	-	
Bendali <i>et al.</i> (1997)	3.80	8.50	
Katikaridis (2000)	-	15.40	
USDA (2002)	62.10	-	
Zarzoso <i>et al.</i> (2002)	2.50	46.00	
Biewer (2001)	-	28.40	
Girnus (2004)	-	47.80	
Singh <i>et al.</i> (2006)	40.22	-	
Brahma and Singh (2007)	-	39.80	
Tikoo <i>et al</i> . (2009)	-	34.80	
Singh <i>et al</i> . (2009b)	-	24.00	
Sreedhar <i>et al</i> . (2010)	39.70	-	

Table 2Incidence of neonatal mortality and morbidity due to
diarrhoea in calves

Table 3Prevalence (%) of rotavirus, coronavirus, *E. coli*,
Cryptosporidium spp. and *Salmonella* spp. in
diarrhoeic calves

Reference	1	2	3	4	5
Yadav and Gupta (1971)	-	-	35.00	-	-
Shalaby <i>et al</i> . (1981)	48.00	-	-	-	-
Simeonov et al. (1981)	42.00	54.00	51.00	-	-
Schulz (1982)	38.80	32.00	-	-	-
Neuvonen et al. (1982)	93.70	-	-	-	-
Fiedler <i>et al</i> . (1982)	1.96	-	-	37.60	0.39
Hasso <i>et al.</i> (1983)	26.00	-	-	-	-
Kaushik <i>et al.</i> (1983)	45.45	-	-	-	-
Sizov et al. (1984)	57.00	-	34.00	-	-
Buhr-Pohlmann (1985)	9.00	-	33.00	3.00	12.00
Bordas <i>et al</i> . (1985)	50.00	20.00	33.00	9.60	-
Nagy et al. (1986)	40.00	15.00	50.00	27.00	0.00
Reynolds et al. (1986)	42.00	14.00	3.00	21.00	12.00
Snodgrass <i>et al.</i> (1986)	35.43	3.64	3.64	12.91	0.66
Debnath <i>et al</i> . (1987)	10.00	-	20.00	-	3.00
Castrucci et al. (1988)	100.00	-	-	-	-
Barrandeguy et al. (1988b)	27.00	-	30.00	5.00	10.00
De-Oliveira <i>et al</i> . (1989)	-	-	72.30	-	10.60
Brenner <i>et al</i> . (1993)	16.50	-	9.00	16.50	20.00
Lofstedt <i>et al</i> . (1999)	12.00	39.00	38.00	33.00	-
Aydin <i>et al</i> . (2001)	-	-	92.07	5.94	0.99
Wani <i>et al</i> . (2003)	-	-	63.68	-	-
Temesgen (2004)	-	-	-	7.20	3.60
Gupta <i>et al.</i> (2006)	-	-	23.72	-	0
Das et al. (2006)	-	-	-	32.90	-
Asati <i>et al</i> . (2008)	-	31.81	-	-	-
Pandey <i>et al</i> . (2009)	-	-	-	26.60	-
Roy et al. (2009)	-	-	31.81	-	-
Prakash et al. (2009)	-	-	-	9.05	-
Kumar et al. (2010)	-	-	72.22	-	-
Arora <i>et al.</i> (2010)	-	-	62.36	-	4.30
Palanivel et al. (2011)	-	-	-	9.05	-

1: Rotavirus; 2: Coroavirus; 3: *E. coli*; 4: *Cryptosporidium* spp.; 5: *Salmonella* spp.

It was the reason for higher incidence of diarrhoea in calves born during first or second lactation.

Shrivastva and Sharma (1980) estimated the mortality rate in zebu cattle calves and buffalo calves as 16.5 % and 30 %, respectively within first year of life. The death was mainly due to gastroenteritis, colisepticaemia, colibacillosis, pneumonia and lung congestion. The mortality rate was higher in male calves than female, showing higher in first four weeks after birth.

Khera (1981) reported that the calf mortality decreased with advancement of age. Maximum 50% mortality occurred within first month of age. Mortality of 32.21 % and 49.66 % was associated with digestive disorders in cow calves and buffalo calves, respectively.

Fischer (1982) found cryptosporidia in 62.6 % of calves aged 1 to 47 days.

Palasek (1982) detected cryptosporidia in calves first time in Czechoslovakia and found them in as many as 100 % of calves between 6 and 13 days of age, kept in large-capacity calf houses.

Fallon and Harte (1983) observed that diarrhoea in calves was significantly influenced by calf source (environment from where calves were purchased), serum immunoglobulin levels and weight at the time of purchase.

Krogh and Sherwood (1983) reported that the prevalence of *E.coli* in diarrhoeic calves varies widely depending on the geographical location, herds and age of the animals.

Sherwood *et al.* (1983) observed only 5-8 % prevalence of *E.coli* in diarrhoeic calves under 3 days of age in some countries.

Kaushik *et al.* (1983) reported the prevalence of rotavirus antibody in 91 (41.75 %) buffalo calves and 56 (30.36 %) cattle calves from Haryana, using immunodiffusion test. At 2–8 weeks of age, 45.45

% buffalo calves and 36.36 % cattle calves were positive for rotavirus antibody.

Tripathi and Soni (1984) isolated *E.coli* strains from crossbred calves suffering from neonatal calf diarrhoea without showing signs of septicaemia.

Contrepois and Vallet (1984) reported a high rate of diarrhoea in calves of a housed herd. Total 71 calves were found affected and 15 died. Faecal samples were collected from 28 affected calves. Out of them, *E.coli* was recovered from 19 calves, corona like virus from 19, rotavirus from 5 and cryptosporidia from 24.

Haggard (1985) reported that colibacillosis which is commonly referred as calf scours, is the principal cause of neonatal calf diarrhoea which maximally accounts for calf mortality.

Hunt (1985) reported that *Salmonella* spp. of bacteria have been associated with calf enteritis but like the septicemic *E. coli*, *Salmonella* has a strong tendency to spread beyond the gut and cause widespread disease.

Oveido *et al.* (1986) recorded that out of 101 diarrhoeic faecal samples collected from calves under 2 months of age, 25 faecal samples were positive for *Eimeria* spp., 22 for *cryptosporidium* spp., 18 for enterotoxigenic *E.coli*, 13 for strongyle, 11 for *strongyloides* spp. and one for giardia.

Peters (1986) reported 1.45 % mortality in calves due to salmonellosis.

Snodgrass *et al.* (1986) reported that *Cryptosporidium* occurs in diarrhoeic calves and more than 10 % of all the scouring calves excrete cryptosporidia at the same time as rotavirus.

Levine (1987) reported *E. coli* as the most important groups of bacteria causing diarrhoea in humans and animals. In debilitated or

immuno-suppressed host or when gastro-intestinal barriers are violated even normal nonpathogenic strains of *E. coli* can cause infection.

Shimizu and Nagatomo (1987) reported 20 % morbidity of salmonellosis in calves and 12.6 % mortality due to this disease in calves in Japan.

Maarof *et al.* (1987) observed highest mortality (34.08%) in the first month of life due to scours and enteritis in calf.

Korinek and Chroust (1988) examined 1577 faecal samples of sixty calves daily from 1 to 28 days after birth for cryptosporidia. Oocysts of *Cryptosporidium* spp. were found in 36.65 % of samples. The excretion of oocysts was first observed as early as 5 days after birth (1.7 % out of 60 calves), rose substantially from about 1 week of age and continued till the end of the observation period. Maximum values of extensity (i.e. per cent of infected animals out of the number of animals under consideration) and intensity (i.e. severity) of infection were found between 9 and 14 days of age. Investigation also revealed cryptosporidia in 30.4 % of healthy no diarrhoeic calves; in diarrhoeic calves the proportion of animals excreting cryptosporidia was 82.2 %. From the epizootiological point of view, this period poses a particular danger as regards the spread of infection. A direct relationship between the intensity of cryptosporidial infection and diarrhoea was demonstrated. They concluded that the incidence of cryptosporidia and their effect on the development and course of diarrhoeal disease in calves up to 28 days of age should not be underestimated.

Barrandeguy *et al.* (1988) detected virus, bacteria and parasites from the calves below 45 days age, affected with diarrhoea. Rotavirus and *E.coli* were most frequently detected pathogens in these calves. Mixed infections of rotavirus, *E.coli*, enterovirus, *Salmonella* spp., *Campylobacter jejuni, Clostridium perfringens*, other enterobacteria and coccidia were detected at low frequency.

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Curtis *et al.* (1988) studied the seasonal effect on the incidence of calf scours and found that the incidence was higher in winter as compared to summer. It was highest with in 14 days of birth. Risk of clinical signs was highest in the first week of life, while risk of death was highest in the third week.

Singh and Tomer (1988) examined incidence of diarrhoea in calves from birth to six months of age and reported that age of calf had significant effect. The incidence of diarrhoea was higher during the first month of age and thereafter it decreased with the increase in age. About 40 % of the calves were affected with diarrhoea either once or more times up to the age of six months.

Singh and Pandey (1988) reported that in India, the incidence of infection with rotavirus ranges from 10 % to 52 % in cow calves and 11 % to 24 % in buffalo calves.

Rao and Deorani (1988) recorded that 19.6 % cattle and 13.6 % buffaloes were infected with Strongyles, 2.9% and 4.1% with *Strongyloides* spp., 9.3 % and none with *Ascaris* spp., 36.6 % and 23.6 % with *Paramphistomum* spp., respectively in the Andaman Islands.

De-Oliveira *et al.* (1989) isolated enteric pathogens from 94.5 % calves that included *E. coli* from 72.3 %, *Salmonella* spp. from 10.6 %, *Proteus* spp. from 8.2 % and pseudomonas from 3 % calves.

Anderson (1989) reported 3.3 % and 1.03 % prevalence of *Cryptosporidium* spp. in adult and weaned calves in USA, respectively.

Pal and Baxi (1990) observed that out of 40 faecal samples from diarrhoeic buffalo calves, 30 had *E. coli* infection.

Khan and khan (1991) reported very high mortality in cow and buffalo neonates. This mortality has mostly been attributed to infectious agents, i.e. rotavirus, corona virus, enteropathogenic *Escherichia coli, salmonella* spp. and *cryptosporidium*. Other important causes of calf mortality include immunodeficiency, seasonal effects, parity of the dam, difficult parturition, sex and birth weight of the neonate and faulty management conditions. Of the infectious agents, rotavirus and *E. coli* are mainly involved in the causation of neonatal calf diarrhoea which leads to high mortality and morbidity in young calves. *E. coli* mainly plays its role up to second week of life while rotavirus up to third week.

Nouri and Toroghi (1991) reported the asymptomatic form of *Cryptosporidium* in 4-30 days of age groups. The high prevalence rate was reported in male calves.

Basoglu *et al.* (1992) reported a mortality rate of 5 to 25 % in calves affected with calf scours caused by enterotoxigenic *E. coli.*

Nappert *et al.* (1992) isolated *Cryptosporidium*, rotavirus and corona virus from diarrhoeic calves. Carbohydrate metabolism was found a significant problem in calves.

Heath (1992) reported that inclement weather was a prime factor in encouraging the spread of calf diarrhoea. Cold wet weather not only stresses calves, but also increases exposure to diarrhoeacausing organisms that spread via faecal contamination of muddy areas. Shelters designed to afford protection for calves may contribute to the problem if insufficient space is provided or the shelters are not cleaned or moved periodically. While providing protection from the elements, such shelters also increase crowding and spread of infectious disease among calves.

Levin (1992) observed that food deprivation for a period as short as 48-72 hours had adverse effects on enteric function, depressing the electrolyte concentration and enhancing net fluid and electrolyte secretion in response to secretagogues causing diarrhoea.

Frank and Kaneene (1993) reported that the incidence of diarrhoea in calves decrease with age.

Garry (1993) reported that the microbes may act individually to cause diarrhoea but frequently when a disease is found in a herd, two

or more of these agents can be identified in affected individuals or in the group of diarrhoeic calves.

Brenner *et al.* (1993) conducted an epidemiological study on neonatal calf diarrhoea in Israel and recorded *Salmonella* spp. (20 %), *Cryptosporidium* spp. and rotavirus (16.5 %), enterotoxigenic *E. coli* K99 (9 %) in diarrhoeic young calves. Further 98 % of salmonellosis cases were found in calves less than three weeks of age.

Garcia and Lima (1993) reported 7-9% incidence of bovine Cryptosporidiosis in young calves less than 2 months of age in Brazil.

NAHMS (1993) reported that *Cryptosporidium parvum* is highly prevalent in the cattle population on US dairy farms and a prevalence of 90 % is reported with almost half of the 1 to 3 weeks old calves shedding oocysts.

Veerapandian *et al.* (1993) recorded that the calf mortality varies between 6.17 and 22.34 % during different age. It was found maximum during first week of age and then declined as age progressed. Enteritis was found to be major cause.

Xiao and Herd (1994) collected faecal samples weekly over a 3 months period from 0-20 week old calves and detected *Cryptosporidium* oocysts and *Giardia* cysts as early as 4 days of age. *Cryptosporidium* oocysts peaked at 1 week and were low by 3 weeks. Some calves however continue to pass low number of oocysts. Shedding of Giardia cysts peaked at 2 weeks of age with high levels maintained until 7 weeks of age. Cumulative infection rate for both *Cryptosporidium* and *Giardia* were 100%.

Sadiek and Schlerka (1995) isolated *E.coli, Campylobacter spp., Eimeria bovis*, rotavirus and corona virus from 36 milk fed calves affected with moderate to severe diarrhoea whereas *E.coli* and *Campylobacter spp.* was also isolated from severely dehydrated calves. Clement *et al.* (1995) identified the risk factors associated with the development of diarrhoea in calves in five beef breeds and reported a higher incidence of diarrhoea in calves born to heifers than in calves born to cows. Bull calves were at higher risk than heifer calves. The case fatality rate was less than 9 %.

Olson *et al.* (1995) observed that Cryptosporidiosis was high in 8 and 30 days of age groups.

Dixit and Sahasrabudhe (1995) reported that the incidence of *Toxocara* spp. was highest in buffalo calves and *Trichuris* spp. in the cross-bred calves. The incidence of *Strongyle* spp. was highest in adult native cattle. It was high during rainy season but low during winter.

Aly *et al.* (1996) detected 12 isolates of enteropathogenic *E.coli*, 5 isolates of *Salmonella* spp. and 8 isolates of *Pseudomonas* spp. in the faecal samples taken directly from rectum of diarrhoeic new born buffalo calves.

Chauhan and Singh (1996) detected rotavirus infection in 42 % diarrhoeic calves in Uttar Pradesh by using ELISA, EM and RNA-PAGE. Diarrhoea associated with rotavirus was observed mainly during winter months. Calves of both sexes were affected. Calves of crossbred or exotic breeds were more susceptible than indigenous animals.

Bendali *et al.* (1997) observed 447 diarrhoea cases in 3157 calves from 94 beef herds, yielding an overall diarrhoea incidence rate of 0.40 cases per 100 beef calf-days at risk. Risk of diarrhoea was highest at 1 week of age and decreased quickly after 3 weeks of age. The cumulative risk of diarrhoea until 30 days of age varied between herds, it ranged from 0 to 70 %, with a median of 8.5 %. The mortality rate on all herd averaged 3.8 % and the case fatality rate for diarrhoea was 4 %. They also observed a significant association between season of birth and the incidence rate of diarrhoea. The incidence rate was lower in April and January and increase on February, December and

March. The univariate and multivariate screening procedures identified 20 variables associated with diarrhoea incidence rate eg. month of birth, housing type, cow feeding practices, cleaning and disinfection of housing etc.

Gopalnath (1997) reported that the seasonal differences of Cryptosporidiosis observed were not marked and clinical disease could be noticed during all seasons.

Mtambo et al. (1997) determined prevalences of Cryptosporidium spp. oocysts in cattle in Tanzania using the modified Ziehl-Neelsen staining technique. Of 486 bovine faecal samples, 5.3 % positive for *Cryptosporidium* spp. The prevalence of were Cryptosporidium was higher in calves less than 3 months of age compared to weaned calves and adults. Cryptosporidium spp. oocysts were detected in both diarrhoeic and non-diarrhoeic animals, but there was a significantly higher prevalence (P < 0.001) of oocyst shedding in diarrhoeic than in non-diarrhoeic animals.

Jhala and Raghvan (1997) examined 123 diarrhoeic faecal samples of calves in Bangalore by RNA-PAGE for rotavirus. Four (3.25 %) samples were found positive. All the seventy samples from clinically normal calves were detected negative.

Grover *et al.* (1997) examined 71 faecal samples collected from organized dairy farms in Ambala and Meerut by RNA-PAGE for presence of rotavirus. Fifteen (21.1 %) were found positive.

Gulati *et al.* (1997) reported that the rotavirus infection was prevalent during winter months i.e. November to February.

Fuente and Luzon (1999) detected *Cryptosporidium* in 43.8 %, 71.9 %, 63.2 %, and 6.9 % of faecal samples, respectively, in the first four weeks of life in diarrhoeic calves. Mixed infections with other enteropathogens were also common.

Klingenberg *et al.* (1999) studied the incidence of diarrhoea among calves after strict closure and eradication of bovine viral diarrhoea virus in a dairy herd and reported that there was no association of sex, group size and birth weight with the development of diarrhoea in calves.

Lofstedt *et al.* (1999) examined 252 calves < 28 days old with diarrhoea on Prince Edward Island, Canada. The faeces of diarrhoeic calves were examined for enteric pathogens and were positive for corona virus (39 %), enterotoxigenic *E. coli* (38 %), cryptosporidia (33 %), and rotavirus (12 %). Forty-one percent (103/252) of the calves had failure of passive transfer of colostral immunoglobulin and 31% (78/252) of the calves were bacteremic predominantly with *E. coli*. Bacteremia was detected in a significantly (P<.0001) greater proportion of calves with failure of passive transfer (47/103 = 46 %) than in calves with adequate passive transfer (21/116 = 18 %) and calves \leq 5 days old.

Ryan *et al.* (1999) observed that 24 % of calves of less than one month age groups were more susceptible than adult.

Gulati *et al.* (1999) reported the prevalence and relative frequencies of G and P types of rotavirus during 1994 to 1997 from diarrhoeic cow and buffalo calves, less than 1 month of age from organized dairy farms and concluded that bovine rotavirus may cross the host species barrier and may be circulating among neonates in India.

Hussain and Saikia (2000b) isolated 101 strains of *E. coli* from 93 faecal samples of 1 to 35 days old diarrhoeic calves. Diarrhoea was more prevalent in 3 to 4 weeks age group of calves than in other age groups.

Garcia *et al.* (2000) reported that several enteropathogens were recovered from neonatal calf with diarrhoea, their relative prevalence varies geographically but the most common prevalent infections in most areas were *Escherichia coli,* rotavirus, corona virus, *Cl. perfringens*, *Salmonella* spp. and *Cryptosporidium* spp.

Jindal et al. (2000) observed that the overall prevalence of infection with rotavirus in both cow and buffalo calves, as recorded by RNA-polyacrylamide gel electrophoresis (PAGE) and ELISA in the organised dairy farm was 27.02 % (10/37). In unorganised dairy herds, the prevalence was 26.08 % (11/41) and 19.5 % (8/41) by RNA-PAGE and ELISA, respectively. In the organised dairy farm, 41.6 % (5/12) of cow calves tested positive for rotavirus infection by RNA-PAGE and 33.3 % (4/12) by ELISA. In buffalo calves, 20 % (5/25) tested positive by RNA-PAGE and 24 % (6/25) by ELISA. In unorganised dairy herds, RNA-PAGE detected 6 rotavirus-positive cow calves from a total of 24 animals (25 %) and 5 rotavirus-positive buffalo calves from a total of 17 (29.4 %). Using ELISA, 20.8 % (5/24) of cow calves and 17.6 % (3/17) of buffalo calves were positive for rotavirus. The infection was more prevalent during the first week of life (12/25) and in female calves (13/37) than male calves (8/41). Calves with liquid faeces were more often positive (12/30) for rotavirus than calves with semi-liquid faeces (9/48).

Aydin *et al.* (2001) found 92.07 % cases positive for *E. coli*, 1.98 % for *Campylobacter jejuni*, 0.99 % *Salmonella* spp., 5.94 % *Eimeria*, 9.90 % *T. vitulorum*, 5.94 % cryptosporidiosis and 5.94 % samples revealed neither parasitic nor bacterial infection in calves (2-30 days age) having diarrhoea.

Chowdhury and Das (2001) recovered 137 isolates of *E.coli* in pure cultures from 198 faecal samples of calves suffering with diarrhoea and from 8 calves which had been died after showing the symptoms of diarrhoea in two districts of West Bengal.

McClure (2001) reported that neonatal diarrhoea was major cause of illness and death of calves below one month of age.

Silva *et al.* (2001) studied the effect of the management practices on the calf mortality rate and found that implementation of simple management practices viz. establishment of maternity paddock, assistance during delivery, naval disinfection, administration of colostrum to new born calves, feed supplementation and training of the workers reduced the calf mortality rate on the farm from 18.65 % in 1994 to 9.39 %, 6.45 % and 4.67 % in 1995, 1996 and 1997, respectively.

Khurana and Pandey (2001) described the presence of rotavirus A, for 1st time in India in diarrhoeic neonatal calves. Out of 150 faecal samples collected from 27 symptomatic calves and 123 asymptomatic calves, less than 30 days of age, bovine rotavirus was found in 14 samples (5 symptomatic and 9 asymptomatic calves).

Chaurasia (2001) reported 87.00 % occurrence of *Escherichia coli* in the faecal samples of diarrhoeic calves.

USDA (2002) reported that the overall mortality in preweaned heifer calves was 10.5 % of heifer calves born alive in 2002 and diarrhoea accounted for 62.1 % of calf losses.

Zarzoso *et al.* (2002) evaluated a vaccination strategy and found that vaccinating pregnant dams with local strains of bovine rotavirus and *E.coli* reduced the morbidity rate of neonatal calf diarrhoea from 46 % to 3 % and mortality rate from 2.5 % to nil.

Shaheen *et al.* (2002) observed *E.coli, Proteus* and *Salmonella* spp. in 28 diarrhoeic calves of 7 to 35 days of age.

Okada and Matsumoto (2002) examined 1481 faecal samples from diarrhoeic calves in Japan between 1987 to 2000. Rotaviruses were isolated in 142 samples by cell culture and latex agglutination test.

Svensson *et al.* (2003) reported that diarrhoea was one the most common diseases reported in calves up to 3 months old. In general,

the quality of colostrum increased with the lactation number of the dam which explains the higher risk of disease in calves born to first-lactation cows.

<u>VLA (2003</u>) reported that most cases of calf diarrhoea were likely to be mixed infections, where more than one of the pathogenic agents was present. Mixed infections with rotavirus and *Cryptosporidium* appeared to be most common.

Stoltenow and Vincent (2003) reported E. coli as the single most important cause of bacterial scours in calves. Single infections were common but mixed infections (eg, *E. coli* + *Cryptosporidium* or Corona virus + Salmonella etc.) were often observed. The damage caused by either corona or rotavirus was often compounded by bacterial infections. Cryptosporidia can be a primary pathogen but they were often found to be part of a mixed infection in combination with corona virus, rotavirus, and/or *E. coli*. Calves infected with *Cryptosporidium* ranged from one to three weeks in age. The risk for fatal diarrhoea was increased when mixed infections occur. Calves as young as one or two days old scour from corona or rotavirus; however, most outbreaks occured when calves were near a week of age and older. The morbidity (number of sick calves) ranged from one to two percent up to 20 to 30 % of the herd. Mortality (death) rates were quite variable. Conversely, up to 25 % mortality had been reported, particularly when bacteria compounded either corona or rotavirus infections. Death losses were consistently associated with pronounced dehydration. Coccidiosis could be a very serious disease in weaned calves, but was seldom a problem in young calves. However, outbreaks in calves three to four weeks of age and older had been reported. Most outbreaks were associated with stress, poor sanitation, overcrowding, or sudden changes in feed.

Wani *et al.* (2003) concluded that 249 (63.68 %) and 60 (59.4 %) strains of *E. coli* were isolated from 391 bovine and 101 ovine

diarrhoeic faecal samples, respectively. STEC were detected in 9.73 % and 6.66 % of the calves and lambs studied, respectively whereas EPEC in 9.73 and 26.66 % of the calves and lambs, respectively.

Constable (2004) reported that calves with diarrhoea often had small intestinal overgrowth with *Escherichia coli* bacteria, regardless of the inciting cause for the diarrhoea, and 30 % of systemically ill calves with diarrhoea had bacteremia, predominantly because of *E coli*. Further, he also reported that Mixed infections with enteric pathogens were commonly diagnosed in calves with naturally acquired diarrhoea and the clinical signs and pathologic damage associated with rotavirus infection were more severe when *E coli* was present than when it was absent. Primary viral morphologic damage to the small intestine also facilitated systemic invasion by normal intestinal flora, including *E. coli*.

Temesgen (2004) observed that the overall incidences of crude morbidity and crude mortality in calves up to the age of 6 months were 61.5 % and 18.0 %, respectively. The most frequent disease syndrome was calf diarrhoea with the incidence of 42.9 %. The incidence of calf diarrhoea and crude morbidity were apparently higher in large dairy farms than in the market oriented smallholder farms. However, the mortality was higher in the later. Risk factors with significant association to calf diarrhoea were age, condition of birth and cleanness of calf house. Older age was again associated with low risk of diarrhoea as compared with younger age. Calves from prolonged labour or dystocia and housed at unclean house were at greater risk of diarrhoea than those calves from normal delivery and in clean house, respectively. Based on laboratory examination, Salmonella and Cryptosporidium were detected from diarrhoeic calves at rate of 2/55 (3.6 %) and 4/55 (7.2 %), respectively. The serotypes of Salmonella identified were Salmonella typhimurium and Salmonella heidelberg both of which were susceptible to commonly used antibiotics.

Acha *et al.* (2004) observed a low overall prevalence (5 %) of diarrhoea in calves up to the age of 6 months in different farms in Mozambique. *Salmonella spp.* was isolated in only 2 % of calves whereas *Campylobacter* was isolated in 11 % of calves. ETEC strains were not found, but K99 antigen was more prevalent in *E. coli* strains from diarrhoeic calves. The incidence varied between farms and between sampling occasions from 0 % to 39 %. The number of cases of diarrhoea was higher during the rainy seasons.

Wani et al. (2004b) examined one hundred and twenty nine faecal samples collected over a period of one year from 96 diarrhoeic and 33 non diarrhoeic lambs aged between 0 and 3 months for the presence of rotavirus and E. coli. Group A rotavirus was detected in 25 % of diarrhoeic lambs using ELISA and RNA-PAGE. Statistically no significant relation was found between rotavirus infection and age of the lambs. The prevalence of rotavirus was more related to meteorological changes than age of the lambs as the number of diarrhoeic lambs with rotavirus infection was found to increase in spring months during which temperature and humidity ranged between 7.34 and 28.9°C and 34.28 and 82.58 %, respectively. None of the lambs without diarrhoea carried rotavirus infection. Bacteriological examination revealed 56 strains of E. coli out of 96 diarrhoeic and 4 strains out of 33 non diarrhoeic faecal samples. Mixed infections were most common.

Sharma (2004) reported higher prevalence of rotavirus in buffalo calves (40 %) and cow calves (34.78 %) in Central India. Male calves were found more susceptibility than female calves.

Wani *et al.* (2005) reported an outbreak of diarrhoea in 4-7 week old crossbred calves in an organized dairy farm in Kashmir, India. The outbreak began in the first week of January 2003 and continued for two weeks. The nine affected calves were diarrhoeic for four to five days and voided liquid, malodorous faeces. Other signs included lethargy and dehydration. Total 11 strains of *E. coli* were recovered from nine diarrhoeic and two apparently healthy faecal samples. None of the faecal samples were positive for Salmonella or group A rotavirus.

Sevinc *et al.* (2005) demonstrated that 5-15 days old goat kids were more often infected with *Cryptosporidium* spp. infection than other age groups. *Cryptosporidium* infection was detected in 19.56 %, 9.72 % and 5.93 % of kids in age groups of 5-15, 16-30 and 31-90 days, respectively.

Gupta *et al.* (2005) recorded that a total of 1684 calves were born during the period 1993-2002 and out of them 650 died. Overall calf mortality was 38.59 %. Mortality for male and female calves was 40.90 % and 36.29 %, respectively. There was no significant difference between sexes. Among different age, the highest mortality (5.99 %) was recorded in 121-150 days age group followed closely in 91-120 days age groups (5.23 %). No significant different among age group was found. Highest mortality (90.15 %) was observed during rainy season followed by summer (36.74 %), winter (30.46 %) and autumn (18.33 %). A significant difference was found among seasons.

Minakshi *et al.* (2005) found that out of 380 diarrhoeic calf samples, 138 (44.81 %) were positive for rotavirus infection in nucleic acid hybridization assay in Haryana and adjoining areas in India.

Lorenz (2006) reported rotavirus and cryptosporidia as major pathogens to be found in faecal samples of diarrhoeic calves and a low prevalence of corona virus and enterotoxic *E. coli* in different European countries.

Gupta *et al.* (2006) reported 23.72 % prevalence of *E.coli* in neonatal diarrhoeic calves aged 0-7 days. *Salmonella* spp. could not be isolated in any of the faecal samples examined.

Kumar and Verma (2006) screened the faecal samples of 250 calves of up to 6 months of age and reported that 72.8 % samples

showed presence of either single or mixed helminthic ova. The single infection was more common than mixed infection. *Neoascaris vitulorum, Trichuris, Strongyloides, Strongyle, Monizia* and Amphistomes were present in 38.8, 11.2, 9.6, 8.8, 3.2 and 1.2 % calves while mixed infection was noticed in 16.8 % calves. Incidence of *N. vitulorum* infection decreased as the as of the calves increased while Strongyle infection increased with increase in age.

Singh *et al.* (2006) observed enteritis (40.22 %) as the major cause of calf mortality. Calves up to 1 month of age, low birth weight and females were found to have significantly higher mortality rate.

Shobhamani and Alahasingari (2006) reported prevalence of *Cryptosporidium* in calves in Andhra Pradesh and found that the prevalence of Cryptosporidiosis was highest (48.69 %) in young calves in the age group of 31- 60 days followed by 1-30 days (43.24 %) and lowest (13.09 %) in adult calves. The prevalence was higher in cross bred calves (31.80 %) than in indigenous breeds (13.00 %).

Das *et al.* (2006) found that overall 20.80 % animals were affected with diarrhoea, out of which cryptosporidiosis was found in 32.9 %. Percentage of samples positive for *Cryptosporidium* in farm 1 was 35.8 % and 8.1 % for diarrhoeic and non diarrhoeic calves, respectively. At farm 2 these percentages were 26.9 % and 4.7 %, respectively.

Garaicoechea *et al.* (2006) reported an incidence of 62.5 % of rotavirus diarrhoea in bovines by examining faecal samples collected during a 10-year period (1994–2003) from beef and dairy herds in Argentina.

Kusumakar (2006) reported 24.12 % prevalence of rotavirus in central India in calves, piglets and children. Maximum prevalence was observed among diarrhoeic children (36.36 %) and least in piglets (25.71 %). The young ones below 2 months in animals and below 2 years in children were more susceptible to rotavirus infection.

Chase (2007) reported diarrhoea causes 60.5 % of neonatal mortality in unweaned dairy heifer calves.

Odde (2007) on the basis of epidemiological data indicated that calves born to first-calf heifers have a higher incidence of calf scours. Heifers produced lower quantities of colostrum. Therefore, their calves had lower concentrations of serum antibody levels.

Brahma and Singh (2007) investigated the disease incidence pattern among young calves over different seasons, age groups and sexes. Diarrhoea showed highest (39.85 %) incidence followed by fever / pneumonia and lameness. The overall incidence of diarrhoea was significantly higher (P<0.01) in buffalo calves (48.16 %) than either crossbred or indigenous cattle breeds. Crossbreds had a significantly higher (P<0.01) incidence of diarrhoea than indigenous calves. Age significantly (P<0.01) influenced the occurrence of diarrhoea, being significantly highest in the first month of life and gradually reduced thereafter with the advancement of age. Maximum incidence of diarrhoea was recorded in rainy season followed by autumn and winter.

Manya *et al.* (2007) reported that coccidiosis mostly occurred in calves of 3 weeks to 9 months of age in overcrowded, stressed and malnourished animals at all the times of the year and prevalent of 65 % population of calves at clinical and subclinical level.

Bardhan (2007) reported 14.29 % prevalence of rotavirus in central India in bovine calves.

Wani *et al.* (2007) screened 406 and 142 diarrhoeic faecal samples from calves and lambs respectively, by RNA-PAGE and ELISA for rotavirus. Seventy six (18.71 %) samples from calves were detected positive while fourteen (9.85 %) samples from lambs were detected positive.

Sharma and Soni (2008) reported that 3 out of 34 faecal samples of enteritic calves (1 day to 4 week age) yielded isolates

typical of genus Salmonella. Two isolates were confirmed as *S. typhimuriam* and one as *S. waltevreden*.

Asati *et al.* (2008) isolated *Escherichia coli* from faecal samples of diarrhoeic calves and recorded 31.81 % overall prevalence of neonatal calf diarrhoea due to colibacillosis.

Manuja *et al.* (2008) examined 455 faecal samples from buffalo calves in north India for rotavirus by RNA-PAGE and ELISA, 21 samples (4.61 %) were found positive.

Chandrashekar (2008) examined 345 diarrhoeic faecal samples, collected from cattle and buffalo calves in M.P., India, for rotavirus infection by RNA-PAGE and found an overall prevalence of 11 % with a higher prevalence (17.2 %) in buffalo calves than cow calves (6.45 %) in the region.

Kaur and Kaur (2008) studied prevalence of gastro-intestinal parasites among cattle including calves of Patiala and its adjoining areas. The GI parasites detected in cow/buffalo were *Toxocara vitulorum* (78.57 %), *Haemonchus* spp. (57.14 %) followed by *Cryptosporidium* spp. (50 %), *Eimeria* spp. (50 %), *Oesophagostomum* spp. (42.86 %) and *Trichuris* spp. (14.29 %).

Barua *et al.* (2009) examined 115 goat and 60 cattle faeces for GI parasites and observed that incidence of *Fasciola* spp. was 8.33 %, *Amphisome* spp. 18.33 %, Strongyle spp. 21.66 %, *Strongyloides papillosus* 1.66 %, *Toxocara vitulorum* 5.00 %, *Moniezia expansa* 5.00 % and *Eimeria bovis* in cattle calves.

Jeyakumar *et al.* (2009) collected 45 faecal samples from cattle including calves of an institutional herd during peak monsoon i.e. August to September in Andaman and Nicobar Islands and observed that all the samples (100 %) were found positive for various parasites such as Strongyle (88.88 %), *Trichuris* spp. (15.55 %), Amphistomes

(8.88 %), *Strongyloides* spp. (42.22 %), *Eimeria* spp. (77.77 %) and mixed parasitic infection (86.66 %).

Tikoo et al. (2009) recorded 34.80 % as an overall incidence of diarrhoea in neonatal calves. They suggested that season, age, managemental practices and breed of the calves had a direct bearing on the incidence of the neonatal calf diarrhoea. Diarrhoea in calves of organized and unorganized sectors was 31.50 and 35.90 %, respectively. The incidence was highest in monsoon (38 %) as compared to winter (35 %) and summer (31 %) seasons. Maximum E.coli and Salmonella spp. isolates were recorded from diarrhoeal cases in monsoon season. The incidence of diarrhoea in cow and buffalo calves was 39.30 and 28.50 %, respectively. The incidence of diarrhoea in Holstein Friesian and crosses, Red Sindhi and cross, Jersey and cross and non-descript local calves were 31.80, 27.80, 48.80 and 33.30 %, respectively. Among buffalo calves, incidence was 29.10 % in Murrah and cross and 27 % in non-descript local calves. Age group wise, 41.30 % in 1 to 10 days, 56.60 % in 11 to 20 days, 28.40 % in 21 to 30 days and 12.80 % in 31 to 40 days of age were seen.

Fernandes *et al.* (2009c) reported that out of 70 clinical cases of neonatal bovine entero-colibacillosis, 48 tested positive for *Escherichia coli*. These belonged to nine different serotypes, the most predominant being enero-toxigenic *E. coli* (ETEC). Non lactose fermenting members of the entero-bacteriacae family were also present. Compared to soil, water and environment (atmosphere air) were the more important sources of the *E.coli* infection.

Sahu and Maiti (2009) found 27.27 % and 20.09 % prevalence of cryptosporidiosis in bovine calves in Durg and Rajnandgaon districts of Chhatisgarh state, respectively. The prevalence rate was maximum (50 %) in 1-2 weeks, followed by 27.27 % in 2-3 weeks age group. The youngest calves affected with Cryptosporidiosis were 4 days old. The breed wise prevalence of cryptosporidiosis showed 33.91 % in crossbreds and 18.06 % in indigenous calves. The season wise prevalence was 36.84 % during winter, followed by 27.69 % in rainy, 23.08 % in autumn and 15.38 % in summer.

Pandey *et al.* (2009) found 26.6 % and 27.1 % overall incidence of *Cryptosporidium* spp. oocyst in young calves and goat kids respectively in Zambia. Maximum percentages of positive cases were in 2-4 weeks old calves (36.3 %). The youngest calf showing oocyst was 6 days old.

Panchasara *et al.* (2009) reported that calf scour diarrhoea was the predominant cause of mortality in Mehsana buffalo calves. Out of total deaths, 35.71 % died due to calf scours. The deaths were more in monsoon followed by winter and least in summer. Mortality was higher in male than female calves. The mortality percentage reduced with the age advancement, the maximum being during first month of age.

Singh *et al.* (2009a) reported calf diarrhoea as most important infectious cause of mortality in neonatal calves and rotavirus, corona virus, *E.coli, Salmonella* spp. and *Cryptosporidium* as the infectious etiological agents of calf diarrhoea. Rotavirus was detected mostly in the faeces of diarrhoeic calves up to 3rd week of life whereas *E. coli* up to 2 week of life with highest frequency in calves younger than 3 days.

Singh *et al.* (2009b) reported that out of total calves presented to a hospital for various ailments, 24.05 % were affected with enteritis which included both cow and buffalo calves. The common causative agents were excessive milk ingestion (dietary diarrhoea), bacterial infection (colibacillosis), viral infection (rota viral diarrhoea) and parasitic infestation (ascariasis, coccidiosis).

Singh *et al.* (2009c) examined 124 faecal samples of buffalo calves and found 70.16 % positive for single and mixed infections of strongyles (49.19 %), followed by amphistomes (32.26 %), coccidia

(28.23 %), ascarids (7.25 %) and *Strongyloides* (0.1 %). The mixed parasitic infestation was observed in 22.58 % samples.

Roy *et al.* (2009) examined 150 diarrhoeic calves and found 31.81 % positive for colibacillosis. The highest prevalence were recorded up to 1 week of age (54.7 %), followed by 1 to 2 weeks (37.7 %) and lowest in 2-3 weeks of age (7.5 %). Calves of the both sexes were equally affected. The breed wise prevalence was higher in crossbreed (66.0 %) than indigenous (33.9 %). Highest occurrence was recorded during post monsoon (39.6 %).

Abe *et al.* (2009) found 9 faecal samples positive for rotavirus, out of 171 faecal samples collected in Japan between 2006-2007.

Gulati *et al.* (2009) reported 35.4 % prevalence of equine rotavirus in diarrhoeic foals. The prevalence ranged between 36-45 % during 2004-2006 with no significant variation. It was more in foals below 2 weeks of age (44.2 %) than in older foals (20 %). The prevalence was significantly higher in March- April as compared to subsequent months of foaling.

Prakash et al. (2009) observed that overall prevalence of Cryptosporidiosis in young calves in Chennai was 9.05 %. The incidence was highest (14.66 %) in young calves in the age groups of 5-30 days followed by 9.38 % in 31-60 days age group, 6.06 % in older calves (3-6 months) and the lowest 3.23 % in adult calves (7-12 months). Eighteen calves above 1 year were negative for Cryptosporidiosis. Analysis of data with regard to season wise prevalence indicated no significant difference in susceptibility (summer-9.46 %; rainy-10.14 % and winter- 7.69 %). A significantly higher incidence of Cryptosporidiosis was observed in crossbred calves (10.95 %) than indigenous breeds (5.95 %). Females (11.76 %) were more susceptible than male calves (4.71 %). The clinical signs observed were malabsorptive diarrhoea, profuse watery diarrhoea which was pale yellow in colour with a distinctive offensive smell and

rarely blood tinged semi-solid faeces. They inferred that very young calves reared in unhygienic farm premises were highly susceptible to Cryptosporidial infections.

Sharma *et al.* (2009) detected a prevalence of 43 % and 30 % of rotavirus diarrhoea in cattle and buffalo calves in Jammu.

Niture *et al.* (2009) detected 6 (7.22 %) faecal samples positive for bovine rotavirus by RNA-PAGE, out of 83 faecal samples collected between 2008 to 2009 in Maharashtra. All the samples showed 4:2:3:2 migration patterns typical of rotavirus A.

Ingle *et al.* (2009) examined 34 faecal samples collected from diarrhoeic calves in Maharashtra by RNA-PAGE and reported that all the 34 samples were found negative for rotavirus.

Bilal *et al.* (2009) reported high (69.05 %) prevalence of GI parasites in calves between 1 to 6 months age whereas in the age group of 7 to 12 months, 42.10 % calves were found positive. Prevalence was higher for nematodes followed by mixed infection and cestodes.

Devkate *et al.* (2010) reported an overall occurrence of 1.49 % for bovine diarrhoea. Species wise occurrence of diarrhoea was found to be higher in cattle (2.16 %) than in buffaloes (1.36 %). Age wise young animals < 12 months of age (10.0 %) were found more susceptible than adult animals (0.84 %). Sex wise higher occurrence in males (3.16 %) as compared to females (0.99 %) was recorded. Season wise highest occurrence was recorded during winter (2.03 %) followed by summer (1.88 %) and least in monsoon (1.65 %).

Vagh and Jani (2010) found that out of total 286 cattle calves and 677 buffalo calves, 122 (42.65 %) and 314 (46.38 %) calves were found positive for calf scours, respectively in Anand district of Gujarat. All the isolates of *E. coli* were typed for 'O' antigen. In diarrhoeic cattle calves, the prevalence of serotype O56 and O82 was highest. In diarrhoeic buffalo calves, the prevalence of serotype O56 was highest followed by serotypes of O82.

Kumar *et al.* (2010) found bacterial diarrhoea in 30 calves out of 56 calves below one month of age having diarrhoea. The bacteria isolated include *E.coli* (72.22 %), *Proteus* spp. (16.66 %), *Klebsiella* spp. (8.33 %) and unidentified organisms (2.77 %). The incidence of diarrhoea was observed higher in colostrum deprived calves.

Arora *et al.* (2010) revealed that 62.36 % diarrhoeic samples were positive for *E.coli* followed by *Proteus* spp. (9.67 %), *Klebsiella* spp. (6.45 %), *Streptococcus* spp. (5.37 %), *Salmonella* spp. (4.30 %) and *Staphylococcus* spp. (4.30 %). Remaining 7.52 % samples were negative for bacterial culture.

Bora *et al.* (2010) observed that out of 157 faecal samples collected from diarrhoeic piglets 68 (43.31 %) were positive in sandwich ELISA while 61 (38.85 %) were positive in SDS-PAGE for rotavirus.

Sreedhar *et al.* (2010) accounted enteritis for highest mortality (39.7 %) in buffalo calves followed by pneumonia (23.8 %), anaemia (12.5 %), pneumo-enteritis (11.3 %), toxaemia (6.8 %), septicaemia (3.4 %) and tympany (2.2 %). The overall mortality in indigenous buffalo calves from birth to one year age was 22.0 % with male calves having higher mortality than female calves. The calves born with lower body weight had significantly high mortality. As the age of the calves increased, the mortality percentage was decreased. Highest mortality (19.5 %) was observed in calves below one month of age. The calves born during summer (18.2 %) and autumn (16.5 %) season had significantly lower mortality than those born during winter (23.8 %) and spring (32.7 %) seasons.

Swiatek *et al.* (2010) reported a prevalence of 26 % for bovine rotavirus A out of 100 faecal samples collected between 2004 to 2005 from calves with and without diarrhoea in Australia.

Beg *et al.* (2010) examined 200 faecal samples from calves aged 0 to 6 months, in Kashmir and detected 31 samples positive for rotavirus by RNA-PAGE.

Manuja *et al.* (2010) reported that out of 455 faecal samples screened, 33 (7.25 %), 14 (3.08 %) and 15 (3.35 %) were positive for rotavirus by monoclonal antibody based ELISA, RNA-PAGE and RT-PCR assays, respectively.

Basera *et al.* (2010) screened 128 diarrhoeic faecal samples from cattle and buffalo calves by RNA-PAGE from Uttarakhand region and found thirteen (10.15 %) samples positive for rotavirus.

Palanivel *et al.* (2011) reported 9.05 % prevalence of *Cryptosporidium* spp. oocyst in calves. The prevalence was significantly higher in young calves in the age group of 5-30 days (14.66 %), followed by age group of 31-60 days (9.38 %) and older calves of above 3 months (6.06 %). In adults it was lowest (3.23 %).

Baruah *et al.* (2011) reported prevalence of *E. coli* in different animals and man. They recovered 144 *E. coli* isolates from faecal samples of diarrhoeic piglets (43), calves (19), buffalo calves (14), goats (9), pups (10), yaks (7), poultry (15) and humans (27). As many as 12.5 % Shiga toxin producing *E. coli* (STEC) were detected among the 144 *E.coli* isolates which assumed a special significance in terms of potential public health hazards as these strains may be cross transferred to man via food chain.

Badiei *et al.* (2011) collected 661 rectal faecal samples from natural cases of diarrhoeic calves in Iran and found that all herds (high producing dairy cows and average producing dairy cows) had *E.coli* infected diarrhoeic calves in their population. The proportion of *E. coli* infected diarrhoeic HPDC and APDC Holstein calves of younger dams (>2 to 3 years) were higher than HPDC and APDC calves of older dams (3 or greater than 4 years). There was no difference among the

occurrence of *E.coli* infection in diarrhoeic HPDC and APDC calves of different herd size groups.

Nourmohammadzadeh *et al.* (2011) collected 100 faecal samples of diarrhoeic calves over a period of 12 months in Iran and observed rotavirus in 34 % of cases and a significant difference was found between the prevalence rate and the season (P<0.05), the highest prevalence was recorded in winter time (48 %) and the least during the summer season (16 %). Statistically significant difference was also found between the two age groups (P<0.05); the highest prevalence rate was seen at 2–4 weeks of age (47.61 %) and the lowest in the first week (20 %). It seems that the presence of a high rate of maternal antibody in colostrum during the first week after parturition can protect the newborn calves against rotavirus.

Gupta *et al.* (2011) reported highest incidence of amphistomiasis in buffaloes (92 %) followed by goat (88 %), cattle (70 %) and sheep (60 %) which includes their offspring also in Udaipur region.

Mondal *et al.* (2011) screened 63 diarrhoeic faecal samples of 38 buffalo calves and 25 cow calves collected from organized dairy farms in Mumbai, by RNA-PAGE. Among the cow calves 1 (4 %) sample and among the buffalo calves 4 (7.89 %) samples showed migration pattern of 4:2:3:2 typical for rotavirus A.

Chitambar *et al.* (2011) found three samples positive by ELISA out of 78 faecal samples for bovine rotavirus A in Pune, Western India.

Malik *et al.* (2012) found that out of total 930 cases of calves recorded in different veterinary hospitals of north western U.P., 53.66 % were of diarrhoea. The incidence rate ranged between 48.68 % and 57.68 % at different places. The incidence rate of calf diarrhoea in cattle calves (52.51 %) was almost similar to that in buffalo calves (54.37 %). Statistically, there was no significant difference with regards to monthly distribution of diarrhoea cases. However, there was

significant difference in the incidence of calf diarrhoea between winter and summer months. The maximum number of calf diarrhoea cases were reported after the onset of rains and continued till the end of winter and autumn which appears to be related to post calving season and climatic stress.

Hansa *et al.* (2012) reported first time in India bovine corona virus with an incidence rate of more than 14 % (15/101) with ELISA and about 20 % (20/101) by RT-PCR. The syndromes associated with bovine corona virus include respiratory and intestinal tract infections in young calves. The virus has specific tropism for intestinal and pulmonary epithelial cells.

Jyoti *et al.* (2012) observed that the overall prevalence of GI parasitic infections in calves was 71.18 % which was higher than adults (32.77 %). Prevalence was higher in buffalo calves (73.22 %) than cattle calves (67.45 %). Mixed infections were 29.43 %. Coccidiosis was the predominating parasitic disease of calves followed by *Strongyle* spp., *Strongyloides* spp., *Moniezia* spp. and *Toxocara vitulorum*.

2.2 Clinical and haemato-biochemical characterization

McSherry and Grinyer (1954) reported that diarrhoeic calves became unable to stand and show significant dehydration and hypoglycemia. The calves also had low blood pH, decreased bicarbonate concentration, hyponatraemia, hypo or hyperchloraemia, azotaemia, hyperkalaemia and acidosis.

Roy *et al.* (1959) reported that there was loss of body weight, negative sodium and potassium balance, hyponatraemia, hyperkalaemia, uraemia and metabolic acidosis in diarrhoeic calves.

Fey *et al.* (1963) reported that out of 88 calves which died due to coli-septicaemia, 80 calves had no gamma globulin in their blood although they received colostrums on their day of birth.

Gay *et al.* (1965) reported association of colibacillosis with the deficiency of plasma immunoglobulins.

Dalton *et al.* (1965) found that calves affected with diarrhoea developed dehydration along with metabolic changes like hyponatraemia, hypo or hyperchloraemia, azotaemia, hyperlactataemia and acidosis.

Selman *et al.* (1971) reported significantly higher immunoglobulin levels in calves that were fed colostrum with in 6 hours after birth than those who received colostrum later.

Fisher and deLa Fuente (1972) reported raised PCV, plasma urea and potassium and low blood pH and bicarbonate in the calves died from neonatal diarrhoea.

Talos and Roth (1972) found decrease in blood glucose and sodium content and an increase in haematocrit value and blood potassium content in 30 calves between 1 to 7 days of age, suffering with enteritis.

Thronton *et al.*, (1972) reported that calves died due to diarrhoea had lower gamma globulin concentration. The concentration of serum albumin and alpha globulin was high and that of gamma globulin was low in the severely diarrhoeic and dehydrated calves.

Tennant *et al.* (1972) observed dehydration in all the calves with enteric infection and haemoconcentration, metabolic acidosis, hyponatraemia, hyperkalaemia, increased blood urea nitrogen and serum inorganic phosphorus in most of the cases.

Manoiu *et al.* (1972) recorded that haemoglobin and haematocrit values were increased in 74 dehydrated calves suffering with enteritis. In biochemical changes, there was decrease in sodium and albumin-globulin ratio with hypo or agammaglobulinaemia.

Penhale *et al.* (1973) observed that the efficiency of absorption of immunoglobulins from the intestine was directly proportional to the age but shut down was different for each class of immunoglobulin i.e. IgG can be absorbed for 27 hours, IgA for 23 hours and IgM for 16 hours.

Mauten *et al.* (1974) reported that there may be villous atrophy and cellular infiltration of lamina propria with lymphocytes and plasma cells and lesser number of neutrophils and eosinophils in cryptosporidiosis in a calf.

Boyd (1974) concluded that the calves with lower amounts of passive immunity had higher rates of diarrhoea than the calves with higher amount of the passive immunity.

Logan (1974a) observed that colostrum principally acted as prophylactic in action against diarrhoea and had little influence once diarrhoea commenced.

Logan *et al.* (1974b) studied the immunity of calf to colibacillosis and prophylactic use of a pooled serum IgM - rich fraction under field conditions and reported that serum IgM - rich fraction could be used as a colostral substitute.

Logan *et al.* (1974c) studied the serum immunoglobulin levels in suckled calves from birth to five weeks and found that calves having high levels of immunoglobulins survived whereas calves with intermediate levels of immunoglobulins died due to non septicaemic diarrhoea and calves with low levels of immunoglobulins died due to colisepticaemia.

Lewis *et al.* (1975) observed hypoglycemia with glucose concentration less than 40 mg/dl of plasma in experimentally induced viral diarrhoea in calves. This change in glucose concentration took place during 24 hours proceeding death of the affected calves.

Fisher and Martinez (1976) concluded that colostral immunoglobulins also exhibited a local activity in the intestine against *E. coli.* Therefore colostrum feeding was beneficial even after

absorptive phase of the intestine (up to 12 hours of birth). They also reported that survival from diarrhoea could be a function of high IgG concentration.

Wilson and Jutila (1976) concluded that IgA, IgM and anti Kimmunoglobulins seemed to be most important immunoglobulins in protecting the calves against colibacillosis.

Johnston *et al.* (1977) reported that colostrum fed to calf soon after birth provided protection from colisepticaemia but did not prevent the diarrhoea due to colibacillosis.

Pohlenz *et al.* (1978) reported that signs are usually unapparent but chronic diarrhoea has been associated with cryptosporidiosis especially in neonatal calves.

Bakheit and Greene (1981) observed that 5.3 % of calves which had more than 20 zinc sulphate turbidity test (ZSTT) units died compared to 5.7 % those with less than 20 ZSTT units and opined that ZSTT was an unreliable guide to the future validity of any individual calf.

Zepperitz and Seidal (1982) observed in field conditions that unweaned calves with severe diarrhoea had low blood gamma globulin content, mean haematocrit value 45.7 %, plasma urea 112 mg/dl and plasma creatinine 1.3 mg/ dl. Improvement in the clinical status as well as haematological values in survivor calves occurred 48 hours of treatment with dextran / sodium chloride, plasma extender and an electrolyte solution containing sodium, potassium, magnesium, chloride, acetate ions and sorbitol.

Jones and Hunt (1983) reported that *Salmonella* infections are most frequent and of great concern to young animals. These rodshaped, gram negative organisms are usually motile and produce gastroenteritis with nausea, vomiting, cramps and diarrhoea.

Roy and Sinha (1984) reported symptoms of varying degree of dehydration and loss of body weight, depression, coma, sunken eye ball, loss of skin elasticity, dryness of the mouth, thirst, coldness of extremities, subnormal temperature, rapid pulse and drowsiness exaggerated as the duration of diarrhoea prolonged. Death was due to peripheral circulatory failure and shock.

<u>Norheim and Simensen (1985</u>) reported that there were seasonal differences in the absorption of immunoglobulins. Calves born during the summer months in temperate/cold climates achieved higher levels of serum immunoglobulins than calves born during the winter months.

Kasari and Naylor (1985) reported metabolic acidosis as sequelae to diarrhoea induced dehydration.

Levine (1985) reported that the most common clinical manifestations of coccidiosis include inappetance, weakness, loss of weight, diarrhoea, depression and anaemia.

Sangwan and Anand (1985) noted that the mean plasma immunoglobulin levels at 24 hours of birth in crossbred calves were significantly higher in healthy calves (28.77 q 0.44 mg/ml) and sick survived calves (31.54 q 1.89 mg/ml) than the calves died (14.90 q 0.65 mg/ml).

Hancock (1985) reported that there were differences between herds in the average serum Ig concentration as well as in the number of hypogamma-globulinemic calves (Ig < 5mg/ml).

Wijerante (1986) found that Friesian and Jersey calves with low serum gamma globulin levels had high mortality rate.

Bijwal and Misra (1987) observed a decrease in blood pH, glucose, bicarbonate and sodium and increase in haemoglobin, haematocrit, blood lactate and blood urea nitrogen values in experimental enteric colibacillosis in calves.

Booth and Naylor (1987) reported depression and dehydration as the notable symptoms in diarrhoeic calves infected with rotavirus and corona virus along with an increase in PCV and decrease in blood pH and bicarbonate levels.

Nooruddin *et al.* (1987) reported decreased Hb %, PCV %, TEC, total protein and increase TLC concentration in cow calves suffering with trichuriasis.

Sridhar *et al.* (1988) reported debility, anorexia, lethargy, elevation of body temperature, pulse and respiratory rates in diarrhoeic calves. There was increased Hb, PCV, TEC, TLC, serum albumin and alpha globulin, potassium and chloride values along with hypogammaglobulinaemia and hyponatraemia.

Dubey (1989) evaluated the clinico-biochemical changes in induced diarrhoea in calves. The concentration of serum electrolytes Na^+ , K^+ and CI^- declined significantly at 12 to 36 hr post-infection concurrent with increased total protein concentration.

Groutides and Michell (1990) observed dehydration, metabolic acidosis, prerenal uraemia, hyponatraemia and hypoglycemia as an important blood profile in *E. coli* induced diarrhoea in calves. They found variability in the concentration of serum potassium. Diarrhoeic calves which survived show hypokalaemia whereas those which died had severe metabolic acidosis and hyperkalaemia.

Radhakishan *et al.* (1991) conducted study on 50 neonatal diarrheic buffalo calves. Hematological examination revealed significant elevation of PCV i.e. 43.7 % in diarrheic calves against 35 % in normal calves.

Srivastava *et al.* (1991) observed that serum sodium and potassium concentration (mEq/L) were 133.3 ± 1.75 and 5.0 ± 0.10 , respectively in calves suffering from diarrhoea, compared to 147.0 ± 2.66 and 5.0 ± 0.14 , respectively in the healthy calves. Chloride

concentrations (mEq/L) averaged 81.4 ± 1.73 respectively in diarrheic calves, as compared to 95.7 ± 2.10 respectively in the healthy calves.

Michell *et al.* (1992) observed increased PCV level, hyponatraemia and hyperkalaemia in cases of induced diarrhoea in calves.

Deshpande *et al.* (1992) studied on 46 buffalo calves with diarrhoea and 5 apparently healthy control of similar age. In diarrheic calves they revealed significant increase in PCV level.

Maach *et al.* (1992) found extreme metabolic acidosis, haemoconcentration, hypoglycemia, hyponatremia, hypochloraemia and hyperkalaemia in calves suffering from acute diarrhoea.

Grove-White and White (1993) observed that metabolic acidosis was wide spread and severe among the older diarrhoeic calves as compared to younger calves.

Bicknell and Noon (1993) reported that neonatal calf with scours had watery yellow, gray or greenish diarrhoea containing varying amounts of mucus which may be tinged with blood. Soiling of the hindquarters and tail with the diarrhoeic feces was common. At first, the animals appeared alert and otherwise normal, but soon refused feed and became depressed, weak, and unable to stand. Dehydration characterized in the calf by sunken eyes, dry skin and weakness. Body temperature readings varied, depending to some extent on the disease agents involved. One consistent fact, however, was a subnormal body temperature in the terminal stages of the disease.

Sahal *et al.* (1993) reported that the diarrhoeic calves aged 1 to 15 days showed hyperkalaemia, metabolic acidosis and azotaemia. These calves were successfully treated with intravenous electrolyte solution containing 1.3 % sodium bicarbonate.

Michell (1994) recognized metabolic acidosis as a potential life threatening consequence of diarrhoea.

Crawford et al. (1995) investigated immunoglobulin concentration in serum in response to injectable immunoglobulin in neonatal dairy calves and found that serum IgG and IgM concentration in colostrum deprived dairy calves at 48 hour after birth was 1.1 and 0.4 g/L, respectively while 28 days of age was 5.3 and 0.8 g/L, respectively, whereas Serum IgG and IgM concentration in colostrum fed calves at 48 hour after birth was 14.6 and 1.0 g/L, respectively while 28 days of age was 12.4 and 0.5 g/L, respectively. Mean IgG and IgM in serum of calves injected with Ig were 4.2 and 0.7 g/L, respectively, and were higher than in calves receiving no Ig. Mean IgG (14.6 g/L) and IgM (1.0 g/L) concentrations in serum of calves fed colostrum were higher than in other calves. Subcutaneous Ig provided moderate amounts Ig in serum.

Verma *et al.* (1995) observed that calves below 14 days of age affected with colibacillosis exhibited the clinical signs as elevated to subnormal temperature, increased heart rate, watery to semisolid faeces and dehydration.

Aly *et al.* (1996) observed anorexia, elevated body temperature and depression with varying degree of dehydration in 28 diarrhoeic buffalo calves passing faeces with offensive odour and bloody mucoid discharge. Haemato-biochemical studies revealed a significant increase in PCV, Hb and serum potassium and a significant decrease in serum total protein, glucose, sodium and chloride.

Constable *et al.* (1996) induced diarrhoea by administering milk replacer, isotonic sucrose solution and furosemide and observed a moderate dehydration, marked lethargy, decreased cardiac output and plasma volume, increased blood lactate, PCV, serum albumin, creatinine, sodium and phosphates.

Talukdar (1996) examined goats showing stunted, pot bellied anaemic and hidebound with diarrhoeic faeces and stated that these symptoms were suggestive of helminth infection in them. Bouda *et al.* (1997) reported that metabolic acidosis, partial compensatory mild pre-renal uraemia, hypoglycemia, hyponatraemia and haemoconcentration were the most important findings in mild dehydrated diarrhoeic calves.

Michell (1998) opined that hyper/hyponatraemia, hyper/hypokalaemia and severity of acidosis were particularly important in relation to the optimum composition of fluids used for therapy.

Walker *et al.* (1998) reported profuse watery diarrhoea, severe dehydration, increase in total serum protein, albumin, creatinine and decrease in venous blood pH, base excess, bicarbonate concentration, mild metabolic acidosis and hyperkalaemia as the major clinicobiochemical alterations in experimentally induced diarrhoeic calves.

Lorenz *et al.* (1998) conducted study on 50 calves suffering from diarrhoea and observed increased potassium and decreased sodium level.

Raman *et al.* (1999) studied the haemato-biochemical changes in cross-bred calves experimentally infected with *Haemonchus contortus*. A significant reduction in PCV% and Hb values with lymphopaenia, neutrophilia and mild eosinophilia were noticed.

Baber *et al.* (2000) induced *E.coli* diarrhoea in newborn buffalo calves and observed green to yellow white colour faeces with watery to semi-solid consistency, weakness, dullness, depression, emaciation and dehydration. The haematological findings included increased haemoglobin concentration and PCV value.

Kalita *et al.* (2000) noted symptoms of diarrhoea in kids such as passage of light yellow watery faeces, inappetance, loss of skin elasticity, dryness of muzzle, coldness of extremities and drowsiness. The respiration and heart rates increased significantly on day 2nd onwards. There was also significant reduction in body temperature.

Rajora and Pachauri (2000) evaluated 36 diarrhoeic calves and six apparently healthy controls. In diarrhoeic calves they revealed significant increase in packed cell volume.

Bali *et al.* (2000) recorded changes in electrolytes in diarrhoeic calves and observed decreased concentration of serum sodium and increased concentration of serum potassium and increased serum protein levels in diarrhoeic calves.

Devi *et al.* (2000) conducted clinico-haematological studies in bovine calves naturally infected with *Toxocara vitulorum*. Hb %, PCV %, and TEC in the affected calves were significantly reduced. Increased MCV and decreased MCHC indicated macrocytic, hypochromic anaemia.

Shrivastava *et al.* (2001) reported hyponatraemia, isokalaemia, hyperchloraemia and hypoproteinemia in diarrhoeic calves.

Kalita *et al.* (2001) reported that reduction of body temperature in diarrhoea might be due to decreased availability of lipid and glycogen exhaustion which were the major energy substrates for heat production.

Gutzwiller (2002) found in a survey which lasted one year and included data of 73 dairy cows with their calves that colostrum immunoglobulin G (IgG) of 22 primiparous cows and serum IgG of their calves were lower than the corresponding IgG levels of 51 multiparous cows and their calves. Serum IgG concentration was not correlated with diarrhoea incidence. Although there were no seasonal differences in the IgG concentration of colostrum and calf serum, neonatal diarrhoea incidence was higher in calves which were born in winter than in calves which were born in summer (P < 0.01). Thus the high diarrhoea incidence in winter was not a consequence of an insufficient IgG transfer to the calves.

Santos *et al.* (2002) evaluated hematological and serum biochemical changes in *Salmonella* infected diarrheic calves and reported increased PCV%, TEC and Hb concentration, concomitant with a transitory leucopenia.

Kumar and Mandial (2002) observed a significant increase in PCV, hyponatraemia, hyperkalaemia, hyperproteinemia, in clinical colibacillosis in calves. They also noticed profuse diarrhoea, dehydration, depressed appetite, dullness and depression without any variation in body temperature, heart rate and respiratory rate.

Shaheen *et al.* (2002) recorded signs of depression, unthriftiness, partial refusal to suckling and moribund state leading to severe diarrhoea. The faeces were pasty semiprofuse and pale yellow in calves below 10 days of age and watery and profuse with pale yellow in calves of 20-30 days of age.

Stoltenow and Vincent (2003) reported that clinical signs associated with *Salmonella* infection include diarrhoea, blood and fibrin in the faeces, depression and fever. The disease is more severe in young or debilitated calves whereas in coccidiosis, occasionally, affected calves may exhibit signs of brain damage but tarry or bloody scours are commonly observed.

Halmandge *et al.* (2005) found that diarrhoeic buffalo calves affected with ascariasis exhibited the clinical signs of weakness, pot belly, rough hair coat, mild to moderate diarrhoea and congested mucous membranes.

Das *et al.* (2006) observed that the temperature and pulse and heart rates in calves affected with *Cryptosporidium* related diarrhoea varied significantly as per degree of severity of the cases. Body temperature increased at the onset and declined with the advancement of infection whereas heart and pulse rates were higher at the early stages and became thread subsequently. There was varying degree of anorexia, dehydration and weakness and emaciation.

Kaur et al. (2006) reported that mean PCV values (pretreatment) were significantly higher (46.28 ± 1.71 %) as compared to healthy control calves (32.14±2.53 %). Following therapy, there was a gradual and significant decline in the PCV values by 48 hr (41.90±1.44 %). Mean albumin levels in untreated calves were significantly higher (3.42±0.10 g/dl) than the healthy control group (3.05±0.15 g/dl) which significantly declined (3.01±0.09 g/dl) at 48 hr post treatment. Plasma sodium was higher in the untreated group as compared to control group. A significant increase in mean plasma potassium level was seen 96 hr post treatment. There was no significant change in total plasma protein and globulin levels of diarrhoeic calves as compared to healthy control calves. The treatment measures instituted were antimicrobial drugs and oral rehydration solution comprising NaCl, NaHCO₃, KCL and glucose. Intravenous fluids of NaCl, ringer's lactate or 7.5 % NaHCO₃ were added depending on the severity of dehydration and acid base status. Thus colibacillosis results in significant haemoconcentration and PCV estimation was most sensitive indicator for assessing dehydration.

Sena *et al.* (2006) reported that serum immunoglobulin status at 0 hours (immediately after birth) was very low and it increased significantly after 12 hours and later up to 72 hours. The increasing trend was drastic till 72 hours and later slight increase in trend was noticed up 3 months. The Ig and serum protein profile revealed highly significant changes in the newly born calves till 3 months of age. The mean total protein and globulin concentration showed a highly significant increasing trend up to 3 months. The mean albumin and A/G ratio showed a decreasing trend till 72 hours and later albumin concentration revealed an increasing trend till 3 months whereas A/G ratio showed almost constant variation in camel calves.

Roy and Fernandes (2007) observed that serum sodium had reduced significantly (hyponatraemia) in diarrhoeic cow and buffalo calves concomitant with perceptibly increased potassium concentration (hyperkalaemia), compared to the respective normal values. Hyperchloraemia persisted erratically in the untreated diarrhoeic calves.

Shobhamani *et al.* (2007) conducted haemato-biochemical studies on 30 crossbred calves suffering from cryptosporidiosis. Hb, PCV and TLC were significantly decreased with mild neutropenia, lymphocytopenia, monocytosis and eosinophilia in affected calves. Serum glucose, total protein, albumin globulin ratio, serum sodium and serum potassium values were significantly reduced in affected animals.

Manya *et al.* (2007) reported that coccidiosis in calves was characterized by sudden onset of diarrhoea watery to bloody with or with out mucous, straining /rectal tenesmus, dehydration, weight loss, depression, loss of appetite and occasionally nervous signs.

Asati et al. (2008) reported dullness and depression, reduced appetite, mild to profused watery diarrhoea having offensive odour in calf patients affected with diarrhoea due to colibacillosis. The faeces were semisolid to watery, greenish to yellow white in colour and some times even blood stained. There was an increase in degree of dehydration, sunken of eye ball socket, cervical skin tent duration and dryness of mucous membrane in calves. The affected calves showed higher mean value of Hb, PCV, TLC and lower mean value of TEC. There were no significant differences in eosinophil, monocyte and basophil percentage. Blood picture of affected calves showed lymphocytosis and neutropenia, whereas MCV and MCH increased and MCHC profile were decreased during infection. There was an increase in total serum protein and globulin, whereas mean blood glucose level, serum albumin and A/G ratio was decreased. Increase blood urea nitrogen, potassium and usually a decrease in serum sodium are the major consequence of diarrhoea.

Bandyopadhyay *et al.* (2008) revealed a slight reduction of Hb level and rise of PCV in diarrhoeic yalk calves. However leucocytosis was evident with marked neutrophilia.

Sinha *et al.* (2008) observed that in naturally acquired fascioliosis and amphistomiosis in buffaloes, mean haemoglobin (Hb) value (ranged between 7.52 to 7.66 g/dl), PCV (ranged between 32.16 to 32.22%) and TEC (ranged between 5.04 to 5.11×10^{6} /µl) was significantly low whereas TLC (ranged between 11.57 to 11.50×10^{3} /µl) and lymphocyte, eosinophil and monocyte counts were increased.

Kamal (2008) studied the serum parameters in 30 diarrhoeic calves of the Swiss Holstein breed and found that there was decrease in values of serum sodium, potassium, bicarbonate, glucose and total protein.

Bender and Bostedt (2009) determined the IgG and IgM levels in sera of newborn calves by ELISA and reported that throughout the colostrum administration period until the 12th living hour, IgG and IgM levels remarkably increased. The correlation between IgG concentrations in sera determined 24 hours post natum and the IgG content of the colostrum administered was highly significant while the correlation of serum IgM levels 24 hours post natum and the IgM content of the foremilk was significant. The sum of the IgG and IgM concentrations in calf serum 24 hours post natum was significantly correlated with the neonatal plasma protein level.

Fernandes *et al.* (2009a) observed subnormal temperature (99.5 to 100.8° F), increased respiration rate (24.7/min) and reduced pulse rate (30.5 to 35.7/min) in the diarrhoeic neonatal calves, while in control calves, the corresponding normal values were 102.1° F, 16.7/min, and 52.0/min.

Fernandes *et al.* (2009b) revealed increased Hb concentration, PCV %, TEC, MCH and TLC concurrent with decreased MCV in diarrhoeic bovine calves. Moderate leucocytosis was also observed. Roy *et al.* (2009) reported that mean Hb, PCV, total serum protein and serum potassium of colibacillosis affected diarrhoeic calves increased whereas serum glucose and serum sodium level decreased in comparison to healthy calves.

Tikoo and Soodan (2009) observed foul smelling, profuse watery, pale yellow coloured diarrhoea with tail and perineum soiled with faeces, loss of appetite, weakness, sunken eyes, dry mucous membrane, cold extremities, dehydration ranging from 8-12 % (skin tent), depression and loss of suckle reflex in diarrhoeic calves affected with colibacillosis. Temperature was normal (101^oF-102^oF) or sub-normal (98-99^oF) and heart rate increased (110bpm). The haemato-biochemical examination revealed increased value of Hb, PCV, TEC, protein, globulin and BUN from that of healthy control calves.

Ghoke *et al.* (2009) reported that clinical profile of coccidiosis in calf included mild fever, diarrhoea with foul smelling faeces containing blood and mucous, perineum and tail smudged with blood stained faeces, moderate to severe straining, pale mucous membrane, weakness, moderate dehydration and dyspnoea. The blood picture revealed decreased Hb level (6 gm/dl) and PCV (20 %).

Singh *et al.* (2009) recorded rough body coat, emaciation, anorexia, diarrhoea and mud coloured foul smelling faeces as main clinical signs of parasitic infestation in buffalo calves. Haemorrhagic diarrhoea due to coccidian oocyst was also recorded.

Nagalakshmi (2009) demonstrated the effect of serum immunoglobulin concentration on calf survival and reported that calves (age 0-56 days) having IgG > 10 mg/ml had higher percentage of survival than calves having IgG < 10 mg/ml.

Pal and Pachauri (2009) observed that the average serum immunoglobulin concentration in calves ranged 13.93 ± 0.50 to 15.20 ± 0.25 ZST units in different groups which were slightly below the normal

level and made the calves susceptible to be diarrhoea. There was significant loss of the body weight in calves after diarrhoea.

Singh *et al.* (2009c) observed rough body coat, emaciation, anorexia, diarrhoea, mud coloured foul smelling faeces in GI parasitic infection in buffalo calves. The cases with heavy presence of coccidian oocysts also showed symptoms of haemorrhagic diarrhoea.

Kertz (2010) reported serum IgG concentrations in dairy calves at 0, 24 and 48 hours after birth as 0.0, 20.2 and 19.1 g/ L, respectively after feeding unheated low bacteria colostrum. Heat-treated colostrum raises IgG absorption.

Kumar *et al.* (2010) observed dullness, depression with lethargy and anorexia. The faeces was semisolid to watery with offensive odour, yellowish white in colour and some times blood stained in cases of bacterial diarrhoea in calves. The haemato-biochemical changes include increase in PCV %, mean Hb value, TEC, TLC, mean potassium value, total protein and decrease in mean sodium value.

Asati *et al.* (2010) reported that diarrhoeic calves affected with colibacillosis exhibited the signs of sunken eyeball, dull and depressed with reduced appetite, mild to profuse watery diarrhoea having offensive odor and progressive dehydration. The haemoglobin concentration, mean PCV, TLC value, mean value of total serum protein and potassium level of diarrhoeic calves increased in comparison to healthy calves whereas blood glucose and sodium level decreased in diarrhoeic calves.

Arora *et al.* (2010) observed a wide variety of clinical manifestation in cases of calf diarrhoea. Faecal consistency varied from semisolid (9 %), loose (59.09 %) to watery faeces (31.81%). The changes in faecal consistency in diarrhoea were due to malabsorption of fluids, electrolytes and other nutrients by small intestine. The clinical examination revealed emaciation, weakness, loss of appetite, dryness of mucous membrane, sunken eyes, sternal recumbency , loss of skin

elasticity and cold extremities of varying degrees. These changes were attributed to changes in metabolism and loss of fluid from interstitial and intracellular spaces. Total deficit (TFD) was found to be 1105.28 \pm 131.3 ml and 1293.5 \pm 215.58 ml on the basis of clinical and laboratory methods, respectively.

Devkate *et al.* (2010) reported that bovine suffering from specific bacterial diarrhoea showed significantly increased body temperature, respiration and pulse rate. The consistency of faeces varied from loose (56.0 %) to semisolid - pasty (40.0 %). In adult animals, the faeces were usually blackish, bloody (56.0 %), whereas in calves, the faeces were yellowish white. The conjunctivae were normal to pale or congested depending upon severity of toxaemia and blood loss. There was moderate dehydration (52.0 %), anorexia, dullness, depression, weakness and suspended rumination.

Chand and Pandey (2010) reported that suckling method of colostrum feeding predisposed calves for development of diarrhoea and other calf hood diseases. The mean (\pm SE) concentration of serum IgG in calves fed colostrum with suckling or nipple bottle were found to be 14.61 \pm 2.62 mg/ml and 20.08 \pm 1.04 mg/ml, respectively. The serum IgG concentration of calves before any colostrum feeding was found undetected. Five out of 12 calves which were fed colostrum with natural suckling revealed failure of passive transfer of maternal immunoglobulins (IgG < 10 mg/ml).

Mir *et al.* (2010) compared the haemato-biochemical parameters of diarrhoeic and healthy calves and reported that PCV, TEC and TLC values in diarrhoeic calves were significantly higher than that of healthy calves whereas non significant increase in mean Hb values. There was significant decrease in values of MCV, MCH and MCHC in diarrhoea. Biochemical parameters such as serum potassium, chloride, total protein, globulins and albumin significantly increased in diarrhoeic calves whereas fall in serum sodium and AG ratio.

Pal and Pachauri (2011) induced diarrhoea in 24 neonatal calves by oral administration of enterotoxigenic *E. coli*. Mean values of plasma glucose concentration recorded prior to induction of diarrhoea in all calves showed significant reduction after onset of diarrhoea; whereas mean values of plasma urea concentration, plasma creatinine and total plasma protein concentration elevated significantly (p < 0.01) in all the calves except in case of total plasma protein in one group where increase was not significant. After administration of ORSs all calves showed significant increase in plasma glucose concentration and significant decrease in plasma urea, plasma creatinine and total plasma protein concentration.

Sahu Maiti (2011) and conducted а study on haematobiochemical profile in 24 bovine calves naturally infected with cryptosporidiosis and observed significantly ($p \le 0.05$) lowered mean total erythrocyte count (TEC), haemoglobin (Hb), lymphocyte percentage but significantly higher packed cell volume (PCV), Total leucocyte count (TLC), neutrophil and eosinophil percentage than the healthy calves. The biochemical study revealed significantly lower mean total protein, albumin, blood glucose, calcium, inorganic phosphorus, serum sodium, potassium and chloride and higher serum urea and aspartate transaminase (AST) in cryptosporidic calves.

Jozica *et al.* (2012) measured IgG, IgM and IgA concentrations in the serum samples of the calves with the quantitative ELISA and found lower concentrations of all the investigated Ig classes in healthy calves at the age of 4 weeks (IgG 20.55±12.96; IgM 0.88; IgA 0.10 g/L) in comparison with values established at the age of 1 week (IgG 26.23±15.30; IgM 1.13; IgA 0.54 g/L). Statistically significant positive correlations were established between Ig concentration in the colostrum and serum of calves.

Morrill and Tyler (2012) analyzed two hundred serum samples of one day old Holstein calves and found that 15 samples had IgG concentrations less than 3.43 mg/ml. The mean IgG concentration of the remaining 185 serum samples was 19.0 mg/ml (SD = 9.7) with a range of 3.5 to 47.0 mg/ml. A total of 150 (65 %) of the samples had IgG concentrations greater than 10 mg/ml indicating adequate passive transfer had occurred, while 50 samples (25 %) had IgG concentrations less than 10 mg/ml indicating failure of passive transfer.

2.3 Antibiotic sensitivity pattern of bacterial isolates

White *et al.* (1970) found chloramphenicol and furadantin to be most effective in inhibiting the growth of *E.coli* cultures. Most strains were found resistant to neomycin, oxytetracycline and sulfonamides.

Sanford (1976) reported that the use of any antimicrobial agent had a selective effect on the bacterial population favouring the survival of those organisms, which were least susceptible to it. Unnecessary use of such agents on large scale would increase the problem of drug fastness among society.

Lintermans *et al.* (1981) compared the antibiotic resistant pattern of *E. coli,* isolated from diarrhoeic calves before 1970 with strains isolated in 1979 and 1980. There was a marked evolution of multiple resistant patterns. One third of recent strains were sensitive to six antibiotics as compared to only 3 % in the 1970 isolates. Trimethoprim resistant strains always showed a multiple resistant pattern and all *E.coli* remained sensitive to polymixins and gentamicin.

Tripathi and Soni (1982) performed antibiotic sensitivity tests in cases of diarrhoea and reported that about 75 % of isolates were sensitive to nalidixic acid, ampicillin and chloramphenicol. Neomycin and polymixin B had intermediate inhibitory effect.

De-Lopez *et al.* (1982) observed that more than 70 % of bovine diarrhoeic *E. coli* samples were resistant to doxycycline, oxytetracycline, streptomycin, tetracycline and all of these strains were found sensitive to gentamicin, nalidixic acid and nitrofurazone.

Mehrotra *et al.* (1984) conducted antibiotic sensitivity tests on 60 *E. coli* isolates of cattle, 60 isolates of poultry, 68 isolates of goats and 28 human isolates against 9 antibiotics and furazolidine. Two hundred seventeen of 248 strains were resistant to tetracycline and oxytetracycline, 207 to erythromycin, 204 to doxycycline, 114 to streptomycin. All were sensitive to nalidixic acid.

Yadav (1985) concluded that majority of calf diarrhoeic *E.coli* strains showed resistant to tetracycline, oxytetracycline, doxycycline and chloramphenicol and most of the strain were sensitive to nalidixic acid and gentamicin.

Fetisova (1988) found that strains of *E. coli* from calves were relatively sensitive to gentamicin, nalidixic acid, furazolidine and colimycin and were resistant to benzyl penicillin, oxacillin, sulphaguanidine and tetracycline.

Vihan and Singh (1988) evaluated the antibiotic sensitivity of *E. coli* isolated from heart, blood and faecal samples of kids and found that the most effective drugs were gentamicin, furadantine, nalidixic acid and cephalexin.

Panwar *et al.* (1990) documented the percent susceptibility of 224 *E. coli* isolated from rectal swabs of diarrhoeic calves in decline order as nalidixic acid (91.0 %), nitrofurantoin (83.0 %), cephaloridine (61.0 %) and gentamicin (56.0 %).

Shah and Jhala (1990) conducted in vitro antibiotic sensitivity of 71 *E. coli* isolates obtained from 116 neonatal calves having diarrhoea. Multiple drug resistant was exhibited by *E. coli*. Gentamicin was found to be the most effective antibiotic having all the isolates sensitive to it.

Alban *et al.* (1991) reported that 100 % of enterotoxigenic *E. coli* isolates from newborn calves showed resistant to at least one drug. More than 60 % isolates showed resistant to sulfonamides, dihydrostreptomycin, tetracycline and ampicillin. Kaura *et al.* (1991) reported that 77.78 % *E.coli* strains isolated from faeces of diarrhoeic calves showed multiple drug resistant patterns. The maximum percent resistant was against tetracycline (83.3 %), followed by streptomycin (52.78 %), soframycin (44.44 %), furazolidine (41.67 %), trimethoprim and augmentin (36.11 %).

Singh *et al.* (1992) studied 154 isolates of *E. coli* (17 from poultry septicaemia, 75 from bovine diarrhoea, 14 from ovine diarrhoea and 48 from equine metritis) for their susceptibility to various antibiotics. Most of the resistant isolates were recovered from diarrhoea. Total 25.5 % animal isolates were resistant to one or more drugs.

Blanco *et al.* (1993) observed that 80.0 % percent of the 713 *E. coli* strains, isolated from 214 calves with diarrhoea and 112 healthy control calves were resistant to at least one of the 18 antimicrobial agents tested. Strains were resistant to sulfadiazine (73.0 %), tetracycline (67.0 %), streptomycin (61.0 %), ampicillin (37.05 %), chloramphenicol (36.0 %), kanamycin (31.0 %), neomycin (29.0 %), amoxicillin + clavulanic acid (7.0 %), gentamicin (3.0 %), cefoxitin (2.0 %), tobramycin (2.0 %), nalidixic acid (1.0 %), amikacin (0.4 %) and polymyxin B (0.31 %). However, all the strains were sensitive to cefotaxime. *E. coli* strains isolated from calves with diarrhoea were more (85 %) resistant to antibiotics than those isolated from healthy calves (73 %).

Krishnamohan *et al.* (1995) conducted the in vitro sensitivity test of 50 *E.coli* isolates to the quinolone antibiotics and found that all the isolates were sensitive to nalidixic acid, 48 to pefloxacin and 17 to flumequine.

Galiero *et al.* (1997) reported that out of 110 *E.coli* strains isolated from buffalo calves 97.0 % of the strains were sensitive to enrofloxacin and flumequine, 89.0 % to pipemidic acid, 81.0 % to

gentamicin, 67.5 % to nalidixic acid, 62.0 % to nitrofurantoin, 46.0 % to trimethoprim and suphamethoxazole and 46.0 % to apramycin.

Bradford *et al.* (1999) studied the antibiotic resistance among *Escherichia coli* isolates from bovine calf diarrhoeal disease and found that many of the isolates were multiply resistant to β -lactams, including expanded-spectrum cephalosporins, aminoglycosides, sulphonamides, tetracycline and fluoroquinolones. In many of the isolates, IEF revealed a strong β -lactamase band compatible with overexpression of the AmpC β -lactamase, either alone or in addition to TEM-type enzymes. Several of the isolates also possessed genes encoding virulence factors associated with animal and human diarrhoeal diseases. These results suggest that the use of antibiotics in animals could lead to a reservoir of antibiotic-resistant bacteria that could potentially infect humans.

Kumar (1999) observed the susceptibility pattern of *E. coli* isolates, in decreasing order as ofloxacin, ciprofloxacin and gentamicin (100 % each), enrofloxacin (95.83 %), amoxycillin (41.67 %), nalidixic acid (37.5 %), cloxacillin (33.33 %), chloramphenicol and ampicillin (25.0 % each), streptomycin (20.83 %) and tetracycline (16.67 %).

Hussain and Saika (2000a) studied antibiogram of *E.coli* strains isolated from diarrhoeic calves and found that sensitivity was highest to gentamicin, nalidixic acid and norfloxacin (100 %) followed by neomycin (98.02 %), chloramphenicol (95.04 %), streptomycin (91.08 %) and furazolidine (80.17 %). Most frequently used tetracycline and co-trimoxazole were found to be moderately effective against *E. coli*.

Chattopadhyay *et al.* (2003) showed that 68.2 %, 61.5 %, 46 % and 30.76 % diarrhoeic *E.coli* isolates 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) evaluated in vitro resistant to 8 antimicrobials among ETEC from calves and piglets over a period of 13 years. The percentage of resistant of bovine isolates in ascending order in the first eight years were ceftiofer (4 %), gentamicin (6 %), spectinomycin (44 %), trimethoprim-sulphonamide (46 %), neomycin (64 %) and oxytetracycline (81 %). For the last five years period least resistant was seen against ceftiofer, followed by florfenicol, trimethoprim-sulphonamide and tetracycline, the resistant rates being 8 %, 11 %, 48 % and 75 %, respectively.

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 ofloxacin (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 %).

Sylvester *et al.* (2006) found that *E. coli* isolates from poultry were fairly resistant to antibiotics like ampicillin, colistin, tetracycline and norfloxacin. Gentamicin and amoxyclav had above 80 % sensitivity followed by ciprofloxacin (66 %) and cefotaxim (62 %).

Sharma and Soni (2008) found that *S. typhimurium* isolates of diarrhoeic faeces of calves were highly sensitive to ciprofloxacin, ofloxacin, enrofloxacin, moderately sensitive to gentamicin and resistant to oxytetracycline, tetracycline and sulphadimidine, while *S. waltevreden* slightly varied in susceptibility and found highly sensitive to ciprofloxacin, enrofloxacin, ofloxacin, moderately sensitive to gentamicin, ampicillin and resistant to oxytetracycline, sulphadimidine and tetracycline.

Ghosh *et al.* (2008) reported that *E. coli* isolates of diarrhoea in dogs were sensitive to ceftriaxone (80.95 %), ciprofloxacin (80.95 %) followed by gentamicin (76.19 %) and cefixime (66.66 %) and intermediately sensitive to amikacin.

Bandyopadhyay *et al.* (2008) revealed highest sensitivity of *E. coli* isolates from diarrhoeic yalk calves to norfloxacin, cefotaxim, gentamicin followed by ciprofloxacin, tetracycline and chloramphenicol.

Tikoo and Soodan (2009) found that *E. coli* isolated from diarrhoeic calves were sensitive to very few antibiotics. Highest resistance in *E. coli* was observed against sulphadiazine and sulphamethoxazole followed by streptomycin, kanamycin, gentamicin, ampicillin/cloxacillin. Enrofloxacin and ciprofloxacin was found to be least resistant.

Sharma *et al.* (2009) determined the antimicrobial resistance of *Escherichia coli* and *Salmonella* spp. from diarrhoeal faeces in calves (0-1 month). From 107 faecal samples collected, 180 *E.coli* and 7 *Salmonella* spp. isolates were obtained. Out of 180 *E.coli* isolates, 80 were serogroup for O antigen. O15, 0123 and O128 (12.7 % each) were the predominantly occurring *E. coli* serogroups. Most of the *E. coli* was resistance to sulphamethoxazole (66.6 %) and oxytetracycline (61.7 %) followed by streptomycin (25.9 %), gentamicin (16.0 %), ampicillin (12.3 %), nitrofurazone (9.9 %), enrofloxacin (6.1 %), ciprofloxacin (2.5 %), norfloxacin (2.5 %) and chloramphenicol (3.7 %). *Salmonella* spp. isolates showed resistance to ampicillin (85.5 %), norfloxacin (14.3 %) and chloramphenicol (14.3 %). However all the *Salmonella* spp. isolates were 100 % sensitive to Ciprofloxacin, enrofloxacin, nitrofurazone and sulphamethoxazole.

Rajkhowa *et al.* (2009) conducted antibacterial susceptibility testing of *E. coli* isolates recovered from faecal samples of neonatal mithun calves with diarrhoea and found that the isolates were 100 % sensitive to amikacin and gentamicin followed by streptomycin, kanamycin, enrofloxacin, ciprofloxacin, norfloxacin, amoxyclav, chloramphenicol and ceftriaxone.

Dubal *et al.* (2009) isolated *E. coli* from the faeces of the diarrhoeic calves and conducted antibiotic sensitivity test. Highest sensitivity was recorded against sparfloxacin and ciprofloxacin (100 %) followed by nitrofurantoin (92.00 %), chloramphenicol (88.00 %) and tetracycline (80.00 %).

Devkate *et al.* (2010) revealed that *E. coli* and mixed bacterial isolates of bovine diarrhoea showed maximum sensitivity for ciprofloxacin (84.0 %) followed by chloramphenicol (80.0 %), enrofloxacin (72.0 %), ceftriaxone (64.0 %), gentamicin (64.0 %), cloxacillin (40.0 %), amoxicillin (32.0 %), oxytetracycline (20.0 %), sulphadiazine (12.0 %), furazolidine(8.0 %), metronidazole (4.0 %) and trimethoprim (4.0 %).

Kumar *et al.* (2010) reported that isolates of *E. coli* from diarrhoeic calves were sensitive to ciprofloxacin (96.15 %), norfloxacin (92.31 %), pefloxacin (92.31 %), cotriamoxazole (84.61 %), sparfloacin (80.76 %), ofloxacin (80.76 %), amoxicillin (76.92 %) and chloramphenicol (15.38 %). These isolate were resistant to gentamicin, cephalexin and furazolidine. The isolates of *Proteus* spp. and *Klebsiella* spp. were sensitive to ciprofloxacin, norfloxacin and pefloxacin.

Mondal *et al.* (2010) conducted antibiogram of the *E. coli* isolated from rabbit and revealed that the isolates were highly sensitive to pefloxacin, ofloxacin, ciprofloxacin, nalidixic acid and chlortetracycline (88-96 % sensitivity) while organisms showed medium sensitivity with ampicillin and gentamicin (57 % sensitivity) The isolated *E. coli* showed resistant to penicillin and lincomycin.

Pan and Bhatia (2010) isolated *E. coli* from diarrhoeic and non diarrhoeic faecal samples from neonatal calves maintained in different farms belonged to three different agro-climatic zones of India and performed the antimicrobial susceptibility test against ten commonly used antibiotics. Oxytetracycline, tetracycline and ampicillin were found to be most resistant and gentamicin and ciprofloxacin were found most

susceptible drugs. Chloramphenicol, amoxicillin, norfloxacin, amikacin and cephalexin were moderately susceptible drugs.

Panda *et al.* (2010) conducted antibiotic sensitivity study of the *E. coli* isolates from ducks and reported that *E. coli* showed high sensitivity to amikacin, amoxicillin, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, kanamycin, levofloxacin and neomycin. They were resistant to ampicillin, cephodoxime, cloxacillin, erythromycin, nalidixic acid, oxytetracycline, tetracycline, sparfloxacin and furazolidine.

Gupta *et al.* (2011) recovered 32 isolates of *E. coli* from diarrhoeic samples of cow calves. The in vitro sensitivity of the isolates to antimicrobial drugs was found 100 % to ciprofloxacin and sulphadiazine, followed by ceftriaxone and ceftriaxone/tazobactam (96.88 %), cefotaxime (71.90 %), amoxyclav (68.75 %) amikacin (46.9%).

2.4 Evaluation of therapeutic regimens

Talos and Roth (1972) suggested the parenteral administration of fluid containing sodium, glucose and corticosteroids to correct the enteritis in calves. Hypoglycemia, hyponatraemia, hyperkalaemia and haemoconcentration were corrected by the above treatment.

Glantz *et al.* (1972) found nifuraldezone reasonably effective for prevention and cure of diarrhoea in calves.

Braun (1975) reported that antimicrobial therapy alone often failed to prevent high death losses due to diarrhoea in calves and this mortality could be reduced by feeding an electrolyte source in the form of a special dietary food.

Kirtikar and Basu (1975) reported that *Dalbergia sissoo* (Shisham) was used for dysentry, boils, eruptions, leprosy and nausea.

Dallenga (1975) reported that diarrhoeic calves with cold muzzle and extremities, subnormal body temperature, sunken eyes, disturbed locomotion and watery faeces indicated the need of electrolyte treatment. An electrolyte solution containing 30 gm sodium bicarbonate and 9 gm sodium chloride in 3 litres of distilled water @ 150 drops/min followed by another electrolyte solution containing 14 gm sodium bicarbonate, 12 gm sodium chloride, 8 gm potassium chloride and 100 gm glucose in 3 litres of distilled water @ 60 drops per minute was suggested.

Bywater (1977) treated experimental induced diarrhoeic calves with glucose-glycine electrolyte solution (GGES) and 400 mg amoxicillin for 4 days and concluded that these were an effective therapeutic agent for diarrhoeic calves. Mean PCV and venous blood pH in treated animals returned to normal more rapidly than in untreated control animals.

Bakheit and Greene (1981) reported that electrolyte therapy alone was at least as effective as the combination of electrolytes and triple sulfonamides with streptomycin for treating calves with diarrhoea.

Shillinger (1982) recorded a significant increase in blood glucose level within one and half hour after oral administration of an electrolyte solution containing sodium bicarbonate, sodium chloride, potassium chloride, calcium phosphate, magnesium sulphate, glucose and protein hydrolysate @ 2 litres per 3 hours in diarrhoeic calves up to 10 days. A significant decrease in haematocrit and urea level was also recorded.

Jones *et al.* (1984) found that hyper-osmotic oral electrolyte solution provided greater nutritional support than iso-osmotic solutions. The glucose to sodium ratio in these oral electrolyte solutions was kept less than 3:1.

Rademacher (1985) observed that diarrhoeic calves treated with Baytril (Enrofloxacin) showed quick recovery (4.9 days) than those treated with other conventional antibacterial drugs (6.2 days). A recovery rate of 94 % was observed. Sinha *et al.* (1985) reported no mortality in the group treated with oral rehydration therapy containing sodium, calcium, magnesium, potassium, glycine and glucose. The mortality in the untreated group was observed as 30 %.

Naylor and Forsyth (1986) reported that acetate and propionate could be used for treatment of calves suffering with dehydration and metabolic acidosis which acted as alkalizing agents and energy producers. The calves were unable to metabolize fully the D-isomer of lactate.

Popovici *et al.* (1986) recorded that intravenous administration of a solution containing sodium bicarbonate and sodium chloride alongwith oral administration of 5.7 gm of a mixture containing sodium chloride, potassium chloride, sodium bicarbonate and potassium hydrogen phosphate in one litre of hay infusion and 5 % glucose was found better than intravenous infusion of sodium bicarbonate @ 13 g/L or isotonic sodium bicarbonate with sodium chloride @ 8.5 g/L in resuscitation of diarrhoeic neonatal calves.

Fujuhara *et al.* (1987) observed that oral administration of glucose-glycine electrolyte solution (GGES) gave better result than administration of saline alone in terms of correcting PCV, sodium and chloride levels in induced calf diarrhoea.

Carpenter (1987) stated that success of any oral rehydration solution mainly on its formulation rather than the type of the diarrhoea.

Booth and Naylor (1987) observed that electrolyte solution containing bicarbonate restored acid base balance and corrected depression better than electrolyte solution devoid of bicarbonate in diarrhoeic calves. There was no difference in both the electrolyte solutions in correcting dehydration.

Naylor (1990) reported that diarrhoeic calves can routinely be saved if treatment includes sufficient fluid and electrolyte to counter balance the diarrheic losses. Optimum oral electrolyte solution should provide sodium (60 to 120 mM/L), potassium (10 to 20 mM/L), chloride (40 to 80 mM/L), metabolizable (non bicarbonate) base such as acetate or propionate (40 to 80 mM/L) and glucose as energy source.

Avery and Snyder (1990) observed that combined administration of sodium and glucose was beneficial because glucose facilitated sodium absorption via small intestine sodium glucose co-transport mechanism.

Roussel and Kasari (1990) reported that the major goal of treating neonatal diarrhoea was correction of dehydration that often involved administration of an OES. Further, high potassium concentration was needed in OES to correct whole body potassium depletion. The acetate and propionate in OES were metabolized by peripheral tissues. They were not produced endogenously in shock and dehydration (as is lactate) and did not have an unmetabolized isomer D- lactate.

Michell *et al.* (1992) observed that oral rehydration solution containing high sodium and glucose was most effective in induced calf diarrhoea.

Signorini *et al.* (1992) found colistin sulfate effective in the treatment of diarrhoea due to colibacillosis when administered orally @ 120 ppm in reconstituted milk for 7 days.

Das *et al.* (1992) reported that leaves of shisham has high content of tannin due to which it has astringent property and given in non-specific diarrhoea.

Frankel *et al.* (1993) reported that glutamine had beneficial effects on fluid uptake and was the primary fuel of small intestine.

Vander Hulst *et al.* (1993) reported that glutamine had the potential to promote enteric sodium uptake as well as sustain villous form and function.

Bugant and Bentezac (1993) reported that glutamine despite its high cost not only had potential to promote enteric sodium uptake but had unique importance in sustaining villous form and function.

Bhan *et al.* (1994) reviewed all published trials of amino acid containing ORS in humans and found no benefit from the inclusion of alanine, glycin or glutamine.

Vyhnalek and Hera (1994) observed that neblon powder had good efficacy in treating and preventing diarrhoea among calves, kids and piglets. Neblon was an herbal product formulated from 11 herbs of which bael were one of the constituents.

Sadiek and Schlerka (1995) successfully treated the calves having severe diarrhoea, dehydration and acidosis by administering a continuous drip infusion of 8.4% sodium bicarbonate, tris buffer, 30 % glucose and 4.5 % sodium chloride as an isotonic solution in 5 litres of distilled water in to ear vein.

Verma *et al.* (1995) concluded that a combined therapy with cotrimoxazole @ 200 mg/kg body weight alongwith rehydration therapy using isotonic fluid containing 0.85 % sodium chloride and 1.3 % sodium bicarbonate was effective in the management of calves suffering with colibacillosis.

Seifi *et al.* (1996) conducted a trial to evaluate the effectiveness of ascorbic acid in the prevention of neonatal calf diarrhoea and found that the number of diarrhoeic calves in treatment group (that received ascorbic acid) was significantly (P < 0.005) lower than the number of diarrhoeic calves in the control group that did not receive the drug.

Constable *et al.* (1996) administered hypertonic saline dextran intravenously and oral electrolyte solution in treating hypovolaemic diarrhoeic calves and found it highly efficacious than giving either of the solutions alone. Brooks *et al.* (1996a) found that oral rehydration solution having three times glucose content showed greater ability in correcting changes associated with diarrhoea as compare to the conventional glucose concentration.

Brooks *et al.* (1996b) observed that an oral rehydration solution containing glutamine was better in correcting metabolic acidosis in diarrhoeic calves. This was facilitated by the ability of glutamine to promote Na⁺-H⁺ exchange in enterocytes.

Turvill and Farthing (1996) also reported glutamine containing oral rehydration solution better for diarrhoeic calves.

Mitch and Walser (1996) reported that beneficial effects of glutamine were not only restricted to the gut but it was the primary source of ammonia buffer which was essential for acid excretion. This could contribute to the beneficial effects on acidosis as would any alleviation of prerenal failure.

Bouda *et al.* (1997) found an oral rehydration solution containing 42 gm of sodium chloride, 40 gm of sodium bicarbonate, 18 gm of potassium chloride and 200 gm of glucose dissolved in 10 litres of water effective in rehydrating diarrhoeic calves with mild dehydration.

Brooks *et al.* (1997) evaluated a glutamine containing oral rehydration solution for the treatment of calf diarrhoea using an *E. coli* model and found it more effective in correcting the plasma volume, extracellular fluid, blood volume and PCV than WHO type ORS (glutamine free) in treatment of calf diarrhoea.

Dubey and Rao (1997) observed that pefloxacin @10 mg/kg body weight orally bid alongwith 5 % DNS @ 10 ml/kg body weight i.v. and Neblon orally was more effective in treatment of diarrhoea in buffalo calves than either furazolidine (5 mg/kg body weight) or trimethoprim potentiated sulphadiazine (30 mg/kg body weight) alongwith same supportive therapy.

Geishauser and Thunker (1997) concluded that administration of 1.3 % sodium bicarbonate solution is useful to help diarrhoeic neonatal calves regain the suckling reflex and the ability to stand. The diarrhoeic calves should have milk feeding and free access to fresh water.

Nappert *et al.* (1997) reported that treatment of diarrhoea in calves was primarily based on correction of dehydration and acidosis with the use of oral and intravenous electrolyte solutions. They observed that solutions containing 50 to 80 mmol/L of alkalizing agents produced highest recovery rate.

Walker *et al.* (1998) reported that combination of hypertonic saline dextran i.v. and isotonic electrolyte solution orally provided a rapid and effective method for resuscitation of severely dehydrated calves.

Brooks *et al.* (1998) observed that treatment of calf diarrhoea with conventional oral rehydration solution could be detrimental to the intestinal villous structure because of nutrient deprivation and such effects could be avoided by a nutrient ORS particularly if it contained glutamine.

Sarg *et al.* (1999) conducted phytochemical and pharmacological studies of *Dalbergia sissoo* in Egypt and observed that the alcoholic extract of green branches of aerial parts showed a dose dependant inhibitory effect on the motility of isolated rabbit duodenum, pronounced bronchodilation, significant anti-inflammatory, antipyretic, analgesic and estrogen like activity.

Alone *et al.* (2000) found better therapeutic response of an isotonic ORS for the correction of electrolyte imbalances viz. hyponatraemia, hyperkalaemia and metabolic acidosis in diarrhoeic calves, than ringer's lactate solution i.v. The isotonic ORS contained 113.6 gm sodium chloride, 50.3 gm potassium chloride, 108.9 gm sodium bicarbonate, 535.1 gm glucose and 223 gm glycine; 32.8 gm of this mixture was dissolved in 1 litre of water and used as a drench.

Bali *et al.* (2000) observed that oral pefloxacin was effective in enteric colibacillosis of calves and the results were better when the treatment was supplemented with activated charcoal and ORS.

Kumar *et al.* (2000) reported that rural people in India and Nepal used shisham leaves to treat animals suffering from non specific diarrhoea. They observed that the effectiveness of shisham leaves could be due to its non specific spasmolytic activity.

Nadkarni (2000) observed that the fruit pulp of bael contained reducing sugars, tannins and marmelosin as the active ingredients. The unripe fruit of bael exhibited astringent, digestive, stomachic and little constipative effect.

Bhalerao *et al.* (2000) found therapeutic efficacy of pefloxacin 5 mg/kg body weight orally bid better than neomycin @ 2.5 mg/kg i.m. bid or lincomycin + streptomycin @ 15 mg/kg body weight i.m. bid in treating diarhoeic calves aged between 1 to 30 days of either sex. All calves were given 5 % DNS as supportive therapy depending on its degree of dehydration.

Rajora and Pachauri (2000) assessed the efficacies of different electrolyte solutions in mild, moderate and severe dehydration caused by diarrhoea in calves and reported that mild cases treated with ORS alone (comprising of 20 gm glucose, 3.5 gm sodium chloride, 2.5 gm sodium bicarbonate and 1.5 gm potassium chloride dissolved in 1 litre of water) and moderate cases treated with 5 % sodium bicarbonate solution i.v. followed by ORS exhibited full clinical recovery. The severe cases treated with 5 % sodium bicarbonate solution i.v. followed by ORS exhibited full clinical recovery. The severe cases treated with 5 % dextrose followed by ORS gave disappointing results.

Constable *et al.* (2001) induced diarrhoea in Holstein Friesian calves using sucrose solution, furosemide, spironolactone and hydrochlorthiazide. After 24 hours the calves show severe diarrhoea, moderate dehydration (8-10% b.wt.), azotemia and depression. Calves

were feed milk replacer (2L/12 hr), hyperosmotic oral electrolyte solution (2L/12 hr) and isoosmotic oral electrolyte solution (1.5 L/6-12 hr). Calves treated with hyperosmotic oral electrolyte solution responded better and significant improvement was observed. It also provided greater nutritional support and also prevented the development of metabolic acidosis.

Kalita *et al.* (2001) found better therapeutic efficacy with 5 % dextrose saline solution i.v. along with Pesulin bolus orally and Belamyl i.m. than Lacrivet Inj. Cotrimol bolus and Conciplex induced diarrhoea in kids.

Karademir and Sendil (2001) observed 90 % recovery rate based on clinical and haemato-biochemical profile in *E. coli* infected diarrhoeic calves treated with gentamicin, sulfadiazine and trimethoprim given orally as well as enrofloxacin i.m. The group that did not received any antibiotic medication showed 5 % recovery rate. Calves in all the groups were given supplementary treatment like fluid medication, oral milk and vitamins.

Shrivastava *et al.* (2001) evaluated the efficacies of cefuroxime, neomycin sulfate and sulfadiazine+trimethoprim alongwith a common supportive therapy consisting of 20 % rintose and bismuth carbonate administered 3 days for the treatment of diarrhoea in calves and observed that cefuroxime gave good efficacy followed by neomycin sulfate and sulfadiazine+trimethoprim.

Hazare *et al.* (2001) worked on the anti-inflammatory activity of *Dalbergia sissoo* leaves in rats and found it safe up to 10.125 gm/kg per os. The extract of leaves possessed significant anti-inflammatory activity without any side effects on the gastric mucosa.

Iwata *et al.* (2002) reported that a single shot therapy of baytril (enrofloxacin) @ 7.5 mg/kg i.m. recorded a recovery rate of 98.1 % in calf diarrhoea by 4^{th} day of treatment.

Kumar and Mandial (2002) found that ampicillin-cloxacillin @ 10 mg/kg body weight i.m. daily and ringer's lactate @ 20-40 mg/kg body weight i.v. as and when required administered till recovery checked diarrhoea in 100 % of animals.

Kumar *et al.* (2002) found complete clinical recovery in the crossbred calves suffering from colibacillosis by 5th day post therapy that received enrofloxacin and ringer's lactate.

Rademacher *et al.* (2002) reported that antibiotic therapy was not indicated in uncomplicated cases of neonatal calf diarrhoea. Therapy for diarrhoea could aim at supplementing WHO-ORS and milk at 12% of body weight per day if the calves were able to drink. In severely dehydrated calves i.v. infusions were to be given based on clinical findings.

Shaheen *et al.* (2002) treated diarrhoeic calves with three different drug regimens viz. norfloxacin 250 mg, metronidazole 200 mg/5ml, ampicillin-cloxacillin (250:250) i.v. bid for 5 days, cephalexin @ 25 mg bid orally for 5 days and ciprofloxacin (200 mg/100ml) i.v. daily for 4 days. Cephalexin orally gave better result as there was no relapse of infection and no mortality.

Kaur *et al.* (2002) conducted a therapeutic trial and observed pefloxacin as highly effective followed by amoxycillin, metronidazole and furazolidine combination and haloquinol against colibacillosis in neonatal calves

Kaur *et al.* (2003) revealed that hypertonic oral rehydration solution in all cases of diarrhoea and the hypertonic sodium bicarbonate (7.5 %) solution parenterally in severe cases were useful in treating diarrhoeic calves suffering from colibacillosis.

Constable (2004) reported that Amoxicillin, chlortetracycline, neomycin, oxytetracycline, streptomycin, sulfachloropyridazine, sulfamethazine, and tetracycline administered PO were labeled in the

United States for the treatment of calf diarrhoea. On the basis of published evidence for the oral administration of these antimicrobial agents, only amoxicillin can be recommended for the treatment of diarrhoea. Dosage recommendations are amoxicillin trihydrate (10 mg/kg PO q12h) or amoxicillin trihydrate–clavulanate potassium (12.5 mg combined drug/kg PO q12h) for at least 3 days; the latter constitutes extra-label drug use. Parenteral administration of broad-spectrum b-lactam antimicrobials- ceftiofur (2.2 mg/kg IM or SC q12h) and amoxicillin or ampicillin (10 mg/kg IM q12h)- or potentiated sulfonamides (25 mg/kg IV or IM q24h) is recommended for treating calves with diarrhoea and systemic illness; both constitute extra-label drug use.

Swarup *et al.* (2004) conducted an experiment on efficacy of paste of shisham leaves (*Dalbergia sisoo*) in control of diarrhoea in cattle and buffalo and found that treatment of diarrhoea using paste of shisham leaves was very good with 70 % cure.

Halmandge *et al.* (2005) reported the efficacy of piperazine, doramectin and closantel as 97.4, 99.71 and 0.62 %, respectively against ascariasis in buffalo calves. It indicated that closantel was ineffective in eliminating *T. vitulorum* worms till 7th day after treatment.

Lorenz (2006) observed in seventy-three calves up to three weeks old with acute diarrhoea and base excess values below -10 mmol/l ,correction of acidosis was carried out within 3.5 h by i.v administration of an amount of sodium bicarbonate which was calculated using the formula: HCO3- (mmol) = body mass (kg) * base deficit (mmol/l) * 0.6 (l/kg). He recommended to supply calves (healthy as well as diarrhoeic) with an amount of milk that equals about 12% of their body weight. In case of diarrhoea oral rehydration solutions must be provided additional.

Das *et al.* (2006) gave different line of treatments to diarrhoeic calves affected with cryptosporidiosis and found that line of treatment

comprising of antiprotozoal (furazolidine @ 500 mg orally bid), antibacterial (sulphadimidine @ 5 gm orally followed by 2.5 gm daily) and parenteral fluid (5 % dextrose saline @ 80 ml/ kg b wt) along with alkalizing agent (5 % sodium bicarbonate @ 2 ml/kg b wt) showed better recovery (71.4 %) than only oral fluid therapy.

Spence (2006) reported that the amount of electrolyte solution needed by the calf each day to correct dehydration is calculated by multiplying the weight of the calf by the percentage dehydration. He also correlated clinical signs with the percentage of dehydration viz. Diarrhoea, but no other sign-5 % dehydration; Eyes slightly sunken, skin losing elasticity, but calf still suckling-7 % dehydration; Eyes sunken, skin slow to flatten if pinched, gums sticky, calf depressed- 9 % dehydration; Eyes very sunken, skin 'tents' (won't flatten if pinched), calf can't stand and is severely depressed- 12 % dehydration.

Brijesh *et al.* (2006) conducted a study on *Dalbergia sissoo* leaves for its possible mechanism(s) of action in infectious diarrhoea. Antibacterial, antiprotozoal and antiviral activities of the plant decoction were checked by agar dilution method, tube dilution method and neutral red uptake assay, respectively. This study showed that *D. sissoo* is antidiarrhoeal as it affects bacterial virulence.

Nag *et al.* (2007) evaluated the therapeutic efficacy of polyherbal formulation containing *Casia absus* (4 parts), *Cuminum cyminum* (2 parts), *Kalanchoe pinnata* (5 parts) and *Helicteres isora* (4 parts) in clinical cases of calf diarrhoea. The formulation had 83.33 and 50.0 % efficacy when given @ 15.0 and 7.5 gm, respectively alongwith isotonic normal saline solution and oral rehydration solution according to dehydration stage and response. There was significant improvement in the clinico-haematobiochemical profile in treated calves.

Shobhamani *et al.* (2007) conducted a treatment trial on 30 diarrhoeic calves suffering from cryptosporidiosis using azithromycin @ 20-30 mg/kg b wt orally 2 hrs before feeding for 5-7 days and tylosin @

10-20 mg / kg b wt orally for 5-7 days alongwith ringer's lactate @ 20-30 ml/kg b wt iv in 2-3 divided doses. There was significant improvement in the haemato-biochemical profile on 14th, 21th and 42th day post therapy in both the groups.

Roy and Fernandes (2007) observed that remedial therapy with sulphadiazine-trimethoprim, ciprofloxacin-tinidazole or amoycillin trihydrate along with supportive rehydration therapy including sterile dextrose normal saline / plane normal saline or ringers lactate in cow and buffalo calves suffering with entero-colibacillosis reverted the reduced concentration of serum sodium, increased potassium and chlorine concentration to normalcy at different period of time after treatment with different drugs.

Kumar *et al.* (2008) found that gum acacia suspension of methanolic extracts of pojo (*Litsaea anthapoly*) bark were comparatively more effective against diarrhoea in goats followed by that of urhul (*Hibiscus rosa sinensis*) flower and takala (*Cassia tora*) leaves.

Tripathi *et al.* (2008) reported significant increase in total leukocyte, percent lymphocyte and absolute lymphocyte in diarrhoeic calves treated with *Ocimum sanctum* @ 300 mg and *Emblica officinalis* @ 150 and 300 mg /kg b wt. Serum globulin and total serum immunoglobulins were found significantly elevated in calves fed Ocimum sanctum and *Emblica officinalis* @ 300 mg/ kg b wt. However, *Emblica officinalis* showed better results than *Ocimum sanctum* at the same dose regimen.

Pal and Pachauri (2008) concluded that Ca-Mg nutrient ORS with glutamine was more effective in treatment of neonatal calf diarrhoea induced with *E. coli* infection as compared to other three ORSs viz. conventional ORS, high caloric glutamine free ORS and Ca-Mg nutrient ORS without glutamine.

Sinha *et al.* (2008) conducted the treatment trial on naturally acquired fasciliosis and amphistomiosis in buffaloes and observed that on 12th day post treatment, improvement in haematological parameter was seen and maximum recovery was noted in oxyclozanide+albendazole @ 15 mg/kg b.wt. treated group followed by in oxyclozanide+tetramizole @ 20 mg/kg b.wt. and oxyclozanide @ 10 mg/kg b.wt. alone treated animals.

Fernandes *et al.* (2009a) reported that the clinical profile of diarrhoeic bovine calves revealed subnormal body temperature and increased respiration and reduced pulse rates. Following treatment, these parameters reached near normalcy. The symptoms abated earliest in calves treated with sulphadiazine-trimethoprim, followed by amoxicillin trihydrate group. In calves treated with ciprofloxacin-tinidazole, recurrence of diarrhoea was observed in 2 calves. Thus, sulphadiazine- trimethoprim alongwith rational supportive fluid and electrolyte replacement therapy was adjudged the drug of choice in the present study.

Roy *et al.* (2009) found that colibacillosis affected calves treated with antibiotics (ceftriaxone and tazobactam @ 5-10 mg/kg b wt daily im for 3 days) and oral rehydration solution containing dextrose, glycine, sodium chloride, calcium lactate, magnesium sulphate and potassium dihydrogen phosphate @ 2-4 litres thrice daily for 3-5 days gave better response than treated only with antibiotics. The clinical and haemato-biochemical profile recovered within 7 days.

Tikoo and Soodan (2009) reported that *E.coli* infected diarrhoeic calves responded well to treatment with in 3-4 days with ciprofloxacin plus tinidazole @ 10 mg/kg b wt orally daily for 3-4 days, pulv. Neblon (15 gm po for 2 days), fluid therapy (ringer's lactate and normal saline solution in equal quantities as per the extent of dehydration) and antihistaminics supportive therapy.

Ghoke *et al.* (2009) found that although coccidiosis is a self limiting disease however treatment with parenteral fluid therapy of ringer's lactate solution @ 25 ml/kg b wt, antimicrobial sulphonamide @ 100 mg/kg b wt oral as well as parenteral along with multi-vitamin and iron supplementation for 3 consecutive days responded well with complete recovery in 3 days.

Pal and Pachauri (2009) evaluated the efficacy of four different ORSs in respect of gaining their body weight and reported significant improvement in respect of gaining the body weight in diarrhoeic calves on day 3 and 5 post ORS therapy but the Ca-Mg nutrient ORS with glutamine was found to be superior to other three ORSs during this treatment.

Devkate *et al.* (2010) indicated that ciprofloxacin along with GutLyt, a polyherbal astringent proved superior to ciprofloxacin alone in cases of specific bacterial bovine diarrhoea. After treatment the clinico-haemato-biochemical parameters returned to almost normal.

Tuteja and Dixit (2010) prepared the aqueous extract of shisham (*Dalbergia sissoo*) bark along with some other plants and tested antibacterial sensitivity. They observed only 15.5 % antibacterial activity in *Dalbergia sissoo*.

Asati *et al.* (2010) evaluated the efficacy of amoxicillin and salbactam and oral rehydration solution alone and in combination against natural infection of colibacillosis in calves. It was found that all the treatments were effective but the calves treated with oral rehydration solution and antibiotics in combination gave better response than other groups. The maximum restoration of Hb value, PCV, TLC and total serum protein was observed in treatment group consisting antibiotic and ORS both.

Arora *et al.* (2010) prepared a poly herbal preparation containing equal quantity each of *Terminalia chebula* (harad), *Zingiber officinale* (ginger) and *Aegle marmelos* (bel) and administered @ 10 gm bid for 5 days orally along with fluid and electrolytes as per need. They reported 79.59 % therapeutic efficacy of the preparation. Animals recovered showed warm extremities on day 3 and faecal consistency, appetite, behaviour and skin elasticity normalized on day 5 post treatment. Significant fall was recorded in clinical parameters viz. rectal temperature, respiration rate and pulse rate post treatment in comparison to pretreatment values.

Kumar *et al.* (2010) reported that ciprofloxacin+ lactobacillus sporogenes alonwith parenteral rehydration therapy and astringent powder was found most effective in treatment of neonatal calf diarrhoea of bacterial origin.

Mir *et al.* (2010) found that intravenous fluid (ringer's lactate) and neblon powder followed by intravenous fluid and unripe powdered bael pulp were more efficacious in treatment of calf diarrhoea than the oral fluid with neblon or bael pulp powder.

Kuanr *et al.* (2011) indicated the presence of antibacterial activity in the extracts of *Litsea glutinosa* and *Caryeae arborea* against *E. coli*.

Singh *et al.* (2012) evaluated the therapeutic efficacy of Sulphadiazine-Trimethoprim, Ciprofloxacin-Tinidazole and Norfloxacin-Tinidazole against calf diarrhoea and found all the three antibiotics equally effective.

Kumaresan *et al.* (2012) reported that fluid therapy along with antimicrobial and probiotic administration was the most reliable treatment intervention in calf scours.

Singh and Pachauri (2012) observed that protective deworming with fenbendazole and praziquantel suspension / tablets and micronized albendazole at the age of 10-15 days, 40-45 days and 70-75 days post birth resulted in effective body weight gain and reduction in mortality in buffalo calves.

3. Materials and Methods

3.1 Animals

Under present investigation, total 240 bovine calves (102 buffalo and 138 cow calves including 42 crossbred and 96 Gir / local nondescript cow calves) up to 4 months of age at the organized farm of the university and adjoining area (field condition) were screened to find out the prevalence of calf diarrhoea for a period of one year i.e. July 2011 to June 2012 (Table 4).

The occurrence of different isolates in the faecal samples of the diarrhoeic calves and clinical and haemato-biochemical characterization was undertaken on 100 clinical cases of diarrhoea. The bovine calves belonged to Instructional Livestock Farm Complex,

College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur and adjoining area located in the southern part of Rajasthan.

For therapeutic evaluation, the diarrhoeic calves were divided in to five groups and each group had 15 animals at random.

Healthy control calves

Ten apparently healthy calves were also selected. The clinical parameters viz. rectal temperature, heart rate and respiration rate etc. were recorded. Blood samples were collected from them for same haemato-biochemical parameters as that of diarrhoeic calves to have comparable base line data for clinical and haemato-biochemical characterization of the diarrhoeic calves as well as to evaluate the therapeutic regimens.

Species	Breed	Organised farm	Field	Total
Buffalo	Surti	63	39	102
Cow	Crossbred	26	16	42
	Gir/local non- desript	79	17	96
	Total	105	33	138
Overall	·	168	72	240

Table 4 Number of calves (0-4 months age) screened

3.2 General information

Diarrhoeic calves were identified on the basis of observative clinical signs of diarrhoea at the earliest possible. Information with respect to species, breed, age, sex, parity of dam, season, deworming, vaccination status etc. was recorded. Observation and sampling was done on 0 day (before treatment), 4th day and 7th day (after treatment).

There were four stages of investigations.

- 1) Clinical characterization
- 2) Haemato-biochemical characterization
- 3) Etiological characterization and antibiotic sensitivity of *E. coli* isolates
- 4) Therapeutic regimens

3.3 Clinical parameters

The detailed investigation of each animal patient about clinical signs viz. general condition, rectal temperature, heart rate, respiration rate, appetite, frequency of defaectaion, colour and consistency of faeces, depression, dehydration etc. were recorded.

The clinical scores (0-3 basis) for faecal consistency, clinical depression and dehydration were recorded as per Walker *et al.* (1998) on 0 day (before treatment) and 4^{th} and 7^{th} day (after treatment).

Score	Faecal Consistency Score	Clinical Depression Score	Clinical Dehydration Score
0	Normal, well formed faeces	Normal, vigorous suckling	Normal, bright eyes, pliable skin
1	Pasty faeces	Mild depression, calf suckles but not vigorously	Mild dehydration, eyes not recess into orbits, slight loss of skin elasticity, skin tents<3 seconds
2	Semi liquid faeces still with a solid component	Moderate depression, calf able to stand, suckling is weak or disorganized	Moderate dehydration, eyes slightly recess into orbit, skin tent > 3 seconds but < 10 seconds
3	Watery faeces	Severe depression, unable to stand and suckle	Severe dehydration, eyes markedly recess into orbits, skin tents > 10 seconds

Table 5 Clinical scores in diarrhoeic calves

3.4 Haemato-biochemical parameters

The blood samples were collected from jugular vein in clean glass tubes containing EDTA for estimation of haematological parameters. Further, 5 ml blood samples were also collected in clean glass tube and allowed clotting for separation of serum for biochemical work. The collection and sampling was done on 0 day (before treatment), 4th day and 7th day (after treatment). The serum samples were stored at -20^oC for biochemical analysis at convenience.

3.4.1 Haematological analysis

- i) Haemoglobin Hellige-Sahli's haemoglobinometer (Feldman et al., 2000)
- ii) Packed cell volume- Microhaematocrit method (Feldman *et al.*, 2000)
- iii) Total Erythrocytes Count-Neubaur haemocytometer (Feldman *et al.*, 2000)
- iv) Total Leucocytes Count- (Feldman et al., 2000)
- v) Differential Leucocytes Count- (Feldman *et al.,* 2000)
- vi) Mean Corpuscular Volume(MCV)- (Feldman *et al.,* 2000)
- vii) Mean Corpuscular Haemoglobin(MCH)- (Feldman et al., 2000)
- viii) Mean Corpuscular Haemoglobin Concentration(MCHC)-(Feldman *et al.*, 2000)

3.4.2 Biochemical analysis

- Serum sodium and potassium- Flame Photometric Method (Oser,1965)
- ii) Serum Chloride- Colorimetric Method (Levinson, 1976)

- iii) Total Serum Protein- Biuret and BCG dye binding method (Doumas *et al.*,1971)
- iv) Albumin and globulin ratio- Biuret and BCG dye binding method (Doumas *et al.*,1971)
- v) Serum glucose- GOD/POD Method (Hultman, 1959)
- vi) Serum immunoglobulins- Estimation of serum IgG and IgM by Bovine IgG and IgM ELISA kit, Koma Biotech Inc., USA

3.5 Collection of faecal samples

Faecal samples (5 to 10 gm) from diarrhoeic calves were collected in clean zip lock polythene bags for parasitological examination.

Rectal swabs were collected aseptically in sterile test tubes for bacterial isolation and identification and transported over ice to laboratory immediately.

For detection of rotavirus, 10 ml truly diarrhoeic i.e. watery faeces or 5 gm semisolid faecal samples were collected in clean zip lock polythene bags. The samples were kept on ice. Samples were coated with a layer of glycerol and transported to virology laboratory.

3.6 Parasitological examination

Faecal samples after transportation to the laboratory were subjected to gross examination and microscopic examination. Direct faecal smear examination and concentration techniques as described by Schalm *et al.* (1986) for identification of parasitic ova/ oocyst and modified acid fast staining technique as described by Garcia and Lima (1993) for *Cryptosporidium* spp. were employed.

3.7 Bacteriological examination

The faecal samples were collected aseptically from the rectum of diarrhoeic calves with the help of sterile cotton swabs soaked in normal saline solution. These swabs were taken in sterile test tubes and transported over ice to laboratory immediately.

The Procedure for isolation and identification of bacterial cultures was done as per Cowan and Steel (1975) and Holt *et al.* (1994).

3.8 Antibiotic sensitivity test

(Determination of antibiogram of E. coli isolates)

The antibiotic sensitivity test was determined by disc diffusion method as per technique of Bauer *et al.* (1966).

The following antimicrobial discs were procured from Himedia Laboratories Pvt. Ltd. Mumbai for determination of antibiogram of bacterial isolates of diarrhoeic calves.

1.	Ofloxacin (Of)		5 µg
2.	Ciprofloxacin (Cf)		10 µg
3.	Enrofloxacin (En)		10 µg
4.	Norfloxacin (Nx)		10 µg
5.	Oxytetracyline (O)		30 µg
6.	Sulphadimidine (Sd)		300 µg
7.	Cotrimoxazole (Cm)		25 µg
8.	Erythromycin (E)		10 µg
9.	Gentamicin (G)		30 µg
10.	Trimethoprim (Tr)		10 µg
11.	Doxycycline (Do)		30 µg
12.	Chloramphenicol (C)		30 µg
13.	Nalidixic acid (Na)		30 µg
14.	Kanamycin (K)		30 µg
15.	Streptomycin (S)		25 µg
		170	

3.9 Virological examination (Rotavirus)

Procedure for detection of rotavirus was adopted as described by Herring *et al.* (1982) using silver stained polyacrylamide gel electrophoresis (PAGE).

3.10 Therapeutic Trial

The clinical cases of calf diarrhoea were employed for evaluation of therapeutic regimens comprised of oral or parenteral fluids (Ringer's lactate) with antimicrobial (Ofloxacin) and also along with an indigenous herbal preparation (Shisham leaves powder) to find out the best suitable therapy. The calves showing viral and / or parasitic diarrhoea were excluded from the evaluation of the therapeutic regimens.

The diarrhoeic calves were divided in 5 groups for therapeutic trial and each group had 15 animal patients. All the animals were given free access to water.

Group	Therapeutic agent
I	Ofloxacin + ORS
II	Ofloxacin + Parenteral fluid with sodium bicarbonate
Ш	Ofloxacin + ORS + Shisham leaves powder
IV	Ofloxacin + Parenteral fluid with sodium bicarbonate + Shisham leaves powder
V	Ofloxacin + Shisham leaves powder

 Table 6
 Different therapeutic regimens

3.10.1 Preparation of medicaments and doses

 Antimicrobial therapy: Ofloxacin was given parenterally as an antimicrobial @ 3 mg/kg b wt.

2. **Oral Rehydration Therapy:** Composition of the ORS was based on the WHO formula for humans and was modified for calf patients as per their therapeutic requirement keeping in the mind earlier reports.

Composition of ORS:

Sodium chloride-	3.50 gm	
Potassium chloride-	1.50 gm	
Sodium bicarbonate-	2.50 gm	
Dextrose-	55.00 gm	
Glutamine-	5.00 gm	

Above ingredients were dissolved in one litre of water and was given three times a day orally.

3. Parenteral Fluid Therapy: Ringer'lactate @ 25 ml/kg b wt i.v. alongwith the 7.5 % sodium bicarbonate 1 ml / kg b wt i.v..

4. **Indigenous Therapy:** The indigenous preparation in the present study comprised of Shisham leaves powder. It was prepared by collecting fresh shisham leaves which were dried under shed and grinded to fine powder. It was administered @ 10 gm / 10 Kg body weight orally twice daily for three days.

3.10.2 Evaluation of the therapy

Evaluation of the therapy was done on the basis of clinical recovery and improvement in the altered values of the clinical and haemato-biochemical parameters towards normalcy (at par to the values in healthy control calves) up to day 7th after treatment.

3.11 Statistical Analysis

The statistical analysis of the data was done using statistical methods described by Snedecor and Cochran (1994). The prevalence of diarrhoea and occurrence of different isolates was analyzed by using standard chi² test to test the significance. The clinical and haematobiochemical parameters of diarrhoeic and healthy calves were subjected to least square analysis of variance using mixed least square computer programme (LSMLMW) PC version 2 developed by Harvey (1990). The interpretation of the results in respect to evaluation of different therapeutic regimens was based on analysis of data among different treatment groups using ANOVA.

4. Results and Discussion

The present investigation was undertaken to find out the prevalence and identify different causative agents of calf diarrhoea at the organized farm of the university as well as under field condition in the southern region of Rajasthan. Clinical and haemato-biochemical characterization of calf diarrhoea along with evaluation of different therapeutic regimens have also been undertaken. The investigation will not only help the dairy husbandry planners and policy makers to take account of this issue in the development of the animal husbandry sector but also help the clinicians to understand this ailment and adopt

most suitable package of treatment to provide relief to the sufferings and reduce the losses to the animal owners.

The prevalence of calf diarrhoea in the calves in 0 to 4 month age group was find out at an organized farm of the university as well as in the field condition in both buffalo (Surti breed) and cow calves (crossbred and indigenous breed i.e. Gir at the farm and Gir and nondescript cow calves in the field) for a period of one year. The effect of age, sex, season and parity of dam on prevalence of diarrhoea was also studied. The faecal and blood samples of 100 diarrhoeic calves were collected and subjected to identification of causative agent(s) in the faeces and haemato-biochemical characterization of diarrhoea in calves. Different therapeutic regimens were evaluated on the basis of alterations in clinical and haemato-biochemical parameters towards normalcy after treatment in diarrhoeic calves.

4.1 Prevalence of calf diarrhoea

The species and breed-wise prevalence of calf diarrhoea at organized farm and under field condition is depicted in Table 7. The overall prevalence of diarrhoea in bovine calves (both buffalo and cow calves) of 0 to 4 month age group was found 41.67 % at the organized farm and field collectively.

Almost similar overall prevalence of diarrhoea in calves has been reported by Singh and Tomer (1988) and Brahma and Singh (2007). Tikoo *et al.* (2009) recorded lower prevalence of diarrhoea in calves than present study. They observed 34.80 % overall prevalence of diarrhoea in calves. Lower prevalence has also been reported by Shimizu and Nagatomo (1987), Bendali *et al.* (1997), Katikaridis (2000), Biewer (2001), Das *et al.* (2006) and Singh *et al.* (2009b) at different places in India and abroad. Higher prevalence of calf diarrhoea than present study has been reported by Girnus (2004) and Malik *et al.* (2012). Diarrhoea in calves varies widely depending upon geographical locations and herds (Krogh and Sherwood, 1983).

The overall prevalence of diarrhoea in bovine calves (both buffalo and cow calves) at the organized farm was 35.71 % whereas under field condition was 55.55 %. The prevalence of diarrhoea at the organized farm was found significantly lower than that of field condition (P>0.05) (Figure 1). It is in agreement with the finding of Tikoo *et al.* (2009). The diarrhoea in calves is influenced by housing type, feeding practices, cleaning and disinfection of housing etc. (Bendali *et al.*, 1997). The management practices at the organized farm of the university were far better than that of field condition which attributed to the lower prevalence of diarrhoea in calves at the organized farm.

Species	Breed	Organised farm	Field	Overall
Buffalo	Surti	49.21	61.54	53.92
		(31)	(24)	(55)
Cow	Crossbred	34.61	56.25	42.86
		(9)	(9)	(18)
	Gir / local non-	25.32	41.18	28.12
	descript	(20)	(7)	(27)
	Total	27.62	48.48	32.61
		(29)	(16)	(45)
	Overall	35.71	55.55	41.67
		(60)	(40)	(100)

Table 7Species and breed-wise prevalence of diarrhoea (%) in
calves

Figures in parenthesis indicate number of diarrhoeic calves

The overall prevalence of diarrhoea in buffalo and cow calves was 53.92 % and 32.61 %, respectively. The prevalence of diarrhoea in buffalo and cow calves at the organized farm was 49.21% and 27.62 %, respectively whereas under field condition was 61.54 % and 48.48 %, respectively. The prevalence of diarrhoea was significantly higher in buffalo calves than cow calves (P>0.05) (Figure 1 and 2). Similar finding has been reported by Brahma and Singh (2007) whereas Tikoo *et al.* (2009) reported higher prevalence of diarrhoea in cow calves than buffalo calves. Malik *et al.* (2012) recorded that the incidence rate of calf diarrhoea in cattle calves was almost similar to that in buffalo calves.

The prevalence of diarrhoea in crossbred calves and Gir / nondescript calves was 42.86 % and 28.12 %, respectively. At the organized farm, the prevalence of calf diarrhoea in crossbred and Gir cattle was 34.61 % and 25.32 %, respectively whereas under field condition, the prevalence of diarrhoea in crossbred and Gir / nondescript cow calves was 56.25 % and 41.18 %, respectively. The higher prevalence has been recorded in crossbred calves than indigenous / local non-descript cow calves (Figure 1 and 2). It is in agreement with the finding of Brahma and Singh (2007). Indigenous and local non-descript animals are known to be disease resistant as compared to exotic and crossbred animals.

The age group-wise prevalence of calf diarrhoea at organized farm and under field condition collectively and separately is depicted in Table 8, 9 and 10, respectively. The overall prevalence of diarrhoea in calves in different age groups viz. 0-15 days, 16-30 days, 31-60 days and 61-120 days was 38.00 %, 29.00 %, 20.00 % and 13.00 %, respectively (Table 8). The prevalence of diarrhoea in calves at the organized farm and under field condition in different age groups viz. 0-15 days, 16-30 days, 31-60 days and 61-120 days was 36.67 % and 40.00 %; 30.00 % and 27.50 %; 18.33 % and 22.50 %; and 15.00 % and 10.00 %, respectively (Table 9 and 10). Statistically, there was unequal distribution of data in different age groups. There was highly significant difference in the overall prevalence of diarrhoea in calves in different age groups at the organized farm and field collectively (P>0.01) whereas the significant difference was found in the prevalence of diarrhoea in calves in different age groups at the organized farm and field collectively (P>0.01) whereas the significant difference was found in the prevalence of diarrhoea in calves in different age groups at

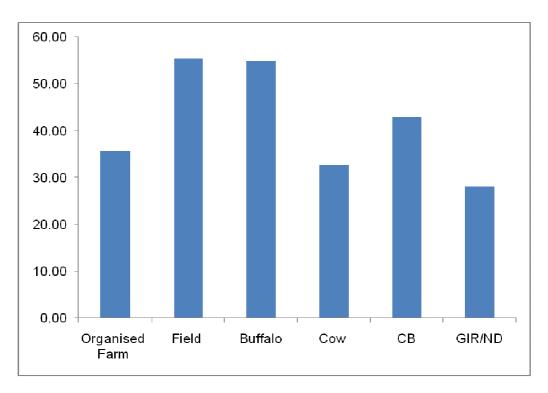


Fig. 1 Overall prevalence (%) of diarrhoea in calves

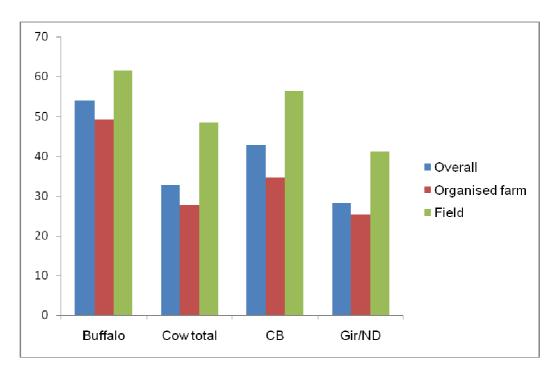


Fig. 2 Species and breed wise prevalence (%) of diarrhoea in calves

the organized farm and under field condition, separately (P>0.05). The prevalence of calf diarrhoea was found decreasing with the advancement of the age in the calves (Figure 3). Similar finding has been reported by Curtis *et al.* (1988), Singh and Tomer (1988), Frank and Kaneene (1993), Bendali *et al.* (1997), Ryan *et al.* (1999), Temesgen (2004) and Brahma and Singh (2007). The finding of Hussain and Saikia (2000b) are in contrast to the present investigation. They observed higher prevalence of diarrhoea in 3 to 4 weeks age group of calves than other age groups. Diarrhoea affects young calves at an age when they have immature immune status, lacks specific antibody, illustrate high metabolism with added stresses and some times deprivation of immune colostrum feeding.

The sex-wise prevalence of calf diarrhoea at organized farm and under field condition collectively and separately is also depicted in Table 8, 9 and 10, respectively. The overall prevalence of diarrhoea in male and female calves was 44.09 % and 38.94 %, respectively (Table 8). The overall prevalence of diarrhoea in male and female calves at the organized farm was 37.23 % and 33.78 %, respectively (Table 9) whereas under field condition was 63.64 % and 48.72 %, respectively (Table 10). The prevalence of diarrhoea was higher in male calves than female calves (Figure 4) but statistically, there was non significant difference in the prevalence of diarrhoea in male and female calves (P<0.05). The trends were similar in buffalo and cow calves. Klingenberg *et al.* (1999) reported that there was no association of sex with the development of diarrhoea in calves. Clement *et al.* (1995) and Devkate *et al.* (2010) reported higher occurrence of diarrhoea in male than female calves.

Season-wise prevalence of diarrhoea in calves is depicted in Table 11 and Figure 5. It showed highest overall prevalence of diarrhoea in bovine

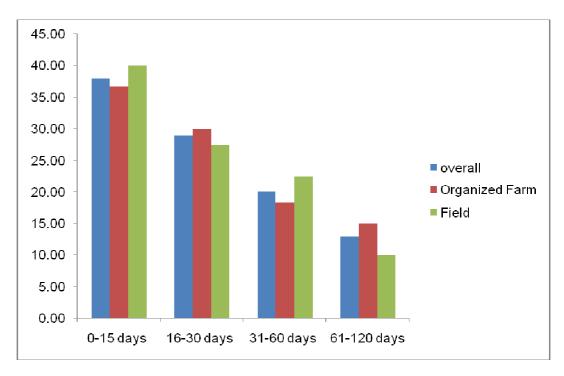


Fig. 3 Age group-wise prevalence (%) of diarrhoea in calves

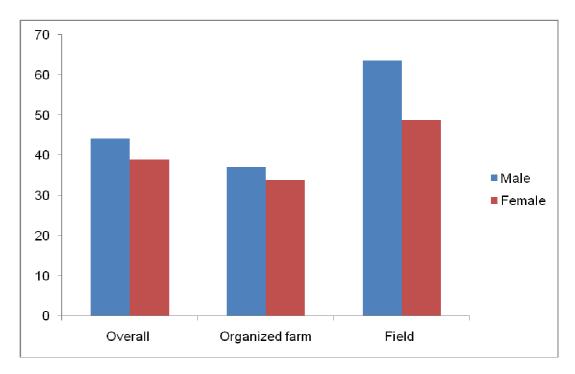


Fig. 4 Sex-wise prevalence (%) of diarrhoea in calves

Species	Breed							A	ge group	S							
		0-15 days			1	16-30 day	s	3	81-60 day	S	6	1-120 day	/S	Overall			
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	
Buffalo	Surti	36.36	36.36	36.36	30.30	27.27	29.09	21.21	18.18	20.00	12.12	18.18	14.54	55.00	52.38	53.92	
		(12)	(8)	(20)	(10)	(6)	(16)	(7)	(4)	(11)	(4)	(4)	(8)	(33)	(22)	(55)	
Cow	СВ	40.00	50.00	44.44	20.00	37.50	27.78	20.00	12.50	16.67	20.00	0.00	11.11	47.62	28.09	42.86	
		(4)	(4)	(8)	(2)	(3)	(5)	(2)	(1)	(3)	(2)	(0)	(2)	(10)	(8)	(18)	
	Gir /	30.77	42.86	37.04	38.46	21.43	29.63	15.38	28.57	22.22	15.38	7.14	11.11	28.26	28.00	28.12	
	ND	(4)	(6)	(10)	(5)	(3)	(8)	(2)	(4)	(6)	(2)	(1)	(3)	(13)	(14)	(27)	
	Total	34.78	45.45	40.00	30.43	27.27	28.89	17.39	22.72	20.00	17.39	4.54	11.11	34.33	30.98	32.61	
		(8)	(10)	(18)	(7)	(6)	(13)	(4)	(5)	(9)	(4)	(1)	(5)	(23)	(22)	(45)	
Overall		35.71	40.90	38.00	30.36	27.27	29.00	19.64	20.45	20.00	14.28	11.36	13.00	44.09	38.94	41.67	
		(20)	(18)	(38)	(17)	(12)	(29)	(11)	(9)	(20)	(8)	(5)	(13)	(56)	(44)	(100)	

Table 8 Age and sex-wise prevalence of diarrhoea (%) in calves at the organized farm and field condition collectively

CB = Crossbred, ND = Non-descript

Figures in parenthesis indicate number of diarrhoeic calves

 χ^2 value (Age group) Buffalo=8.16 ; CB cow=4.67; Gir/ND cow=3.96; cow total=8.25; Overall=13.44

 χ^2 value (Overall sex) Buffalo=2.20 ; CB cow=0.22; Gir/ND cow=0.04; cow total=0.02; Overall=1.44

Table 9Age and sex-wise prevalence of diarrhoea (%) in calves at the organized farm

Species	Breed							Δ	ge group	S						
			0-15 days	;	1	6-30 day	S	3	81-60 day	S	6	1-120 day	S	Overall		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Buffalo	Surti	35.00	36.37	35.48	30.00	27.27	29.03	20.00	18.18	19.35	15.00	18.18	16.13	48.78	50.00	49.21
		(7)	(4)	(11)	(6)	(3)	(9)	(4)	(2)	(6)	(3)	(2)	(5)	(20)	(11)	(31)
	СВ	40.00	50.00	44.44	20.00	50.00	33.33	20.00	0.00	11.11	20.00	0.00	11.11	35.71	33.33	34.61
		(2)	(2)	(4)	(1)	(2)	(3)	(1)	(0)	(1)	(1)	(0)	(1)	(5)	(4)	(9)
Cow	Gir	30.00	40.00	35.00	40.00	20.00	30.00	10.00	30.00	20.00	20.00	10.00	15.00	25.64	25.00	25.32
COW		(3)	(4)	(7)	(4)	(2)	(6)	(1)	(3)	(4)	(2)	(1)	(3)	(10)	(10)	(20)
	Total	33.33	42.86	37.93	33.33	28.57	31.03	13.33	21.43	17.24	20.00	7.14	13.79	28.30	26.92	27.62
		(5)	(6)	(11)	(5)	(4)	(9)	(2)	(3)	(5)	(3)	(1)	(4)	(15)	(14)	(29)
Overall		34.28	40.00	36.67	31.43	28.00	30.00	17.14	20.00	18.33	17.14	12.00	15.00	37.23	33.78	35.71
		(12)	(10)	(22)	(11)	(7)	(18)	(6)	(5)	(11)	(6)	(3)	(9)	(35)	(25)	(60)

CB = Crossbred

Figures in parenthesis indicate number of diarrhoeic calves

 χ^2 value (Age group) Buffalo=2.94; CB cow=3.00; Gir cow=2.00; cow total=4.52; Overall=7.88

 χ^2 value (Overall sex) Buffalo=2.61 ; CB cow=0.11; Gir cow=0.00; cow total=0.03; Overall=1.67

Table 10 Age and sex-wise prevalence of diarrhoea (%) in calves under field condition

Species	Breed							A	ge group	S							
		0-15 days			16-30 days			3	31-60 day	S	6	1-120 day	s	Overall			
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	
Buffalo	Surti	38.46	36.36	37.50	30.33	27.27	29.17	23.08	18.18	20.83	7.69	18.18	12.50	68.42	55.00	61.54	
		(5)	(4)	(9)	(4)	(3)	(7)	(3)	(2)	(5)	(1)	(2)	(3)	(13)	(11)	(24)	
	СВ	40.00	50.00	44.44	20.00	25.00	22.22	20.00	25.00	22.22	20.00	0.00	11.11	71.43	44.44	56.25	
		(2)	(2)	(4)	(1)	(1)	(2)	(1)	(1)	(2)	(1)	(0)	(1)	(5)	(4)	(9)	
Cow	Gir	33.33	50.00	42.86	33.33	25.00	28.57	33.33	25.00	28.57	0.00	0.00	0.00	42.86	40.00	41.18	
COW	/ND	(1)	(2)	(3)	(1)	(1)	(2)	(1)	(1)	(2)	(0)	(0)	(0)	(3)	(4)	(7)	
	Total	37.50	50.00	43.75	25.00	25.00	25.00	25.00	25.00	25.00	12.50	0.00	6.25	57.14	42.10	48.48	
		(3)	(4)	(7)	(2)	(2)	(4)	(2)	(2)	(4)	(1)	(0)	(1)	(8)	(8)	(16)	
Overall		38.09	42.10	40.00	28.57	26.31	27.50	23.81	21.05	22.50	9.52	10.53	10.00	63.64	48.72	55.55	
		(8)	(8)	(16)	(6)	(5)	(11)	(5)	(4)	(9)	(2)	(2)	(4)	(21)	(19)	(40)	

CB = Crossbred, ND = Non-descript

Figures in parenthesis indicate number of diarrhoeic calves

 χ^2 value (Age group) Buffalo=3.33; CB cow=2.11; Gir/ND cow=2.71; cow total=4.50; Overall=7.90

x2 value (Overall sex) Buffalo=0.17 ; CB cow=0.11; Gir/ND cow=0.14; cow total=0.00; Overall=0.10

calves (both buffalo and cow calves) during rainy season (29.58 %), followed by winter (23.12 %) and summer season (11.02 %) at the organized farm and field condition collectively. Statistically, the data were not equally distributed among different seasons and there was highly significant difference in the prevalence of diarrhoea in different seasons (P>0.01). At the organized farm, highest overall prevalence was observed during rainy season (24.79 %), followed by winter (19.30 %) and summer (8.33 %). There was highly significant difference in the prevalence of calf diarrhoea in different seasons at the organized farm (P>0.01). Under field condition also, the overall prevalence of calf diarrhoea was highest during rainy season (41.67 %), followed by winter (32.61 %) and summer (22.73 %) with unequal distribution of data in different seasons (P>0.01). The trends were similar in buffalo and cow calves except in crossbred calves at organized farm and field where lowest prevalence of diarrhoea was observed during winter season which might be attributed due to poor tolerance of heat by the exotic and crossbred animals. Similar finding has been reported by Acha et al. (2004), Brahma and Singh (2007), Tikoo et al. (2009) and Malik et al. (2012). Curtis et al. (1988) and Devkate et al. (2010) reported highest occurrence of diarrhoea in winter season. Cold wet weather not only stresses calves but also increase exposure to diarrhoea (Heath, 1992).

Parity of dam-wise prevalence of diarrhoea in calves is presented in Table 12. The overall prevalence of diarrhoea in bovine calves (both buffalo and cow calves) of first, second, third, fourth and fifth & above parity dams at the organized farm and field condition collectively was 65.91 %, 50.00 %, 36.92 %, 26.31% and 37.50 %, respectively. At the organized farm, the overall prevalence of diarrhoea in bovine calves

Species	Breed	0	rganized Fa	rm	F	ield conditio	on	Both collectively				
		Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer		
Buffalo	Surti	29.09	23.33	14.28	45.83	39.28	25.00	34.18	28.41	20.00		
		(16)	(14)	(1)	(11)	(11)	(2)	(27)	(25)	(3)		
Cow	СВ	25.00	8.33	15.79	41.67	25.00	28.57	31.25	15.00	19.23		
		(5)	(1)	(3)	(5)	(2)	(2)	(10)	(3)	(5)		
	Gir/ND	19.56	16.67	5.71	33.33	20.00	14.28	22.41	17.31	6.49		
		(9)	(7)	(4)	(4)	(2)	(1)	(13)	(9)	(5)		
	Total	22.21	14.81	7.86	37.50	22.22	21.43	25.55	16.67	9.71		
		(14)	(8)	(7)	(9)	(4)	(3)	(23)	(12)	(10)		
Overall		24.79	19.30	8.33	41.67	32.61	22.73	29.58	23.12	11.02		
		(30)	(22)	(8)	(20)	(15)	(5)	(50)	(37)	(13)		

Table 11Season-wise prevalence (%) of diarrhoea in calves

CB = Crossbred, ND = Non-descript

Figures in parenthesis indicate number of diarrhoeic calves

 χ^2 value (Organized farm) Buffalo=12.84; CB cow=2.67; Gir/ND cow=1.90; cow total=6.11; Overall=12.40

 χ^2 value (Field) Buffalo=6.74 ; CB cow=2.00; Gir/ND cow=2.00; cow total=5.88; Overall=9.76

 χ^2 value (Farm and field collectively) Buffalo=19.35; CB cow=4.33; Gir/ND cow=3.56; cow total=6.53; Overall=21.14

Species	Parity		Org	anized	Farm			Field	d condi	tion		Both collectively					
		I	II	III	IV	V & above	I	II	III	IV	V & above	I	II	III	IV	V & above	
	Breed	•															
Buffalo	Surti	61.90 (13)	55.55 (5)	45.45 (5)	33.33 (3)	38.46 (5)	83.33 (5)	83.33 (5)	50.00 (5)	55.55 (5)	50.00 (4)	66.67 (18)	66.67 (10)	47.62 (10)	44.44 (8)	42.86 (9)	
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	СВ	57.14 (4)	40.00 (2)	25.00 (1)	20.00 (1)	20.00 (1)	100.00 (3)	33.33 (1)	75.00 (3)	33.33 (1)	33.33 (1)	70.00 (7)	37.50 (3)	50.00 (4)	25.00 (2)	25.00 (2)	
•	Gir	50.00	33.33	25.00	15.38	33.33	100.00	50.00	50.00	20.00	40.00	57.14	36.36	27.78	16.13	36.36	
Cow	/ND	(3)	(3)	(8)	(4)	(2)	(1)	(1)	(2)	(1)	(2)	(4)	(4)	(10)	(5)	(4)	
	Total	53.85	35.71	25.00	16.13	27.27	100.00	40.00	62.50	25.00	37.50	64.70	36.84	31.82	17.95	31.58	
		(7)	(5)	(9)	(5)	(3)	(4)	(2)	(5)	(2)	(3)	(11)	(7)	(14)	(7)	(6)	
Overall		58.82	43.48	29.79	20.00	33.33	90.00	63.64	55.55	41.18	43.75	65.91	50.00	36.92	26.31	37.50	
		(20)	(10)	(14)	(8)	(8)	(9)	(7)	(10)	(7)	(7)	(29)	(17)	(24)	(15)	(15)	

Table 12Parity of dam wise prevalence (%) of diarrhoea in calves

CB = Crossbred, ND = Non-descript

Figures in parenthesis indicate number of diarrhoeic calves

 χ^2 value (Organized farm) Buffalo=9.81; CB cow=3.78; Gir/ND cow=5.50; cow total=9.59; Overall=9.67

 χ^2 value (Field) Buffalo=3.17 ; CB cow=2.67; Gir/ND cow=2.86; cow total=4.13; Overall=9.00

 χ^2 value (Farm and field collectively) Buffalo=5.82; CB cow=4.78; Gir/ND cow=5.04; cow total=10.32; Overall=9.80

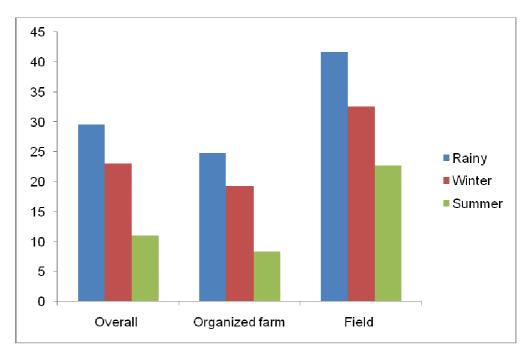


Fig. 5 Season-wise prevalence (%) of diarrhoea in calves

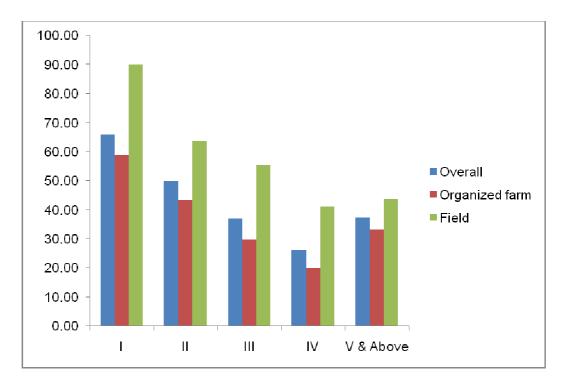


Fig. 6 Parity of dam-wise prevalence (%) of diarrhoea in calves

(both buffalo and cow calves) of first, second, third, fourth and fifth & above parity dams was 58.82 %, 43.48 %, 29.79 %, 20.00% and 33.33 %, respectively. Like wise under field condition, the overall prevalence of diarrhoea in calves (both buffalo and cow calves) of first, second, third, fourth and fifth & above parity dams was 90.00 %, 63.64 %, 55.55 %, 41.18% and 43.75 %, respectively. The highest prevalence of diarrhoea was observed in the calves of first parity dams followed by calves of second parity dams at organized farm, field and both collectively (Figure 6). The trend was similar in different species (buffalo and cow) and breed of cows except in crossbred cows and total cow calves under field condition where highest prevalence of diarrhoea was observed in the calves of first parity dams but it was followed by calves of third parity dams. Thus, in buffalo and cow calves at the organized farm as well as under field condition the highest prevalence of calf diarrhoea was observed in calves of first parity dams. Similar finding has been reported by Clement et al. (1995) and Odde (2007). First and second parity cows have significantly lower levels of immunoglobulins in their colostrum than higher parity cows. It has also been shown that cows that have a dry period of less than four weeks produce colostrum with low immunoglobulin levels. It was the reason for higher incidence of diarrhoea in calves born during first or second lactation (Logan et al., 1981). In other words, quality of colostrum increases with the lactation number of dam which explains the higher risk of disease in calves born to first lactation cows (Svensson et al., 2003).

4.2 Occurrence of different isolates in the faecal samples of diarrhoeic calves

Identification of infectious agents which cause diarrhoea in calves in a herd is essential for implementing effective preventive and treatment measures and may show any potential zoonotic risks, as several organisms causing diarrhoea have the potential to cause severe disease in humans (Bazeley, 2003).

Table 13Sample-wise occurrence of different isolates in the
faecal samples of diarrhoeic calves

S.No.	Isolates	Total no. of samples	Percentage
1	Escherichia coli	76	76
2	Escherichia coli, Rotavirus	6	6
3	<i>Escherichia coli,</i> Rotavirus, <i>Cryptosporidium</i> spp.	3	3
4	Rotavirus, <i>Cryptosporidium</i> spp., <i>Eimeria</i> spp., <i>Toxacara</i> spp.,Amphistomes	3	3
5	Rotavirus, <i>Salmonella</i> spp., <i>Eimeria</i> spp., Amphistomes	3	3
6	<i>Escherichia coli, Eimeria</i> spp., <i>Toxacara</i> spp., Amphistomes, Strongyles	1	1
7	<i>Eimeria</i> spp., <i>Toxacara</i> spp., Amphistomes, Strongyles, <i>Trichuris</i> spp.	5	5
8	<i>Eimeria</i> spp., <i>Toxacara</i> spp., Amphistomes, Strongyles, <i>Strongyloides</i> spp.	3	3

The faeces of diarrhoeic calves were examined for bacteria, rotavirus and parasitic ova or oocyst by different methods as mentioned in Chapter 3. Sample-wise occurrence of different isolates in the faecal samples of diarrhoeic calves is presented in Table 13. The faecal samples were collected from 100 diarrhoeic calves. Out of that, 76 faecal samples were positive for single isolate i.e. Escherichia coli. Remaining 24 faecal samples were found positive for mixed infections and infestations. In mixed infections, E. coli, Salmonella spp., rotavirus, Cryptosporidium spp., Eimeria spp. and other parasitic ova (Amphistomes, Trichuris spp., Strongyles Toxocara spp., and Strongyloides spp.) were observed. Similar finding has been reported by Barrandeguy et al. (1988), Garcia et al. (2000), Aydin et al. (2001) and Stoltenow and Vincent (2003). Gyles (1994) reported that Escherichia coli are the most common causative agent of calf diarrhoea. Microorganisms like Escherichia coli, Salmonella spp., rotavirus and Cryptosporidium spp. causing diarrhoea in neonates are collectively responsible for 75-95% of infection in neonatal calves worldwide (Hansa et al., 2012).

The occurrence of different isolates in the faecal samples of diarrhoeic calves is depicted in Table 14. It showed that occurrence of *E. coli* in diarrhoeic faecal samples of bovine calves was found highest (86.00 %), followed by rotavirus, *Eimeria* spp. and Amphistomes (15.00 % each), *Toxocara* spp. (12.00 %), Strongyles (9.00 %), *Cryptosporidium* spp. (6.00 %), *Trichuris* spp. (5.00 %) and *Salmonella* spp. and *Strongyloides* spp. (3.00 each) at the organized farm and field condition collectively (Figure 7).

E. coli was the major organism observed in the faecal samples of the diarrhoeic calves. It was almost uniformly distributed at the organized farm (88.33 %) as well as under field condition (82.50 %). Further, there was also no significant difference in the occurrence of *E. coli* between buffalo (85.45 %) and cow calves (86.67 %) and also between crossbred

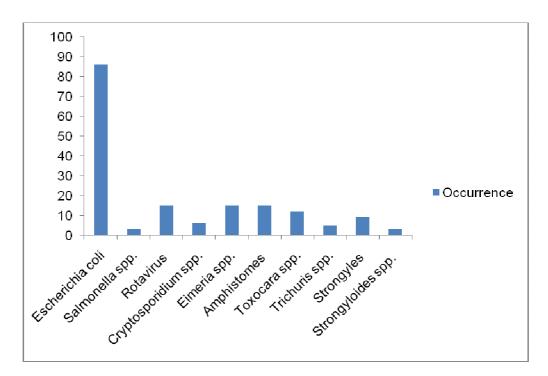


Fig. 7 Overall occurrence (%) of different isolates in the faecal samples of diarrhoeic calves

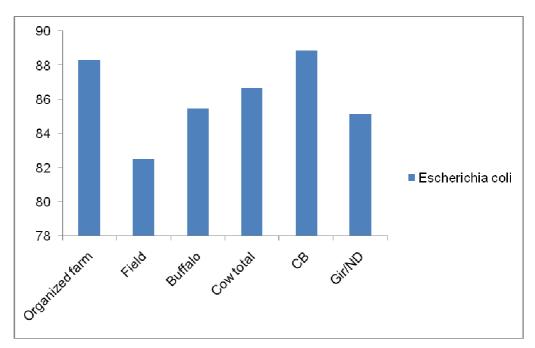


Fig. 8 Occurrence (%) of *E.coli* in the faecal samples of diarrhoeic calves

(88.89 %) and Gir / local non-descript calves (85.18 %) (P<0.05) (Figure 8). It indicated that *E. coli* is the most common organism responsible for calf diarrhoea. Almost similar occurrence of *E. coli* has been reported by Srisuparbh (1978), Barrandeguy *et al.* (1988), Garcia *et al.* (2000), Chaurasia (2001) and Stoltenow and Vincent (2003). Higher occurrence of *E. coli* infection in diarrhoeic calves than present study has been reported by Aydin *et al.* (2001) whereas low occurrence of *E. coli* has been reported by De-Oliveiria *et al.* (1989), Wani *et al.* (2003), Roy *et al.* (2009), Kumar *et al.* (2010) and Arora *et al.* (2010). Occurrence of *E. coli* in diarrhoeic calves varies widely depending upon geographical location (Krogh and Sherwood, 1983).

Salmonella spp. was another bacterial isolate detected in the faecal samples of diarrhoeic calves with the occurrence of only 3.00 %. The organism was isolated only from the faecal samples of diarrhoeic crossbred calves (Figure 9). The occurrence of *Salmonella* spp. was found very low in the faecal samples of diarrhoeic calves. It is in agreement with the earlier reports (Srisuparbh, 1978; Debnath *et al.*, 1987; Barrandeguy *et al.*, 1988; and Temesgen, 2004). Fiedler *et al.* (1982), Nagy *et al.* (1986), Snodgrass *et al.* (1986), Aydin *et al.* (2001) and Gupta *et al.* (2006) reported lower or zero occurrence of *Salmonella* spp. in the faecal samples of diarrhoeic calves than present study. Higher occurrence of this organism has been reported by Buhr-Pohlmann (1985), Reynolds *et al.* (2010).

The occurrence of rotavirus in the faecal samples of diarrhoeic calves (both buffalo and cow calves) in the present investigation was 15.00 % with 13.33 % and 17.50 % at the organized farm and under field condition, respectively. The occurrence in diarrhoeic buffalo and cow calves was 18.18 % and 11.11 %, respectively whereas in diarrhoeic

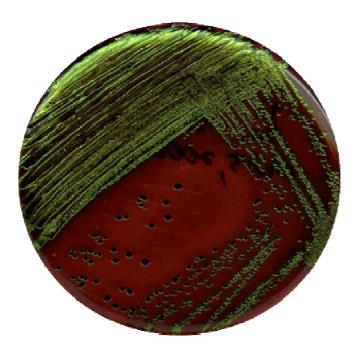


Plate 1 Metallic Sheen of E. coli on EMB Agar



Plate 2 Pink Colony of E. coli on McConkey Agar

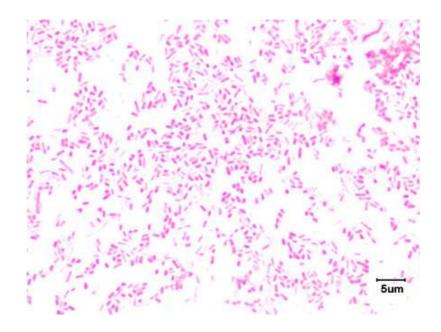


Plate 3 Bacteria morphologically indistinguishable from Enterobacteriae



Plate 4 Pale coloured Colonies of Salmonella spp. on McConkey Agar



Plate 5 Antibiotic sensitivity test on *E.coli* isolates

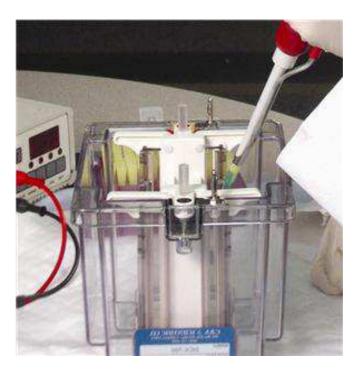


Plate 6 Loading the sample of Rota virus in RNA PAGE

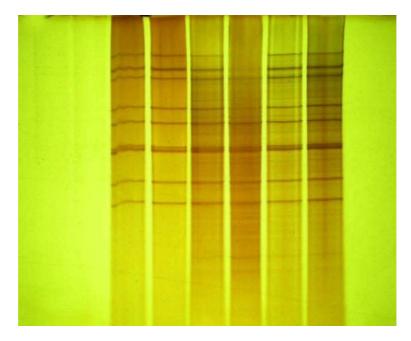


Plate 7 RNA-PAGE showing migration pattern of Rotavirus of bovine calves

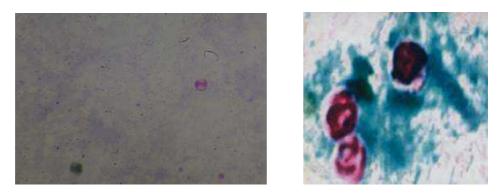


Plate 8 Ooysts of *Cryptosporidium* spp.

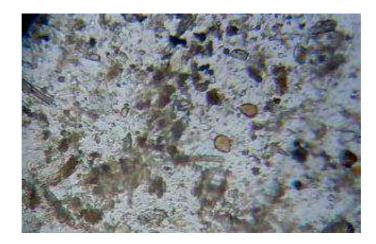


Plate 9 Oocysts of *Eimeria* spp. in faecal samples of diarrhoeic calves



Plate 10 Amphistomes in the diarrhoeic faeces of calves



Plate 11 Amphistomes isolated from the diarrhoeic faeces of calves

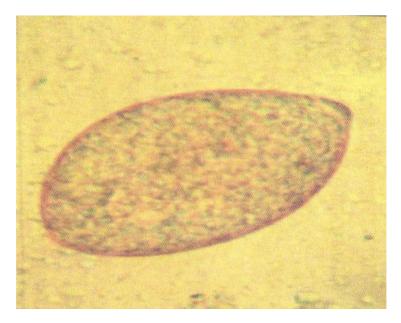


Plate 12 Amphistomes egg in the faeces of diarrhoeic calves

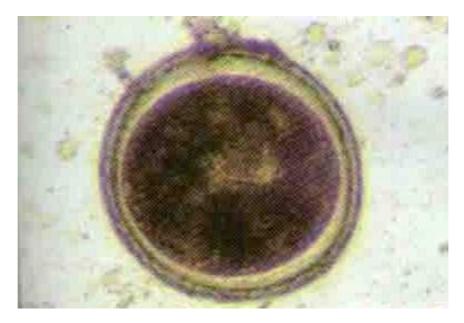


Plate 13 Toxocara spp. egg in the faeces of diarrhoeic calves



Plate 14 Trichuris spp. egg in the faeces of diarrhoeic calves



Plate 15 Strongyle egg in the faeces of diarrhoeic calves



Plate 16 Strongyloides spp. egg in the faeces of diarrhoeic calves

crossbred and Gir / local non-descript calves, the occurrence of rotavirus was found to be 16.67 % and 7.41 %, respectively. There was no significant difference in the occurrence of rotavirus at the organized farm and field (P<0.05). The occurrence of rotavirus was found significantly higher in buffalo calves and crossbred calves than cow calves and Gir/local non-descript calves, respectively (P>0.05) (Figure 9).

Almost similar overall occurrence of rotavirus in diarrhoeic calves has been reported by Brenner et al. (1993), Bardhan (2007) and Beg et al. (2010). Higher occurrence of rotavirus in diarrhoeic calves has been reported by Kaushik et al. (1983), Chauhan and Singh (1996), Grover et al. (1997), Jindal et al. (2000), Sharma et al. (2004), Minakshi et al. (2005), Garaicocoechea et al. (2006), Kusumaker (2006) and Sharma et al. (2009) than the present study at different locations in India and abroad. The occurrence of rotavirus in diarrhoeic calves lower than present study has also been reported (Jhala and Raghvan, 1997; Lofstedt et al., 1999; Manuja et al., 2008; Chandrashekhar, 2008; Niture et al. 2009; Manuja et al., 2010; Basera et al., 2010 and Mondal et al., 2011). Ingle et al. (2009) observed no any diarrhoeic faecal samples of calves positive for rotavirus. Higher occurrence of rotavirus in diarrhoeic faecal samples of buffalo calves than cow calves in present study is in agreement with the reports of Kaushik et al. (1983), Sharma (2004), Chandrashekhar (2008) and Mondal et al. (2011) but in contrast with the report of Sharma et al. (2009). The incidence of infection with rotavirus ranges from 10 % to 52 % in cow calves and 11 % to 24 % in buffalo calves (Singh and Pandey, 1988).

Cryptosporidium spp. was also one of the important isolate in the faecal samples of diarrhoeic calves in the present study. The overall occurrence of this protozoan parasite in diarrhoeic faecal samples of calves (both buffalo and cow calves) was found 6.00 % with

almost equal distribution at the organized farm (5.00 %) and under field condition (7.50 %) statistically. The occurrence of *Cryptosporidium* spp. in diarrhoeic buffalo and cow calves was 7.27 % and 4.44 %, respectively whereas in crossbred and Gir/local non-descript cow calves was 11.11 % and 0.00 %, respectively (Table 14 and Figure 9).

The overall occurrence of *Cryptosporidium* spp. (6.00 %) in faecal samples of diarrhoeic calves in present study is in agreement with that of Barrandeguy *et al.* (1988), Mtambo *et al.* (1997), Aydin *et al.* (2001) and Temesgen (2004). Das *et al.* (2006), Kaur and Kaur (2008), Sahu and Maiti (2009), Pandey *et al.* (2009), Prakash *et al.* (2009) and Palanivel *et al.* (2011) reported higher prevalence of Cryptosporidiosis in diarrhoeic calves ranging from 9.05 % to 50.0 %. The occurrence of *Cryptosporidium* spp. oocyst in the faecal samples of diarrhoeic calves was significantly higher in buffalo calves and crossbred cow calves than cow calves and Gir/non-descript cow calves, respectively (P>0.05). Shobhamani and Alahasingari (2006), Sahu and Maiti (2009) and Prakash *et al.* (2009) also reported higher prevalence of Cryptosporidiosis in crossbred than indigenous breed cow calves.

The occurrence of *Eimeria* spp. in faecal sample of diarrhoeic calves was 15.00 % in both buffalo and cow calves at the organized farm and field collectively. The occurrence of *Eimeria* spp. in the faecal sample of diarrhoeic bovine calves (both buffalo and cow calves) was 10.00 % and 22.50 % at the organized farm and field condition, respectively. The occurrence of *Eimeria* spp. in diarrhoeic buffalo and cow calves was 20.00 % and 8.89 %, respectively whereas in crossbred and Gir/local non-descript cow calves was 16.67 % and 3.70 %, respectively (Table 14). The occurrence of *Eimeria* spp. was significantly higher in buffalo and crossbred cow calves than cow calves and Gir/local non-descript cow calves, respectively (P>0.05)

(Figure 9). Similar finding has been reported by Barrandeguy *et al.* (1988) and Barua *et al.* (2009). Higher occurrence

S.	Name of isolates		Orga	nized fa	rm			Field	d conditi	on		Both collectively					
No.		Buffalo		Cattle		Total	Buffalo		Cattle		Total	Buffalo		Cattle		total	
			СВ	Gir	Total			СВ	ND	Total			СВ	Gir/ND	Total	1	
1	Escherichia coli	87.10	88.89	90.90	89.65	88.33	83.33	88.89	71.43	81.25	82.50	85.45	88.89	85.18	86.67	86.00	
		(27)	(8)	(18)	(26)	(53)	(20)	(8)	(5)	(13)	(33)	(47)	(16)	(23)	(39)	(86)	
2	Salmonella spp.	0.00	11.11	0.00	3.49	1.67	0.00	22.22	0.00	12.50	5.00	0.00	16.67	0.00	6.67	3.00	
		(0)	(1)	(0)	(1)	(1)	(0)	(2)	(0)	(2)	(2)	(0)	(3)	(0)	(3)	(3)	
3	Rotavirus	16.13	22.22	5.00	10.34	13.33	20.83	11.11	14.28	12.50	17.50	18.18	16.67	7.41	11.11	15.00	
		(5)	(2)	(1)	(3)	(8)	(5)	(1)	(1)	(2)	(7)	(10)	(3)	(2)	(5)	(15)	
4	Cryptosporidium	6.45	11.11	0.00	3.45	5.00	8.33	11.11	0.00	6.25	7.50	7.27	11.11	0.00	4.44	6.00	
	spp.	(2)	(1)	(0)	(1)	(3)	(2)	(1)	(0)	(1)	(3)	(4)	(2)	(0)	(2)	(6)	
5	<i>Eimeria</i> spp.	16.13	11.11	0.00	3.45	10.00	25.00	22.22	14.28	18.75	22.50	20.00	16.67	3.70	8.89	15.00	
		(5)	(1)	(0)	(1)	(6)	(6)	(2)	(1)	(3)	(9)	(11)	(3)	(1)	(4)	(15)	
6	Amphistomes	0.00	0.00	0.00	0.00	0.00	41.67	33.33	28.57	31.25	37.50	18.18	16.67	7.41	11.11	15.00	
		(0)	(0)	(0)	(0)	(0)	(10)	(3)	(2)	(5)	(15)	(10)	(3)	(2)	(5)	(15)	
7	<i>Toxocara</i> spp.	6.45	0.00	0.00	0.00	3.33	37.50	11.11	0.00	6.25	25.00	20.00	5.55	0.00	2.22	12.00	
		(2)	(0)	(0)	(0)	(2)	(9)	(1)	(0)	(1)	(10)	(11)	(1)	(0)	(1)	(12)	
8	Trichuris spp.	0.00	0.00	0.00	0.00	0.00	12.50	11.11	14.28	12.50	12.50	5.45	5.55	3.70	4.44	5.00	
		(0)	(0)	(0)	(0)	(0)	(3)	(1)	(1)	(2)	(5)	(3)	(1)	(1)	(2)	(5)	
9	Strongyles	0.00	0.00	0.00	0.00	0.00	29.17	11.11	14.28	12.50	22.50	12.73	5.55	3.70	4.44	9.00	
		(0)	(0)	(0)	(0)	(0)	(7)	(1)	(1)	(2)	(9)	(7)	(1)	(1)	(2)	(9)	
10	Strongyloides	0.00	0.00	0.00	0.00	0.00	8.33	11.11	0.00	6.25	7.50	3.64	5.55	0.00	2.22	3.00	
	spp.	(0)	(0)	(0)	(0)	(0)	(2)	(1)	(0)	(1)	(3)	(2)	(1)	(0)	(1)	(3)	

 Table 14
 Occurrence (%) of different isolates in the faecal samples of diarrhoeic calves

CB = Crossbred, ND = Non-descript

Figures in parenthesis indicate number of positive samples for the respective isolate

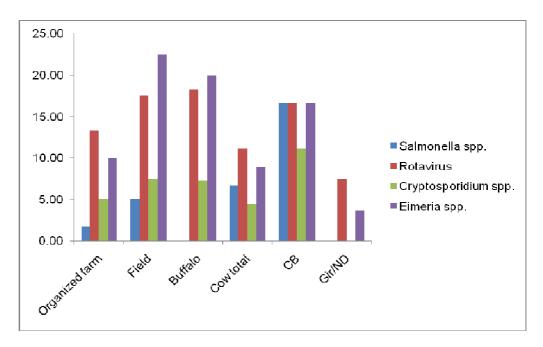


Fig. 9 Occurrence (%) of different isolates in the faecal samples of diarrhoeic calves

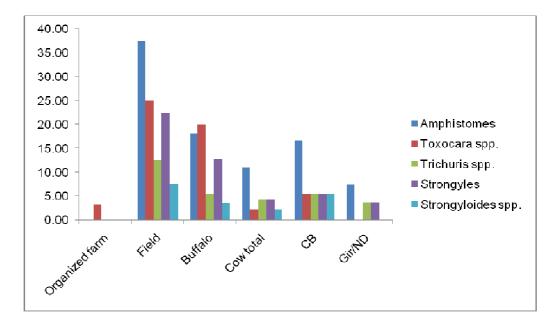


Fig. 10 Occurrence (%) of helminth infestation in the faecal samples of diarrhoeic calves

of *Eimeria* spp. oocyst in the faeces has been reported by Oveido *et al.* (1986), Kaur and Kaur (2008), Jeyakumar *et al.* (2009) and Singh *et al.* (2009c) than present study, whereas lower occurrence has been reported by Aydin *et al.* (2001). The occurrence of coccidiosis at the organized farm was found lower than field condition due to adoption of improved animal husbandry practices. Buffalo and crossbred cow calves were more susceptible to coccidiosis (Figure 9).

The other gastro-intestinal helminth parasites observed in the faeces of diarrhoeic calves (both buffalo and cow calves) were Amphistomes (15.00 %), *Toxocara* spp. (12.00 %), Strongyles (9.00 %), *Trichuris* spp. (5.00 %) and *Strongyloides* spp. (3.00 %) (Table 14). The occurrence of *Toxocara* spp. in diarrhoeic buffalo calves was higher than cow calves and this difference was highly significant statistically (P>0.01) whereas the occurrence of Amphistomes and Strongyles was significantly higher in diarrhoeic buffalo calves than cow calves (P>0.05) (Figure 10). Almost similar finding has been reported by Oveido *et al.* (1986), Rao and Deorani (1988), Dixit and Sahasrabudhe (1995), Aydin *et al.* (2001), Kumar and Verma (2006) and Barua *et al.* (2009). The parasitic infestation was not observed in faeces of diarrhoeic calves of the organized farm except *Toxocara* spp. (3.33 %) (Figure 10). It might be attributed due to regular deworming of the calves at the organized farm.

Several enteropathogens were recovered from the calves with diarrhoea; their relative prevalence varies geographically (Garcia *et al.*, 2000).

Age and sex-wise distribution of occurrence of different isolates in the faecal samples of diarrhoeic calves is depicted in Table 15.

Highest occurrence of *E. coli* was observed in faecal samples of diarrhoeic calves of 0-15 days age group (44.19 %), followed by 16-30 days age group (31.39 %), 31-60 days age group (18.60 %) and 61-120 days age group (5.81 %). Statistically, the data were not equally

distributed in different age groups and the difference was highly significant (P>0.01). It was revealed that the susceptibility of bovine calves for *E. coli* decreased with the advancement of the age (Figure 11). It is in agreement with the reports of Sherwood *et al.* (1983), Frank and Kaneene (1993), Lofstedt *et al.* (1999), Shaheen *et al.* (2002), Gupta *et al.* (2006), Singh *et al.* (2009a), Roy *et al.* (2009) and Kumar *et al.* (2010). The Prevalence of *E. coli* in diarrhoeic calves varies widely depending on the age of the animals (Krogh and Sherwood, 1983).

Highest occurrence of *Salmonella* spp. in diarrhoeic faecal samples of calves was observed in 16-30 days age group (66.67 %), followed by 31-60 days age group (33.33 %). There was no occurrence of *Salmonella* spp. in the faecal samples of diarrhoeic calves of 0-15 days and 61-120 days age group (Figure 12). Radostits *et al.* (2009) reported that diarrhoea caused by *Salmonella* spp. can occur at all ages. Brenner *et al.* (1993) observed that 98.00 % of Salmonellosis cases were found in calves less than three weeks of age whereas Shaheen *et al.* (2002) observed *Salmonella* spp. in faeces of diarrhoeic calves of 7 to 35 days of age. Sharma and Soni (2008) isolated *Salmonella* spp. in the faeces of diarrhoeic calves of 1 to 4 weeks age. In the present investigation, only three isolates of the *Salmonella* spp. were observed in the faecal samples of diarrhoeic calves.

The age-wise occurrence of rotavirus was found maximum in the faecal samples of 0-15 days age group (60.00 %), followed by 16-30 days age group (33.33 %) and 31-60 days age group (6.67 %). Rotavirus was not detected in faecal samples of 61-120 days age diarrhoeic calves. There was highly significant difference in the occurrence of rotavirus in the faecal samples of diarrhoeic calves of different age groups and data were not equally distributed in different age groups (P>0.01). The occurrence

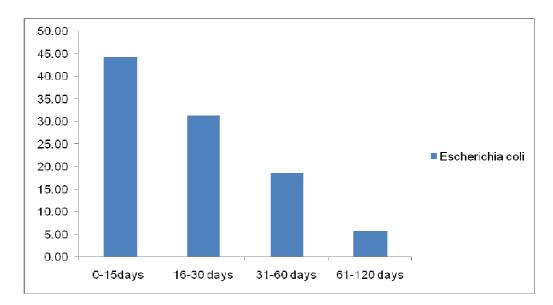


Fig. 11 Age-wise occurrence (%) of *E.coli* isolates in the faecal samples of diarrhoeic calves

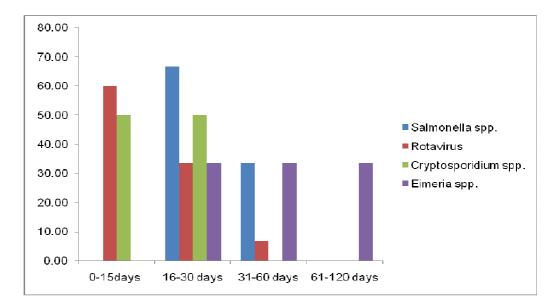


Fig. 12 Age-wise occurrence (%) of different isolates in the faecal samples of diarrhoeic calves

of rotavirus in the faecal samples of diarrhoeic calves was found decreasing with increase in the age of the calves (Figure 12). The finding of present study is in agreement with that of Kaushik *et al.* (1983), Barrandeguy *et al.* (1988), Frank and Kaneene (1993), Lofstedt *et al.* (1999), Jindal *et al.* (2000), Kusumakar (2006) and Singh *et al.* (2009a). Maximum prevalence of rotavirus diarrhoea was observed in 5-21 days old calves (Radostits *et al.*, 2009).

The occurrence of *Cryptosporidium* spp. in diarrhoeic faecal samples of bovine calves was found only in 0-15 days age group (50.00 %) and 16-30 days age group (50.00 %). Cryptosporidium spp. oocysts were not observed in the faecal samples of diarrhoeic calves above 30 days of age (Table 15 and Figure 12). Similar finding has been reported by Korinek and Chroust (1988), Olson *et al.* (1995), Fuente and Luzon (1999), Lofstedt *et al.* (1999), Pandey *et al.* (2009), Prakash *et al.* (2009) and Palanivel *et al.* (2011). The report of Shabhamani and Alahasingari (2006) is in contrast with the present investigation. They observed higher prevalence of *Cryptosporidium* spp. in young calves in the age group of 31-60 days, followed by 1-30 days.

The occurrences of *Eimeria* spp. oocyst were observed in faecal samples of diarrhoeic calves only after the age of 15 days. The occurrence of coccidiosis in 16-30 days, 31-60 days and 61-120 days age group was 33.33 % in each age group, respectively (Figure 12). Almost similar finding has been reported by Manya *et al.* (2007) and Radostits *et al.* (2009). They reported coccidiosis only after three weeks of age in calves. Stoltenow and Vincent (2003) reported that coccidiosis was a seldom problem in young calves.

The occurrence of helminth parasitic ova viz. Amphistomes and *Toxocara* spp. was observed in the faecal samples of diarrhoeic calves of age group 16-30 days and above whereas Strongyles and *Strongyloides*

S.	Name of isolates							Α	ge groups	5						
No.			0-15 days		1	6-30 days			31-60 days		6	1-120 day	'S		Overall	
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
1	Escherichia coli	41.67	47.37	44.19	33.33	28.95	31.39	18.75	18.42	18.60	6.25	5.26	5.81	85.71	86.36	86.00
		(20)	(18)	(38)	(16)	(11)	(27)	(9)	(7)	(16)	(3)	(2)	(5)	(48)	(38)	(86)
2	Salmonella spp.	0.00	0.00	0.00	100.00	0.00	66.67	0.00	100.00	33.33	0.00	0.00	0.00	3.57	2.27	3.00
		(0)	(0)	(0)	(2)	(0)	(2)	(0)	(1)	(1)	(0)	(0)	(0)	(2)	(1)	(3)
3	Rotavirus	50.00	71.43	60.00	50.00	14.28	33.33	0.00	14.28	6.67	0.00	0.00	0.00	14.28	15.91	15.00
		(4)	(5)	(9)	(4)	(1)	(5)	(0)	(1)	(1)	(0)	(0)	(0)	(8)	(7)	(15)
4	Cryptosporidium	33.33	66.67	50.00	66.67	33.33	50.00	0.00	0.00	0.00	0.00	0.00	0.00	5.36	6.82	6.00
	spp	(1)	(2)	(3)	(2)	(1)	(3)	(0)	(0)	(0)	(0)	(0)	(0)	(3)	(3)	(6)
5	<i>Eimeria</i> spp.	0.00	0.00	0.00	44.44	16.67	33.33	22.22	50.00	33.33	33.33	33.33	33.33	16.07	13.64	15.00
		(0)	(0)	(0)	(4)	(1)	(5)	(2)	(3)	(5)	(3)	(2)	(5)	(9)	(6)	(15)
6	Amphistomes	0.00	0.00	0.00	44.44	16.67	33.33	22.22	50.00	33.33	33.33	33.33	33.33	16.07	13.64	15.00
		(0)	(0)	(0)	(4)	(1)	(5)	(2)	(3)	(5)	(3)	(2)	(5)	(9)	(6)	(15)
7	<i>Toxocara</i> spp.	0.00	0.00	0.00	28.57	20.00	25.00	28.57	40.00	33.33	42.86	40.00	41.67	12.50	11.36	12.00
		(0)	(0)	(0)	(2)	(1)	(3)	(2)	(2)	(4)	(3)	(2)	(5)	(7)	(5)	(12)
8	<i>Trichuris</i> spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.0	100.0	100.0	5.36	4.54	5.00
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(3)	(2)	(5)	(3)	(2)	(5)
9	Strongyles	0.00	0.00	0.00	0.00	0.00	0.00	40.00	50.00	44.44	60.00	50.00	55.55	8.93	9.09	9.00
		(0)	(0)	(0)	(0)	(0)	(0)	(2)	(2)	(4)	(3)	(2)	(5)	(5)	(4)	(9)
10	Strongyloides	0.00	0.00	0.00	0.00	0.00	0.00	100.0	100.0	100.0	0.00	0.00	0.00	1.78	4.54	3.00
	spp.	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(2)	(3)	(0)	(0)	(0)	(1)	(2)	(3)

Table 15 Age and sex-wise distribution of occurrence (%) of different isolates in faecal samples of diarrhoeic calves

Figures in parenthesis indicate number of positive samples for respective isolate

 χ^2 value (Age group) *E.coli*=28.14 ; *Salmonella* spp.=3.67; Rotavirus=13.53; *Cryptosporidium* spp.=6.00; *Eimeria* spp.=5.00; Amphistomes=5.00; *Toxocara* spp.=4.67; *Trichuris* spp.=15.00; Strongyles=9.22; *Strongyloides* spp.=9.00

 χ^2 value (Overall sex) *E.coli*=1.16; *Salmonella* spp.=0.33; Rotavirus=0.07; *Cryptosporidium* spp.=0.00; *Eimeria* spp.=0.60; Amphistomes=0.60; *Toxocara* spp.=0.33; *Trichuris* spp.=0.20; Strongyles=0.11; *Strongyloides* spp.=0.33

spp. appeared in the faecal samples of diarrhoeic calves of above 30 days of ages. *Trichuris* spp. eggs were seen in diarrhoeic faeces of calves above 60 days age. It is concluded that the parasitic infestation was observed after 30 days of age in calves except Amphistomes and *Toxocara* spp. which appeared after 15 days of age in diarrhoeic bovine calves (Figure 13). There was significant difference in the occurrence of parasitic infestation (*Trichuris* spp., Strongyles and *Strongyloides* spp.) in different age groups in diarrhoeic calves (P>0.05). Similar finding has been reported by Oveido *et al.* (1986), Aydin *et al.* (2001) and Kumar and Verma (2006).

The sex-wise distribution occurrence of different isolates in the faecal samples of diarrhoeic calves is also presented in Table 15. The occurrence of different isolates in the faecal samples of diarrhoeic calves was found almost uniform in male and female calves (Figure 14). Statistically, there was equal distribution of different isolates in both sexes. In other words, there was no significant difference in the occurrence of different organisms in male and female diarrhoeic calves (P<0.05). It revealed that calves of both sexes were equally susceptible to different causative agents of diarrhoea. It is in agreement with the finding of Chauhan and Singh (1996) and Roy *et al.* (2009).

Season-wise occurrence (%) of different isolates in faecal samples of diarrhoeic calves is depicted in Table 16. The occurrence of *E. coli* in the faecal samples of diarrhoeic calves was found maximum during rainy season (95.00 %), followed by summer (84.61 %) and winter season (83.78 %). Statistically, the occurrence of *E. coli* in faecal samples of diarrhoeic calves was not equally distributed in different seasons (Figure 15) and the difference was highly significant (P>0.01). Acha *et al.* (2004), Tikoo *et al.* (2009), Panchasara *et al.* (2009) and Roy *et al.* (2009) also

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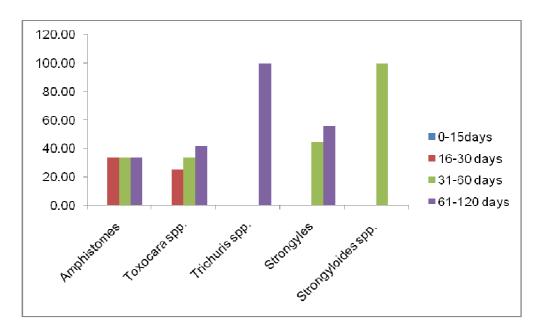


Fig. 13 Age-wise occurrence (%) of helminth infestation in the faecal samples of the diarrhoeic calves

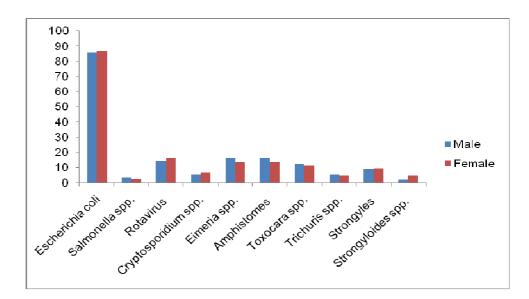


Fig. 14 Sex-wise occurrence of different isolates in the faecal sample of diarrhoeic calves

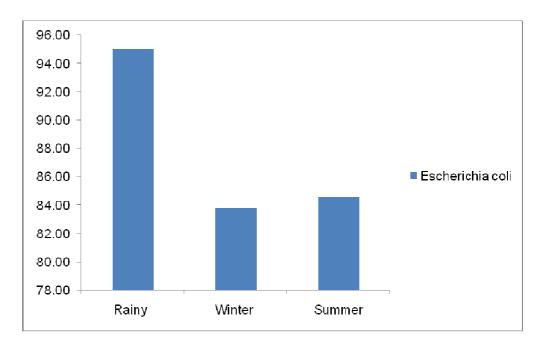


Fig. 15 Season-wise occurrence (%) of *E.coli* isolates in the faecal sample of diarrhoeic calves

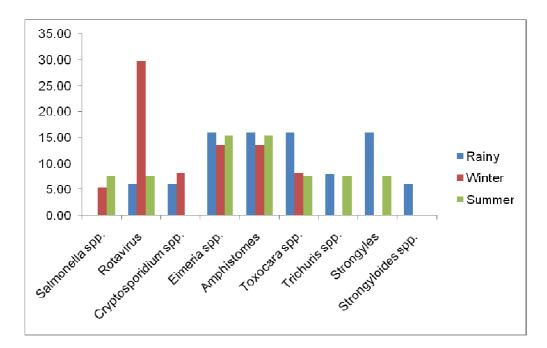


Fig. 16 Season-wise occurrence (%) of different isolates in the faecal sample of diarrhoeic calves

reported higher occurrence of colibacillosis during rainy season whereas Wani *et al.* (2005) reported outbreak of *E. coli* diarrhoea in winter season.

The season-wise occurrence of *Salmonella* spp. in the faecal samples of diarrhoeic calves was 5.40 % and 7.69 % in winter and summer season, respectively. No isolate of *Salmonella* spp. was observed in the faecal samples of calves showing diarrhoea during rainy season (Figure 16). It might be attributed to the fact that there were only three cases of diarrhoea showing *Salmonella* spp. in the diarrhoeic faecal samples. Acha *et al.* (2004) and Tikoo *et al.* (2009) reported occurrence of *Salmonella* spp. in the faecal samples of diarrhoeic calves during rainy season.

The highest occurrence of the rotavirus was observed in the faecal samples of diarrhoeic calves during winter season (29.73 %), followed by summer (7.69 %) and rainy season (6.00 %) (Table 16 and Figure 16). Statistically, the difference in the occurrence of rotavirus in the faecal samples of diarrhoeic calves was found highly significant in different seasons (P>0.01). Similar finding has been reported by (1996),et al. Chauhan and Singh Gulati (1997)and Nourmohammadzadeh et al. (2011).

The occurrence of *Cryptosporidium* spp. in the faecal samples of diarrhoeic calves was observed 8.11 % during winter and 6.00 % during rainy season. Cryptosporidium spp. was not observed in faecal samples of diarrhoeic calves during summer season (Figure 16). Statistically, there was no significant difference in the occurrence of *Cryptosporidium* spp. oocyst in the faecal samples of diarrhoeic calves in three seasons of the year (P<0.05). Gopalnath (1997) and Prakash also reported that seasonal differences et al. (2009)of cryptosporidiosis were not marked. Sahu and Maiti (2009) reported highest incidence of cryptosporidiosis during winter season followed by rainy season.

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S.	Name of isolates		Season	
No.		Rainy	Winter	Summer
1	Escherichia coli	95.00	83.78	84.61
		(44)	(31)	(11)
2	Salmonella spp.	0.00	5.40	7.69
		(0)	(2)	(1)
3	Rotavirus	6.00	29.73	7.69
		(3)	(11)	(1)
4	Cryptosporidium	6.00	8.11	0.00
	spp.	(3)	(3)	(0)
5	<i>Eimeria</i> spp.	16.00	13.51	15.38
		(8)	(5)	(2)
6	Amphistomes	16.00	13.51	15.38
		(8)	(5)	(2)
7	Toxocara spp.	16.00	8.11	7.69
		(8)	(3)	(1)
8	Trichuris spp.	8.00	0.00	7.69
		(4)	(0)	(1)
9	Strongyles	16.00	0.00	7.69
		(8)	(0)	(1)
10	Strongyloides	6.00	0.00	0.00
	spp.	(3)	(0)	(0)

Table 16Season- wise occurrence (%) of different isolates in
faecal samples of diarrhoeic calves

Figures in parenthesis indicate number of positive samples for respective isolate χ^2 value *E.coli*=19.27 ; *Salmonella* spp.=2.00; Rotavirus=11.20;

Cryptosporidium spp.=3.00; *Eimeria* spp.=3.60; Amphistomes=3.60; *Toxocara* spp.=6.50; *Trichuris* spp.=6.18; Strongyles=12.67; *Strongyloides* spp.=6.00

The occurrence of *Eimeria* spp. oocyst were seen in the faecal samples of diarrhoeic calves in rainy (16.00 %), summer (15.38 %) and winter (13.51 %) season almost equally (Figure 16). Statistically, the distribution of occurrence of *Eimeria* spp. in faecal samples of diarrhoeic calves was found equal among three seasons of the year and there was no significant difference in susceptibility of calves to

Eimeria spp. in different seasons (P<0.05). Similar finding has been reported by Manya *et al.* (2007).

The occurrence of helminth ova in the faecal samples of diarrhoeic calves was found highest during rainy season in most of the isolates (Table 16 and Figure 16). There was significant difference in the occurrence of parasitic infestation (*Toxocara* spp., *Trichuris* spp., Strongyles and *Strongyloides* spp.) in calves in different seasons (P>0.05). Similar finding has been reported by Dixit and Sahasrabudhe (1995), Jeyakumar *et al.* (2009) and Gupta *et al.* (2011).

The parity of dam-wise occurrence (%) of different isolates in faecal samples of diarrhoeic calves is depicted in Table 17.

The occurrence of *E.coli* was highest in the faecal samples of the diarrhoeic calves of first parity dams (96.55 %), followed by second parity dams (94.12 %), fifth and above parity dams (80.00 %), third parity dams (79.17 %) and fourth parity dams (73.33%) (Figure 17). There was significance difference in the occurrence of *E. coli* diarrhoea in calves of dams of different parities (P>0.05).

The occurrence of *Salmonella* spp. was maximum (5.88 %) in faecal samples of diarrhoeic calves of second parity dams, followed by third parity dams (4.17 %) and first parity dams (3.45 %). The occurrence of rotavirus in faecal samples of diarrhoeic calves of first, second, third, fourth and fifth and above parity dams was 17.24 %, 17.65 %, 12.50 %, 13.33 % and 13.33 %, respectively. The occurrence of rotavirus in the faecal samples was higher in diarrhoeic calves of first and second parity dams than the diarrhoeic calves of higher parity dams. The occurrence of *Cryptosporidium* spp. in the diarrhoeic faecal samples was highest in the calves of first parity dams (10.34 %) than calves of second (5.88 %), third (4.17 %), fourth (6.67 %) and fifth and above (0.00 %) parity dams (Table 17 and Figure 18).

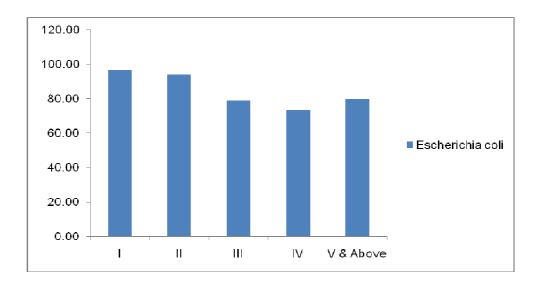


Fig. 17 Parity of dam-wise occurrence (%) of *E. coli* isolates in the faecal sample of diarrhoeic calves

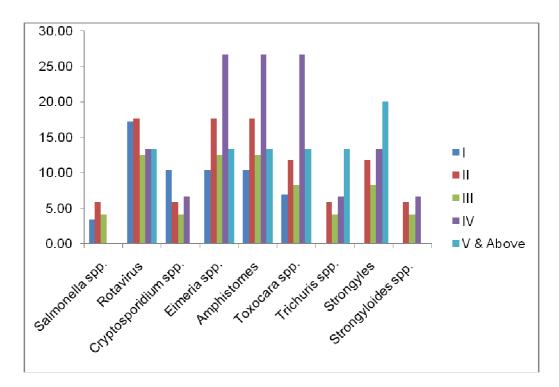


Fig. 18 Parity of dam-wise occurrence (%) of different isolates in the faecal sample of diarrhoeic calves

S.No.	Name of isolates		Pa	arity of d	am	
		I	II	III	IV	V & above
1	Escherichia coli	96.55 (28)	94.12 (16)	79.17 (19)	73.33 (11)	80.00 (12)
2	Salmonella spp.	3.45 (1)	5.88 (1)	4.17 (1)	0.00 (0)	0.00 (0)
3	Rotavirus	17.24 (5)	17.65 (3)	12.50 (3)	13.33 (2)	13.33 (2)
4	Cryptosporidium spp.	10.34 (3)	5.88 (1)	4.17 (1)	6.67 (1)	0.00 (0)
5	<i>Eimeria</i> spp.	10.34 (3)	17.65 (3)	12.50 (3)	26.67 (4)	13.33 (2)
6	Amphistomes	10.34 (3)	17.65 (3)	12.50 (3)	26.67 (4)	13.33 (2)
7	<i>Toxocara</i> spp.	6.90 (2)	11.76 (2)	8.33 (2)	26.67 (4)	13.33 (2)
8	Trichuris spp.	0.00 (0)	5.88 (1)	4.17 (1)	6.67 (1)	13.33 (2)
9	Strongyles	0.00 (0)	11.76 (2)	8.33 (2)	13.33 (2)	20.00 (3)
10	Strongyloides spp.	0.00 (0)	5.88 (1)	4.17 (1)	6.67 (1)	0.00 (0)

Table 17Parity of dam-wise occurrence (%) of differentisolates in faecal samples of diarrhoeic calves

Figures in parenthesis indicate number of positive samples for respective isolate χ^2 value *E.coli*=10.86; *Salmonella* spp.=2.00; Rotavirus=2.00;

Cryptosporidium spp. =9.50; *Eimeria* spp.=0.67; Amphistomes=0.67; *Toxocara* spp.=1.33; *Trichuris* spp.=2.00; Strongyles=2.67; *Strongyloides* spp.=2.00

It is concluded that most of the above mentioned isolates were found highest in the faecal samples of diarrhoeic calves of first or second parity of dams. Logan *et al.* (1981) reported that first and second parity cows have significantly lower levels of immunoglobulins in their colostrum than higher parity cows. It might be the reason for higher occurrence of *E. coli Salmonella* spp., rotavirus and *Cryptosporidium* spp. in the faecal samples of diarrhoeic calves of first or second parity dams. Svensson *et al.* (2003) reported that quality of colostrum increases with the lactation number of dam which explains the higher risk of diseases in calves born to first lactation cows.

The parity of dam-wise occurrence of other parasites viz. *Eimeria* spp., Amphistomes, *Toxocara* spp., *Trichuris* spp., Strongyles and *Strongyloides* in the faecal samples of were not found higher in diarrhoeic calves of first and second parity dam (Table 17 and Figure 18). It might be attributed to the fact that the parasitic infestation was not observed in the diarrhoeic calves in the first 15 days of life, hence there was no role of the colostrum and parity of the dam did not play in role in the occurrence of parasitic infestation.

4.3 Clinical and haemato-biochemical characterization of calf diarrhoea

The values of clinical and haemato-biochemical parameters were determined in diarrhoeic as well as healthy calves. In diarrhoeic calves, *E. coli* as a single isolate was observed in 76 faecal samples whereas mixed infection of bacteria viz. virus, protozoa and/or other parasites was observed in 24 faecal samples. Thus, diarrhoeic calves with colibacillosis and mixed infections were characterized separately.

4.3.1 General and clinical profile

The diarrhoeic calves were found dull and depressed with reduced appetite. The calves had a clinical evidence of mild to moderate diarrhoea with semisolid to watery faeces having offensive odour. The faeces were yellow-white to greenish in a colour, some times blackish, mucoid and even blood stained. The tail and perineum region of the calf patients was soiled with faeces. The frequency of defaecation was found increased. Similar findings have been reported by Bicknell and Noon (1993), Baber *et al.* (2000), Kumar and Mandial (2002), Shaheen *et al.* (2002), Asati *et al.* (2008), Tikoo and Soodan (2009) and Kumar *et al.* (2010).

The mean \pm S.E. values of clinical parameters of diarrhoeic and healthy calves are depicted in Table 18. The mean rectal temperature (°F), mean heart rate (per minute), mean respiration rate (per minute), mean faecal consistency score, mean clinical depression score and mean clinical dehydration score in healthy calves was 101.28 \pm 0.93, 110.20 \pm 0.88, 17.00 \pm 0.78, 0.00 \pm 0.00, 0.00 \pm 0.00 and 0.00 \pm 0.00, respectively whereas corresponding values in colibacillosis affected diarrhoeic calves were 102.40 \pm 0.63, 117.43 \pm 0.82, 22.10 \pm 0.69, 2.55 \pm 0.07, 1.84 \pm 0.09 and 1.80 \pm 0.08, respectively.

It was revealed that there was slight increase in the mean rectal temperature, heart rate and respiration rate in the diarrhoeic calves affected with *E. coli* infection (Figure 19) but the increase in above parameters was non significant, statistically whereas the faecal consistence score, clinical depression score and clinical dehydration score in colibacillosis affected diarrhoeic calves was found significantly higher than healthy calves (Figure 20). Thus, in colibacillosis affected diarrhoeic calves, the clinical manifestations as semisolid to watery consistency of faeces, mild to moderate depression, weak and disorganized suckling, mild to moderate dehydration, eyes not recess or slightly recess in to orbit and loss of skin elasticity were observed. Similar findings have also been reported by Bicknell and Noon (1993), Kumar and Mandial (2002), Tikoo and Soodan (2009), Asati *et al.* (2010), Arora *et al.* (2010), Kumar *et al.* (2010). Sridhar *et al.* (1988),

Aly *et al.* (1996) and Devkate *et al* (2010) reported increase in body temperature in colibacillosis affected calves



Plate 17 Diarrhoeic buffalo calf



Plate 18 Diarrhoeic cow calf



Plate 19 Different types of diarrhoeic faeces of calves



Plate 20 Collection of diarrhoeic faecal samples for cultural examination

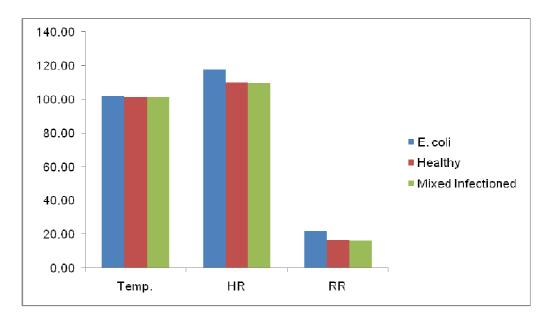


Fig. 19 Comparison of temperature (°F), heart rate (per minute) and respiration rate (per minute) in colibacillosis affected diarrhoeic calves, mixed enteric infection and healthy calves

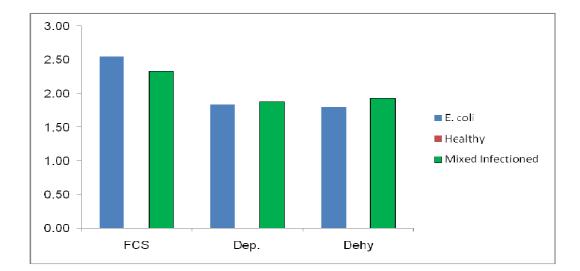


Fig. 20 Comparison of faecal consistency score, clinical depression and dehydration scores in colibacillosis affected diarrhoeic calves, mixed enteric infection and healthy calves

S.	Parameters	Diarrhoei	c calves	Healthy
No.		Colibacillosis (n=76)	mixed infection (n=24)	calves (n=10)
1	Rectal temperature (ºF)	102.40±0.63 ^b	101.20±0.71 ^a	101.28±0.93 ^{ab}
2	Heart rate (per minute)	117.43±0.82 ^b	109.42±0.63 ^a	110.20±0.88 ^{ab}
3	Respiration rate (per minute)	22.10±0.69 ^b	16.08±0.92 ^ª	17.00±0.78 ^{ab}
4	Faecal consistency score	2.55±0.07 [°]	2.33±0.16 ^b	0.00±0.00 ^a
5	Clinical depression score	1.84±0.09 ^b	1.87±0.90 [°]	0.00±0.00 ^a
6	Clinical dehydration score	1.80±0.08 ^b	1.92±0.79 [°]	0.00±0.00 ^a

Table 18Mean ±S.E. values of clinical parameters of diarrhoeiccalves and healthy control

Rows with different superscript differ significantly

whereas Kalita *et al.* (2000) and Fernandes *et al.* (2009) observed subnormal temperature. Sridhar *et al.* (1988), Verma *et al.* (1995), Kalita *et al.* (2000) and Devkate *et al.* (2010) reported increase in respiration rate, heart rate and pulse. Diarrhoea leads to excess loss of intestinal fluid resulting in severe dehydration (Demigne *et al.*, 1980).

There was no significant difference in the mean rectal temperature, mean heart rate and mean respiration rate in healthy calves and diarrhoeic calves affected with mixed infections (Figure 19) but mean faecal consistency score (2.33 ± 0.16), mean clinical depression score (1.87 ± 0.90) and clinical dehydration score (1.92 ± 0.79) was found significantly higher than healthy calves (Figure 20). Thus in diarrhoeic calves affected with mixed infections of bacteria, virus and/or parasites, semisolid to watery faeces, mild to

moderate depression, weak and disorganized suckling and moderate dehydration with eyes slightly recess in to orbit were the main clinical findings. It is in agreement with the reports of Booth and Naylor (1987), Talukdar (1996), Halmandge *et al.* (2005), Das *et al.* (2006), Manya *et al.* (2007) and Singh *et al.* (2009).

4.3.2 Haemato-biochemical profile

The mean \pm S.E. values of haematological parameters of diarrhoeic calves and healthy calves are depicted in Table 19. There was significant increase in the mean Hb value (g/dl) of diarrhoeic calves affected with *E.coli* infection (12.40±0.45). The mean Hb value (g/dl) in healthy calves was found 11.30±0.83 (Figure 21). It is in agreement with the report of Manoiu *et al.* (1972), Bijwal and Misra (1987), Sridhar *et al.* (1988), Baber *et al.* (2000), Asati *et al.* (2008), Roy *et al.* (2009), Fernandes *et al.* (2009b), Tikoo and Soodan (2009) and Kumar *et al.* (2010).

The mean PCV (%) value in the diarrhoeic calves with colibacillosis (46.80±1.52) was found significantly higher than healthy calves (36.40±1.62) (Figure 22). It is in agreement with the earlier reports (Zepperitz and Seidal, 1982; Sridhar *et al.*, 1988; Baber *et al.*, 2000; Kumar and Mandial, 2002; Kaur *et al.*, 2006; Asati *et al.*, 2008; Roy *et al.*, 2009; Fernandes *et al.*, 2009b; Tikoo and Soodan, 2009; and Kumar *et al.*, 2010). Increase in PCV value in colibacillosis affected calves was apparently due to haemo-concentration associated with dehydration and hypovolaemia.

The mean value of TEC $(10^6/\mu l)$ in diarrhoeic calves affected with colibacillosis (7.90±0.42) was significantly higher than healthy control (6.89±0.70) (Figure 21). Similar finding has been reported by Sridhar *et al.* (1988), Tikoo and Soodan (2009), Fernandes *et al.* (2009b), Kumar *et al.* (2010) and Mir *et al.* (2010).

The mean MCV (fl), MCH (pg) and MCHC (g/dl) values in healthy and colibacillosis affected diarrhoeic calves were 59.16 ± 0.57 and 53.36 ± 0.68 ; 18.66 ± 0.94 and 16.40 ± 0.22 ;

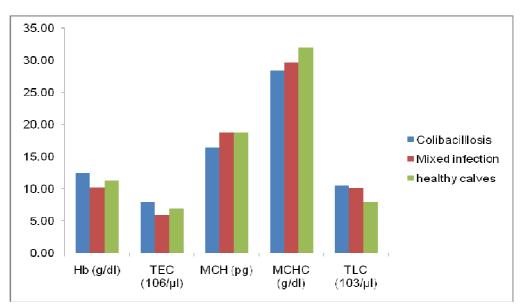


Fig. 21 Comparison of haematological parameters in colibacillosis, mixed enteric infection and healthy calves

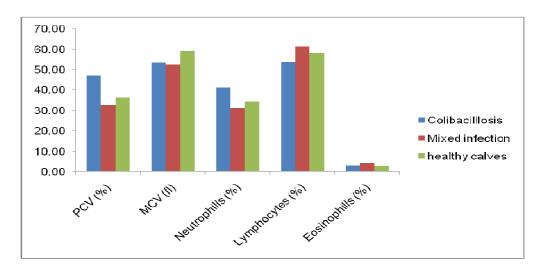


Fig. 22 Comparison of haematological parameters in colibacillosis, mixed enteric infection and healthy calves

and 32.02 ± 7.60 and 28.50 ± 6.28 , respectively. The values of these parameters were found significantly lower in calves affected with *E.coli* infection than healthy calves (Figure 21and 22). It is in agreement reports of Asati *et al.* (2008) and Mir *et al.* (2010).

The mean value of TLC $(10^3/\mu I)$ in *E. coli* infected diarrhoeic calves (10.42 ± 2.82) was significantly higher than healthy calves (7.86 ± 2.12) (Figure 21). Similar finding has been reported by Sridhar *et al.* (1988), Fernandes *et al.* (2009b), Kumar *et al.* (2010), Asati *et al.* (2010) and Mir *et al.* (2010).

The mean value of neutrophils (%) in the healthy control and colibacillosis affected diarrhoeic calves was 34.16±0.54 and 41.11±0.42, respectively. The mean value of neutrophils in colibacillosis affected diarrhoeic calves was significantly higher than healthy calves. The mean lymphocyte (%) in colibacillosis affected diarrhoeic calves (53.52±0.46) was significantly lower than healthy calves (58.18±0.62). There was marked neutrophilia and lymphopenia in diarrhoeic calves affected with colibacillosis (Figure 22). Similar finding has been reported by Bandyopadhyay *et al.* (2008). The finding of Asati *et al.* (2008) was in contrary with the present study.

There was non significant difference in mean eosinophils (%), mean basophils (%) and mean monocytes (%) in calf patients affected with diarrhoea due to *E. coli* infection and healthy calves. Similar finding has been reported by Asati *et al.* (2008).

The mean value of Hb (gm/dl) in diarrhoeic calves, affected with mixed enteric infections was 10.20±0.67 and was found significantly lower than healthy calves (Figure 21). The mean PCV (%) in diarrhoeic calves affected with mixed enteric infections (32.60±2.62) was significantly lower than healthy calves (Figure 22). The mean value of TEC $(10^6/\mu l)$ in diarrhoeic calves affected with mixed infection (5.88±0.62) was significantly lower than healthy calves (Figure 21). The mean MCV (fl) in

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S.	Parameters	Diarrhoe	eic calves	Healthy
No.		Colibacillosi s	mixed infection	calves (n=10)
		(n=76)	(n=24)	
1	Hb (g/dl)	12.40±0.45 ^c	10.20±0.67 ^a	11.30±0.83 ^b
2	PCV (%)	46.80±1.52 ^c	32.60±2.62 ^a	36.40±1.62 ^b
3	TEC (10 ⁶ /μΙ)	7.90±0.42 ^c	5.88±0.62 ^a	6.89±0.70 ^b
4	MCV (fl)	53.36±0.68 ^b	52.48±0.82 ^a	59.16±0.57 ^c
5	МСН (рд)	16.40±0.22 ^a	18.70±0.74 ^b	18.66±0.94 ^b
6	MCHC (g/dl)	28.50±6.28 ^a	29.62±7.18 ^b	32.02±7.60 ^c
7	TLC (10 ³ /μl)	10.42±2.82 ^c	10.12±2.34 ^b	7.86±2.12 ^a
8	DLC			
	(i) Neutrophills (%)	41.11±0.42 ^c	31.25±0.43 ^a	34.16±0.54 ^b
	(ii) Eosinophills (%)	2.88±0.26 ^a	4.02±0.24 ^b	2.84±0.47 ^a
	(iii) Basophills (%)	0.33±0.16 ^a	0.34±0.12 ^a	0.34±0.21 ^a
	(iv) Lymphocytes (%)	53.52±0.46 ^a	61.22±0.76 ^c	58.18±0.62 ^b
	(v) Monocytes (%)	2.56±0.24 ^a	3.06±0.66 ^b	2.68±0.36 ^{ab}

Table 19Mean ± S.E. values of haematological parameters of
diarrhoeic calves and healthy control

Rows with different superscript differ significantly

diarrhoeic calves having mixed enteric infection (52.48±0.82) was significantly lower than healthy calves (Figure 22). There was non significant difference in MCH (pg) in diarrhoeic calves having mixed enteric infection and healthy calves (Figure 21). The MCHC value (g/dl) in calves affected with mixed enteric infection was significantly lower (29.62±7.18) than healthy calves (Figure 21). The TLC (10^3 /µI) was found significantly higher (10.12 ± 2.34) than healthy calves (Figure 21). Marked neutropenia ($31.25\pm0.43\%$), increase in lymphocytes ($61.22\pm0.76\%$) and eosinophilia (4.02 ± 0.24) was observed in calves affected with mixed enteric infections (Table 19 and Figure 22). Almost similar finding has been reported by Nooruddin *et al.* (1987), Devi *et al.*

(2000), Shobhamani *et al.* (2007), Ghoke *et al.* (2009) and Sahu and Maiti (2011) in different viral and parasitic infestations.

The mean \pm S.E. values of biochemical parameters of diarrhoeic calves and healthy control calves is depicted in Table 20. The mean value of serum sodium (mmol/L) in healthy calves and colibacillosis affected diarrhoeic calves was 128.30 \pm 1.24 and 115.40 \pm 1.21, respectively. There was significant decrease in the serum sodium in the colibacillosis affected calves against the normal values of healthy control (Figure 23). It is in agreement with the finding of the earlier workers (Sridhar *et al.*, 1988; Groutides and Michell, 1990; Michell *et al.*, 1992; Aly *et al.*, 1996; Bali *et al.*, 2000; Kumar and Mandial, 2002; Roy and Fernandes, 2007; Asati *et al.*, 2010 and Mir *et al.*, 2010). Hyponatraemia occurs as a result of excessive secretion of the sodium ions by intestinal villus cells which are lost through the intestinal tract (Radostits *et al.*, 2009).

The mean value of serum potassium (mmol/L) in healthy and colibacillosis affected diarrhoeic calves was 4.16 ± 0.16 and 5.1 ± 0.14 , respectively. There was significant increase in the serum potassium in diarrhoeic calves affected with colibacillosis than healthy calves (Figure 24). Similar finding has been reported by Sridhar *et al.* (1988), Michell *et al.* (1992), Aly *et al.* (1996), Walker *et al.* (1998), Bali *et al.* (2000), Kumar and Mandial (2002), Roy and Fernandes (2007), Asati *et al.* (2008), Roy *et al.* (2009), Kumar *et al.* (2010) and Mir *et al.* (2010). Hyperkalaemia observed in the present investigation might be due to increased retention of K⁺ ion by kidney and increased tubular reabsorption of K⁺ ion in response to acidosis and also due to cellular damage. Movement of K⁺ ion

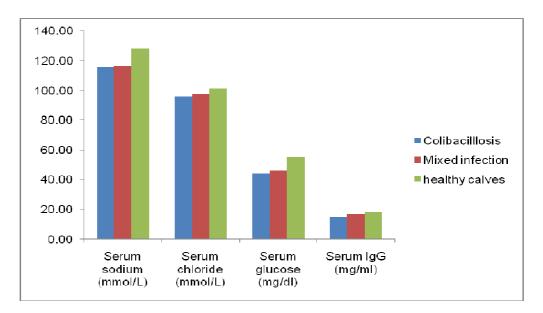


Fig. 23 Comparison of biochemical parameters in colibacillosis, mixed enteric infection and healthy calves

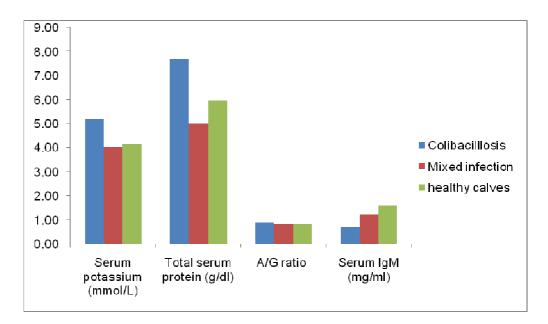


Fig. 24 Comparison of biochemical parameters in colibacillosis, mixed enteric infection and healthy calves

from intracellular to extracellular fluid also contributed to increased serum potassium concentration (Tasker, 1991).

The mean value of serum chloride (mmol/L) in healthy control and diarrhoeic calves affected with colibacillosis was 101.40 ± 0.62 and 96.20 ± 0.46 , respectively. There was significant decrease in the serum chloride concentration in colibacillosis affected diarrhoeic calves than healthy calves (Figure 23). Similar finding has been reported by Dubey (1989), Maach *et al.* (1992) and Aly *et al.* (1996). The finding of Sridhar *et al.* (1988), Shrivastava *et al.* (2001), Roy and Fernandes (2007) and Mir *et al.* (2010) was in contrary to the present investigation. They observed hyperchloraemia in diarrhoeic calves. Hypochloraemia occurred as result of increased loss of chloride ion in the intestinal tract during diarrhoea and failure of gastric H⁺ and Cl⁻ ion to be reabsorbed by the villus of small intestine (Radostits *et al.*, 2009).

The total serum protein (g/dl) in healthy calves and colibacillosis affected diarrhoeic calves was 5.96±0.18 and 7.68±0.27, respectively. There was significant increased in the total serum protein in colibacillosis affected diarrhoeic calves (Figure 24). It is in agreement with the finding of Walker *et al.* (1998), Bali *et al.* (2000), Kumar and Mandial (2002), Asati *et al.* (2008), Roy *et al.* (2009), Tikoo and Soodan (2009) and Kumar *et al.* (2010). Aly *et al.* (1996) reported decrease in total serum protein in colibacillosis. Increase in total serum protein was due to hypovolaemia, haemo-concentration and reduced glomerular filtration rate (Walker *et al.*, 1998).

The A/G ratio in present investigation in the healthy control and *E.coli* infected diarrhoeic calves was 0.85 ± 0.02 and 0.88 ± 0.03 , respectively. There was significant increase in the A/G ratio in diarrhoeic calves affected with colibacillosis (Figure 24). It is in agreement with the finding of Sridhar *et al.* (1988), Constable *et al.* (1996) and Walker *et al.* (1998). Decrease in A/G ratio in colibacillosis has been observed by Asati *et al.* (2008) and Mir *et al.* (2010).

The mean value of serum glucose (mg/dl) in healthy and colibacillosis affected diarrhoeic calves was 54.98±0.90 and 43.80±1.80, respectively. There was marked decrease in serum glucose values in diarrhoeic calves showing *E. coli* in the faeces (Figure 23). This finding is supported by the reports of earlier workers (Groutides and Michell, 1990; Maach *et al.*, 1992; Aly *et al.*, 1996; Roy *et al.*, 2009, and Asati *et al.*, 2010). Hypoglycemia in colibacillosis affected diarrhoeic calves was due to anorexia, decreased intestinal absorption of glucose and reduced rate of conversion of lactic acid to glucose (Morris *et al.*, 1985).

The mean serum IgG (mg/ml) and IgM (mg/ml) values in healthy and colibacillosis affected diarrhoeic calves were 18.16±1.74 and 1.60±0.02; and 14.80±1.82 and 0.70±0.01, respectively. There was significant decrease in the serum IgG and serum IgM values in colibacillosis affected diarrhoeic calves (Figure 23 and 24). Gay *et al.* (1965) reported association of colibacillosis with the deficiency of plasma immunoglobulins. Thronton *et al.* (1972) and Manoiu *et al.* (1972) observed low gamma-globulin in diarrhoeic and dehydrated calves. The risk of development of infectious diseases is greater in calves in which there has been failure of passive transfer of maternal immunoglobulins (Gay, 1983). Gutzwiller (2002) reported that serum IgG concentration was not correlated with diarrhoea. Chand and Pandey (2010) reported that suckling method of colostrum feeding predisposes calves for development of diarrhoea due to lower level of IgG (14.61±2.62 mg/ml) in such calves.

In diarrhoeic calves affected with mixed infection of bacteria, virus and/or parasites, the mean serum sodium (116.40 \pm 1.42 mmol/L), serum potassium (4.04 \pm 0.12 mmol/L), serum chloride (97.62 \pm 0.68 mmol/L), total serum protein (5.02 \pm 0.21 g/dl), A/G ratio (0.83 \pm 0.04), serum glucose

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S. NO.	Parameters	Diarrhoei	c calves	Healthy calves
		Colibacillosis (n=76)	mixed infection (n=24)	(n=10)
1	Serum sodium (mmol/L)	115.40±1.21 ^ª	116.40±1.42 ^b	128.30±1.24 ^c
2	Serum potassium (mmol/L)	5.18±0.14°	4.04±0.12a	4.16±0.16 ^b
3	Serum chloride (mmol/L)	96.20±0.46 ^a	97.62±0.68 ^b	101.40±0.62 ^c
4	Total serum protein (g/dl)	7.68±0.27 ^c	5.02±0.21 ^ª	5.96±0.18 ^b
5	A/G ratio	0.88±0.03 ^c	0.83±0.04 ^a	0.85±0.02 ^b
6	Serum glucose (mg/dl)	43.80±1.80 ^a	46.28±1.20 ^b	54.98±0.90 ^c
7	Serum immunoglobulins			
	(i) Serum IgG (mg/ml)	14.80±1.82 ^a	16.64±1.72 ^b	18.16±1.74 [°]
	(ii) Serum IgM (mg/ml)	0.70±0.01 ^a	1.24±0.03 ^b	1.60±0.02 ^c

Table 20Mean ± S.E. values of biochemical parameters of
diarrhoeic calves and healthy control

Rows with different superscript differ significantly

(46.28 \pm 1.20 mg/dl), serum IgG (16.64 \pm 1.72mg/ml) and serum IgM (1.24 \pm 0.03mg/ml) were found significantly decreased (Table 20 and Figure 23 and 24). Similar findings have been reported by Lewis *et al.* (1975), Shobhamani *et al.* (2007), Manya *et al.* (2007) and Sahu and Maiti (2011) for viral and parasitic infestations.

4.4 Antibiotic sensitivity pattern of *E. coli* isolates

Antibiotic sensitivity pattern of bacterial isolates pave way in suggesting the treatment and control of the disease. It also helps in preventing development of resistant strains against the drugs which may be either intermediate or resistant to the bacteria in antibiotic sensitivity test. It aids the clinician to go for a direct approach of treatment with drugs to which bacteria are highly susceptible.

The percent antibiotic sensitivity pattern of diarrhoeic calves *E.coli* isolates is presented in Table 21.

The antibiotic sensitivity pattern of *E.coli* isolated from the faecal samples of the diarrhoeic calves showed 100.00 % sensitivity to ofloxacin, norfloxacin and chloramphenicol followed by ciprofloxacin and nalidixic acid (98.84 % each), kanamycin (94.19 %), enrofloxacin (90.70%), gentamicin (81.39 %), doxycycline (79.07 %), streptomycin (65.12 %), trimethoprim (58.14 %), sulphadimidine (48.84 %), cotrimoxazole (46.51 %), oxytetracycline (41.86 %), tetracycline (38.37 %) and erythromycin (6.98 %).

The above data suggested that *E.coli* was highly sensitive to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, enrofloxacin and gentamicin. This might be attributed to the fact that these antibiotics were seldom used in treatment of enteric infections in bovine calves. High degree of sensitivity of *E.coli* to these antibiotics have also been reported by Hussain and Saikia (2000), Sharma *et al.* (2004), Bandyopadhyay *et al.* (2008), Dubal *et al.* (2009), Devkate *et al.* (2010), Kumar *et al.* (2010), Pan and Bhatia (2010) and Gupta *et al.* (2011).

The *E. coli* isolates showed maximum resistance against erythromycin (93.02 %), followed by tetracycline (40.70 %),

S.	Name of antibiotic	Escheri	<i>chia coli</i> isolate	s (n=86)
No.	disc	Resistant	Intermediate	Sensitive
1	Ofloxacin	0.00	0.00	100.0
		(0)	(0)	(86)
2	Ciprofloxacin	1.16	0.00	98.84
		(1)	(0)	(85)
3	Enrofloxacin	6.98	2.32	90.70
		(6)	(2)	(78)
4	Norfloxacin	0.00	0.00	100.
		(0)	(0)	(86)
5	Oxytetracycline	34.88	23.25	41.86
		(30)	(20)	(36)
6	Sulphadimidine	33.72	17.44	48.84
		(29)	(15)	(42)
7	Cotrimoxazole	30.23	23.25	46.51
		(26)	(20)	(40)
8	Erythromycin	93.02	0.00	6.98
		(80)	(0)	(6)
9	Gentamicin	0.00	18.60	81.39
		(0)	(16)	(70)
10	Trimethoprim	20.93	20.93	58.14
		(18)	(18)	(50)
11	Doxycycline	9.30	11.63	79.07
		(8)	(10)	(68)
12	Chloramphenicol	0.00	0.00	100.0
		(0)	(0)	(86)
13	Nalidixic acid	0.00	1.16	98.84
		(0)	(1)	(85)
14	Kanamycin	0.00	5.81	94.19
		(0)	(5)	(81)
15	Streptomycin	32.56	2.32	65.12
		(28)	(2)	(56)
16	Tetracycline	40.70	20.93	38.37
		(35)	(18)	(33)

Table 21Percent antibiotic sensitivity pattern of diarrhoeiccalves Escherichia coli isolates

Figures in parenthesis indicate number of *E. coli* isolates

Particulars		Multiple resistance (No. antibiotics)							
	0	1	2	3	4	5	6	7	8
Resistance	0	44	12	1	4	8	9	7	1
Intermediate	9	55	7	9	5	1	0	0	0

Table 22Multiple antibiotic resistance of diarrhoeic Escherichia coli isolates

oxytetracycline (34.88 %), sulphadimidine (33.72 %), streptomycin (32.56 %), cotrimoxazole (30.23 %), trimethoprim (20.93 %), doxycycline (9.30 %), enrofloxacin (6.98 %) and ciprofloxacin (1.16 %). Similar findings have been reported by Kaura *et al.* (1991), Chattopadhyay *et al.* (2003), Hariharan *et al.* (2004), Tikoo and Soodan (2009), Sharma *et al.* (2009) and Pan and Bhatia (2010).

Multiple antibiotic resistance of diarrhoeic *E. coli* isolates is depicted in Table 22. Out of 86 *E. coli* isolates, 30 (34.88 %) showed multiple antibiotic resistance for 3 to 8 antibiotics. Multiple resistance pattern has been recorded among *E.coli* isolates from diarrhoeic calves by Shah and Jhala (1990), Kaura *et al.* (1991) and Sharma *et al.* (2009) also. Multiple resistant pattern is possibly due to indiscriminate use of antibiotic therapy which exerts a selection pressure and leads to the development of multiple drug resistance among different strains of bacteria.

4.5 Evaluation of therapeutic regimens

Total five therapeutic regimens were evaluated for their efficacies in calf diarrhoea caused by *E. coli*. Each of the drug regimen was tried separately on a group of 15 diarrhoeic calves. Efficacy of therapeutic trial was evaluated on the basis of improvement in the clinical profile and return of altered haemato-biochemical parameters towards normalcy (i.e. at par to the values in the healthy control calves). The drug trial(s) showing faster recovery (4th day after treatment) in all the altered values of clinical and haemato-biochemical parameters was established as the best therapeutic regimen.

The mean \pm S.E. values of clinical and haemato-biochemical parameters on 0, 4th and 7th day of treatment in different groups is presented in Table 23 to 27 and Response of different therapeutic regimens on mean \pm S.E. values for different clinical and haemato-biochemical parameters of diarrhoeic calves on 0, 4th and 7th day after treatment is presented in Figure 25 to 40.

4.5.1 Ofloxacin and ORS therapy (Group I)

Mean \pm S.E values of clinical and haemato-biochemical parameters of group I diarrhoeic calves are depicted in Table 23. There was non significant change in the mean rectal temperature, heart rate and respiration rate of diarrhoeic calves before and after treatment. The slightly elevated values of these parameters returned to normal on day 4th after treatment. Significant improvement was observed in the faecal consistency score (2.47±0.75 to 1.00±0.01), clinical depression score (1.80±0.66 to 0.40±0.42) and clinical dehydration score (1.73±0.62 to 0.33±0.38) from 0 day to 4th day after treatment but these parameters returned to complete normalcy (0.00±0.00) on day 7th after treatment.

Like-wise haematological parameters also showed improvement on 7th day (Hb 11.80±0.34 gm/dl; PCV 37.70±0.82 %; TEC 7.08±0.76 x 10^{6} /µl; and TLC 8.09±2.85 x 10^{3} /µl) as compared to 4th day after treatment (Hb 12.00±0.86 gm/dl; PCV 39.80±1.04 %; TEC 7.22±0.82 x 10^{6} /µl; and TLC 8.48±2.80 x 10^{3} /µl) and 0 day (Hb 12.30±1.21 gm/dl; PCV 46.60±2.48 %; TEC 7.92±0.92 x 10^{6} /µl; and TLC 10.32±3.20 x 10^{3} /µl). It was found that there was significant difference in the values of Hb, PCV, TEC and TLC on day 0 (before treatment), 4th and 7th (after treatment).

As regards, effect of the therapy on the biochemical profile of diarrhoeic calves of group I, the significantly higher values of serum potassium (5.22 ± 0.26 mmol/L), total serum protein (7.72 ± 0.80 gm/dI) and A/G ratio (0.88 ± 0.02) reduced towards normalcy (serum potassium 4.26 ± 0.09 mmol/L; total serum protein 6.30 ± 0.32 ; and A/G ratio 0.86 ± 0.01) by day 7th post treatment. On the other hand, as a result of therapy the significantly lowered values of serum sodium (114.80 ± 1.62 mmol/L), serum chloride (96.80 ± 1.52 mmol/L) and serum glucose (43.60 ± 1.28 mg/dI) as recorded on day 0, increased towards normalcy

Table 23	Mean ± S.E. values of clinical and haemato-biochemical parameters
	of Group I diarrhoeic calves (n = 15)

S. No.	Parameters	Before treatment	After treatment		ANOVA	
		0 day	4 th day	7 th day	SEm±	CD (P=0.05)
1	Rectal temperature	102.20±0.83	101.80±0.73	101.40±0.13	0.060	NS

	(°F)					
2	Heart rate (per minute)	114.60±1.42	113.20±1.12	111.00±0.96	0.273	NS
3	Respiration rate (per min.)	22.40±0.92	18.13±0.64	17.87±0.38	0.366	NS
4	Faecal consistency score	2.47±0.75	1.00±0.01	0.00±0.00	0.001	0.003
5	Clinical depression score	1.80±0.66	0.40±0.42	0.00±0.00	0.007	0.002
6	Clinical dehydration score	1.73±0.62	0.33±0.38	0.00±0.00	0.007	0.002
7	Hb (g/dl)	12.30±1.21	12.00±0.86	11.80±0.34	0.032	0.093
8	PCV (%)	46.60±2.48	39.80±1.04	37.70±0.82	0.033	0.093
9	TEC (10 ⁶ /μl)	7.92±0.92	7.22±0.82	7.08±0.76	0.053	0.148
10	TLC (10 ³ /µl)	10.32±3.20	8.48±2.80	8.09±2.85	0.007	0.020
11	Serum sodium (mmol/L)	114.80±1.62	122.70±0.84	124.80±0.40	0.098	0.275
12	Serum potassium (mmol/L)	5.22±0.26	4.96±0.12	4.26±0.09	0.004	0.011
13	Serum chloride (mmol/L)	96.80±1.52	98.02±0.78	99.00±0.42	0.079	0.222
14	Total serum protein (g/dl)	7.72±0.80	6.98±0.45	6.30±0.32	0.067	0.188
15	A/G ratio	0.88±0.02	0.87±0.01	0.86±0.01	0.001	0.0040
16	Serum glucose (mg/dl)	43.60±1.28	48.78±1.22	54.10±1.10	0.040	0.112

(serum sodium 124.80±0.40 mmol/L; serum chloride 99.00±0.42 mmol/L; and serum glucose 54.10±1.10 mg/dl) by day 7th post treatment. However, on day 4th post therapy most of the aforesaid parameters did not have marked improvement (Table 23). It was revealed that the diarrhoeic calves in group I showed complete recovery only on day 7th after treatment.

4.5.2 Ofloxacin and parenteral fluid (Ringer's lactate) with sodium bicarbonate therapy (Group II)

The mean \pm S.E. values of clinical and haemato-biochemical parameters of group II diarrhoeic calves is depicted in Table 24.

The rectal temperature, heart rate and respiration rate of the diarrhoeic calves before and after treatment did not show significant variation but slightly increased values of these clinical parameters returned to normal on day 4^{th} post treatment. Significant improvement was observed in the faecal consistency score (2.40±0.44 to 0.40±0.20), clinical depression score (1.87±0.36 to 0.20±0.32) and clinical dehydration score (1.80±0.46 to 0.20±0.38) from day 0 before treatment to day 4^{th} after treatment. Complete normalcy (0.00±0.00) in the above parameters was observed on day 7th after treatment.

The mean ± S.E. values of haematological parameters viz. Hb (g/dl), PCV (%), TEC $(10^6/\mu l)$ and TLC $(10^3/\mu l)$ on day 0 in group II was 12.50 ± 0.37 , 48.40 ± 1.35 , 7.89±0.96 and 10.36 ± 3.12 , respectively. There was improvement in these parameters on day 4th (Hb 12.00 ± 0.38 gm/dl; PCV 38.10 ± 0.94 %; TEC $7.59\pm0.84 \times 10^6/\mu l$; and TLC $8.32\pm2.82 \times 10^3/\mu l$) but these parameters returned to complete normal (Hb 11.60 ± 0.28 gm/dl; PCV 36.60 ± 0.87 %; TEC $6.92\pm0.62 \times 10^6/\mu l$; and TLC $8.02\pm2.80 \times 10^3/\mu l$) on day 7th after therapy. The differences in Hb, PCV, TEC and TLC were found significant before and after treatment.

There was significant improvement in the biochemical parameters of diarrhoeic calves from day 0 to day 4th after treatment viz. serum

S. No.	Parameters	Before treatment	After treatment				AVOI
		0 day	4 th day	7 th day	SEm±	CD (P=0.05)	
1	Rectal temperature (°F)	101.80±0.37	101.40±0.24	101.40±0.14	0.098	NS	
2	Heart rate (per minute)	117.20±0.70	112.20±0.64	111.13±0.32	0.495	NS	
3	Respiration rate (per min.)	21.67±0.64	17.27±0.42	17.00±0.32	0.382	NS	
4	Faecal consistency score	2.40±0.44	0.40±0.20	0.00±0.00	0.001	0.003	
5	Clinical depression score	1.87±0.36	0.20±0.32	0.00±0.00	0.001	0.003	
6	Clinical dehydration score	1.80±0.46	0.20±0.38	0.00±0.00	0.001	0.003	
7	Hb (g/dl)	12.50±0.37	12.00±0.38	11.60±0.28	0.058	0.164	

Table 24Mean ± S.E. values of clinical and haemato-biochemical parametersof Group II diarrhoeic calves (n = 15)

-				1		
8	PCV (%)	48.40±1.35	38.10±0.94	36.60±0.87	0.038	0.106
9	TEC (10 ⁶ /μl)	7.89±0.96	7.59±0.84	6.92±0.62	0.064	0.179
10	TLC (10 ³ /µl)	10.36±3.12	8.32±2.82	8.02±2.80	0.008	0.023
11	Serum sodium (mmol/L)	113.70±1.54	125.30±0.80	127.80±0.62	0.567	1.596
12	Serum potassium (mmol/L)	5.26±0.28	4.70±0.14	4.22±0.10	0.004	0.012
13	Serum chloride (mmol/L)	95.46±1.64	98.14±0.82	100.14±0.43	0.091	0.255
14	Total serum protein (g/dl)	7.74±0.94	6.62±0.56	6.18±0.48	0.006	0.018
15	A/G ratio	0.89±0.02	0.87±0.02	0.85±0.01	0.003	0.007
16	Serum glucose (mg/dl)	43.86±1.62	46.12±1.72	52.92±1.24	0.043	0.122

sodium (113.70±1.54 to 125.30±0.80 mmol/L), serum potassium (5.26±0.28 to 4.70±0.14 mmol/L), serum chloride (95.46±1.64 to 98.14±0.82 mmol/L), total serum protein (7.74±0.94 to 6.62±0.56 g/dl), A/G ratio (0.89±0.02 to 0.87±0.02) and serum glucose (43.86± 1.62 to 46.12±1.72 mg /dl). These parameters showed further improvement on day 7th after therapy and achieved normal values (serum sodium 127.80±0.62 mmol/L; serum potassium 4.22±0.10 mmol/L; serum chloride 100.14±0.43 mmol/L; total serum protein 6.18±0.48 g/dl; A/G ratio 0.85±0.01; and serum glucose 52.92±1.24 mg/dl) at par with the healthy control. It was concluded that in diarrhoeic calves of treatment group II (ofloxacin and intravenous ringer's lactate with sodium bicarbonate therapy), there was marked improvement in the most of the clinical and haemato-biochemical parameters on day 4th after treatment but complete clinical recovery was achieved on day 7th after treatment.

4.5.3 Ofloxacin, ORS and Shisham leaves powder therapy (Group III)

The Mean \pm S.E. values of clinical and haemato-biochemical parameters of groups III diarrhoeic calves is presented in Table 25.

There was non significant variation in the rectal temperature, heart rate and respiration rate of the diarrhoeic calves under treatment in group III on day 0 (before treatment) and day 4^{th} and 7^{th} (after treatment). There was marked improvement in the faecal consistency score (2.60±0.80 to 0.00±0.00), clinical depression score (1.87±0.28

to 0.13 ± 0.26) and clinical dehydration score $(1.80\pm0.62 \text{ to } 0.13\pm0.38)$ from day 0 (before treatment) to day 4th (after treatment). The faecal consistency became completely normal whereas clinical depression and dehydration score were little bit elevated on day 4th and became normal (0.00±0.00) on day7th after treatment.

The mean \pm S.E. values of haematological parameters viz. Hb (g/dl), PCV (%), TEC (10⁶/µl) and TLC (10³/µl) on day 0 in group III was

S. No.	Parameters	Before treatment 0 day	After treatment		ANOVA	
			4 th day	7 th day	SEm±	CD (P=0.05)
1	Rectal temperature (°F)	102.00±0.64	101.84±0.52	101.33±0.57	0.058	NS
2	Heart rate (per minute)	116.27±0.82	110.47±0.46	110.47±0.28	0.485	NS
3	Respiration rate (per min.)	21.07±0.72	17.57±0.30	17.40±0.28	0.300	NS
4	Faecal consistency score	2.60±0.80	0.00±0.00	0.00±0.00	0.001	0.004
5	Clinical depression score	1.87±0.28	0.13±0.26	0.00±0.00	0.001	0.003
6	Clinical dehydration score	1.80±0.62	0.13±0.38	0.00±0.00	0.001	0.002
7	Hb (g/dl)	12.10±0.64	11.80±0.52	11.30±0.40	0.025	0.070
8	PCV (%)	47.60±1.42	38.90±1.08	37.70±0.84	0.039	0.109
9	TEC (10 ⁶ /μl)	7.88±0.89	7.08±0.90	6.90±0.46	0.057	0.161
10	TLC (10 ³ /µl)	10.52±3.02	8.30±2.98	7.80±2.82	0.008	0.023
11	Serum sodium (mmol/L)	115.90±1.64	126.20±0.91	128.30±0.82	0.115	0.322
12	Serum potassium (mmol/L)	5.08±0.22	4.88±0.14	4.22±0.08	0.004	0.012
13	Serum chloride (mmol/L)	96.20±1.64	100.62±0.82	100.90±0.64	0.091	0.257
14	Total serum protein (g/dl)	7.64±0.80	6.02±0.48	6.00±0.36	0.006	0.018
15	A/G ratio	0.88±0.02	0.85±0.02	0.85±0.01	0.001	0.004
16	Serum glucose (mg/dl)	43.42±1.52	50.26±1.44	54.12±1.04	0.045	0.128

Table 25Mean ± S.E. values of clinical and haemato-biochemical parametersof Group III diarrhoeic calves (n = 15)

12.10±0.64, 47.60±1.42, 7.88±0.89 and 10.52±3.02, respectively. There was improvement in these parameters on day 4th (Hb 11.80±0.52 gm/dl; PCV 38.90±1.08 %; TEC 7.08±0.90 x 10⁶/µl; and TLC 8.30±2.98 x10³/µl) but these parameters returned to complete normal (Hb 11.30±0.40 gm/dl; PCV 37.70±0.84 %; TEC 6.90±0.46 x 10⁶/µl;

and TLC 7.80 \pm 2.82 x10³/µI) on day 7th after therapy. There was significant difference in the values of Hb, PCV, TEC and TLC before and after treatment.

The values of serum sodium on day 0 (before treatment), day 4th and 7th (after treatment) were 115.90 \pm 1.64, 126.20 \pm 0.91 and 128.30 \pm 0.82 mmol/L, respectively. The values for serum potassium were 5.08 \pm 0.22, 4.88 \pm 0.14 and 4.22 \pm 0.08 mmol/L on day 0, 4th and 7th, respectively. The serum chloride concentration in group III on day 0, 4th and 7th was 96.20 \pm 1.64, 100.62 \pm 0.82 and 100.90 \pm 0.64, respectively whereas total serum protein was 7.64 \pm 0.80, 6.02 \pm 0.48 and 6.00 \pm 0.36 g/dl, respectively. The value of A/G ratio on day 0, 4th and 7th was 0.88 \pm 0.02, 0.85 \pm 0.02 and 0.85 \pm 0.01, respectively. The serum glucose values in the diarrhoeic calves in group III on day 0, 4th and 7th were 43.42 \pm 1.52, 50.26 \pm 1.44 and 54.12 \pm 1.04, respectively. There was significant difference in the values of the biochemical parameters before and after treatment. Some of the parameters (serum chloride, total serum protein and A/G ratio) became almost normal on day 4th after treatment but serum sodium, serum potassium and serum glucose could be normal on day 7th after treatment.

In response to ofloxacin, ORS and Shisham leaves powder therapy, faecal consistency became normal on day 4th. The other clinical and haemato-biochemical parameters were found near to normalcy on day 4th but these parameters were completely normal on day 7th after treatment.



Plate 21 Shisham tree (Delbergia sissoo) in college campus



Plate 22 Drying of Shisham leaves in shadow

4.5.4 Ofloxacin, parenteral fluid (Ringer's lactate) with sodium bicarbonate and Shisham leaves powder therapy (Group IV)

The mean \pm S.E. values of clinical and haemato-biochemical parameters of group IV diarrhoeic calves is depicted in Table 26.

Rectal temperature, heart rate, and respiration rate showed non-significant differences before and after treatment. The slightly elevated values of these parameters were completely normal on day 4th post treatment. The values of faecal consistency score, clinical depression score, and clinical dehydration score were 2.60±0.68, 1.87±0.74 and 1.80±0.28, respectively before treatment. These parameters became completely normal (0.00±0.00) on day 4th post therapy with ofloxacin, parenteral fluid with sodium bicarbonate and shisham leaves powder. Thus complete clinical recovery has been observed in this group on day 4th after treatment.

There was a significant difference in Hb, PCV, TEC and TLC before and after treatment (Table 26). The Hb (g/dl) of diarrhoeic calves before treatment was 12.40±0.38 which became normal (11.30±0.30) on 4th day after treatment. The PCV (%) of diarrhoeic calves before treatment was 45.80±1.28 which returned to normal (36.40±0.92 %) on day 4th post therapy in group IV. The TEC ($10^6/\mu$ I) and TLC ($10^3/\mu$ I) was 7.94±0.96 and 10.50±2.90, respectively before treatment. These parameters were found normal (6.90±0.82 and 7.90±2.76, respectively) on 4th day after treatment. There was non significant difference in the values of haematological parameters on day 4th and day 7th after treatment.

The values of biochemical parameters viz. serum sodium (mmol/L), serum potassium (mmol/L), serum chloride (mmol/L), total serum protein (g/dl),

Table 26Mean ± S.E. values of clinical and haemato-biochemical parametersof Group IV diarrhoeic calves (n = 15)

Sr. No.	Parameters	Before treatment	After treatment		ANOVA	
		0 day	4 th day	7 th day	SEm±	CD (P=0.05)

1	Rectal temperature (°F)	101.80±0.48	101.30±0.30	101.24±0.28	0.057	NS
2	Heart rate (per minute)	118.13±0.64	110.47±0.28	110.13±0.28	0.714	NS
3	Respiration rate (per min.)	21.67±0.58	17.07±0.42	17.00±0.56	0.395	NS
4	Faecal consistency score	2.60±0.68	0.00±0.00	0.00±0.00	0.001	0.004
5	Clinical depression score	1.87±0.74	0.00±0.00	0.00±0.00	0.009	0.003
6	Clinical dehydration score	1.80±0.28	0.00±0.00	0.00±0.00	0.009	0.002
7	Hb (g/dl)	12.40±0.38	11.40±0.32	11.30±0.30	0.063	0.178
8	PCV (%)	45.80±1.28	36.40±0.92	36.00±0.84	0.040	0.114
9	TEC (10 ⁶ /μl)	7.94±0.96	6.90±0.82	6.88±0.32	0.066	0.185
10	TLC (10 ³ /µl)	10.50±2.90	7.90±2.76	7.82±2.05	0.008	0.023
11	Serum sodium (mmol/L)	115.20±1.54	128.00±1.10	128.30±0.74	0.115	0.324
12	Serum potassium (mmol/L)	5.16±0.28	4.17±0.16	4.12±0.12	0.004	0.012
13	Serum chloride (mmol/L)	96.18±1.62	100.70±0.80	101.40±0.58	0.092	0.258
14	Total serum protein (g/dl)	7.62±0.82	5.96±0.46	5.96±0.48	0.006	0.017
15	A/G ratio	0.88±0.02	0.85±0.02	0.84±0.01	0.003	0.008
16	Serum glucose (mg/dl)	43.90±1.90	54.24±1.36	54.96±1.24	0.045	0.126

A/G ratio and serum glucose (mg/dl) before treatment were 115.20 \pm 1.54, 5.16 \pm 0.28, 96.18 \pm 1.62, 7.62 \pm 0.82, 0.88 \pm 0.02 and 43.90 \pm 1.90,respectively. The decreased values of serum sodium on day 0 was found normal (128.00 \pm 1.10 mmol/L) on day 4th. The serum potassium concentration (mmol/L) was also normal (4.17 \pm 0.16) on day 4th. The serum chloride (mmol/L) showed significant increase on day 4th after treatment and was almost normal (100.70 \pm 0.80). The total serum protein (g/dl) decreased non-significantly and became normal (5.96 \pm 0.46) on 4th day post therapy. Serum glucose (mg/dl) increased and returned to normal (54.24 \pm 1.36) on 4th day in response to the treatment with ofloxacin, parenteral fluid with sodium bicarbonate and shisham leaves powder. The A/G ratio on day 4th was found normal (0.85 \pm 0.02). There was non significant difference in the values of biochemical parameters on day 4th and day 7th after treatment.

It was revealed that all the clinical and haemato-biochemical parameters returned to normal on day 4th after treatment in group IV in response to ofloxacin, parenteral fluid (Ringer's lactate) with sodium bicarbonate and shisham leaves powder therapy.

4.5.5 Ofloxacin and Shisham leaves powder therapy (Group V)

The mean \pm S.E. values of clinical and haemato-biochemical parameters of group V diarrhoeic calves is depicted in Table 27.

There was non-significant difference in rectal temperature, heart rate, and respiration rate before and after treatment. The slightly increased values of these parameters returned to normalcy on 7th day after treatment. The faecal consistency score, clinical depression score and clinical dehydration score in group V diarrhoeic calves was 2.53±0.52, 1.80±0.82 and 1.80±0.48, respectively on day 0. The values of faecal consistency score, clinical depression score and clinical depression score and clinical dehydration score in day 0. The values of faecal consistency score, clinical depression score and clinical dehydration score on day 4th and 7th after treatment were 0.30±0.00

Table 27Mean ± S.E. values of clinical and haemato-biochemical parametersof Group V diarrhoeic calves (n = 15)

Sr.ParametersBefore treatmentAfter treatmentANOVA
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		0 day	4 th day	7 th day	SEm±	CD (P=0.05)
1	Rectal temperature (°F)	101.80±0.34	101.64±0.28	101.60±0.30	0.080	NS
2	Heart rate (per minute)	117.53±0.32	115.33±0.38	112.60±0.36	0.407	NS
3	Respiration rate (per min.)	22.13±0.66	20.27±0.44	18.93±0.38	0.231	NS
4	Faecal consistency score	2.53±0.52	0.30±0.00	0.00±0.00	0.001	0.003
5	Clinical depression score	1.80±0.82	0.60±0.38	0.13±0.04	0.001	0.003
6	Clinical dehydration score	1.80±0.48	0.93±0.20	0.20±0.06	0.001	0.003
7	Hb (g/dl)	12.30±0.56	12.10±0.52	12.00±0.44	0.011	0.032
8	PCV (%)	44.60±1.44	42.30±1.12	40.40±0.98	0.039	0.111
9	TEC (10 ⁶ /μl)	7.90±0.94	7.28±0.84	7.32±0.85	0.025	0.072
10	TLC (10 ³ /µl)	10.40±3.24	9.48±2.92	8.56±2.94	0.009	0.025
11	Serum sodium (mmol/L)	115.30±1.92	117.40±1.43	121.60±1.00	0.109	0.306
12	Serum potassium (mmol/L)	5.12±0.36	4.90±0.42	4.78±0.34	0.006	0.016
13	Serum chloride (mmol/L)	96.26±1.82	97.82±1.20	98.86±0.82	0.090	0.254
14	Total serum protein (g/dl)	7.66±0.86	7.42±0.64	6.74±0.52	0.007	0.019
15	A/G ratio	0.87±0.02	0.86±0.02	0.86±0.01	0.001	0.002
16	Serum glucose (mg/dl)	43.82±2.32	45.20±2.16	49.24±1.80	0.043	0.120

and 0.00 ± 0.00 , 0.60 ± 0.38 and 0.13 ± 0.04 and 0.93 ± 0.20 and 0.20 ± 0.06 , respectively. It is apparent that faecal consistency score became completely normal on day 7th post therapy whereas clinical depression and dehydration scores were almost normal on day 7th after treatment.

The difference in the values of haematological parameters was significant on 0 day (before treatment) and 4^{th} and 7^{th} day (after treatment). The Hb, PCV, TEC and TLC in diarrhoeic calves of group V was 12.30 ± 0.56 , 44.60 ± 1.44 , 7.90 ± 0.94 and 10.40 ± 3.24 , respectively before treatment. These parameters were recorded as

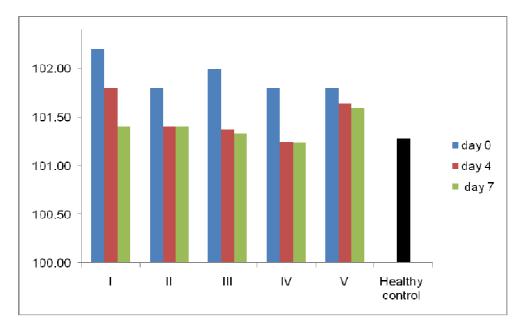
 12.00 ± 0.44 , 40.40 ± 0.98 , 7.32 ± 0.85 and 8.56 ± 2.94 , respectively on 7th day post therapy which were still higher than corresponding value in the healthy control calves.

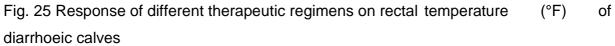
The value of serum sodium (mmol/L) on 0 day, 4th day and 7th day was 115.30 \pm 1.92, 117.40 \pm 1.43 and 121.60 \pm 1.00, respectively. The corresponding values of serum potassium (mmol/L) and serum chloride (mmol/L) were 5.12 \pm 0.36 and 96.26 \pm 1.82; 4.90 \pm 0.42 and 97.82 \pm 1.20; and 4.78 \pm 0.34 and 98.86 \pm 0.82, respectively. The serum glucose (mg/dl) in diarrhoeic calves of group V was 43.82 \pm 2.32, 45.20 \pm 2.16 and 49.24 \pm 1.80 on day 0 (before treatment), 4th and 7th (after treatment), respectively. The values of total serum protein (g/dl) and A/G ratio on day 0(before treatment), 4th and 7th (after treatment) were 7.66 \pm 0.86 and 0.87 \pm 0.02; 7.42 \pm 0.64 and 0.86 \pm 0.02; and 6.74 \pm 0.52 and 0.86 \pm 0.01, respectively. It was revealed that the values of biochemical parameters in diarrhoeic calves group V could hardly reach the normalcy on day 7th post therapy. There was significant difference in the values of biochemical parameters on day 4th and day 7th after treatment.

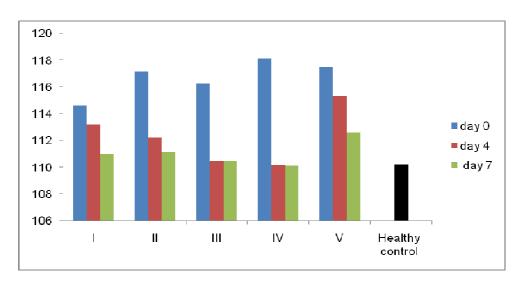
The changes in different parameters in response to five therapeutic regimens on 0 (before treatment), 4th and 7th day (after treatment) in diarrhoeic calves are shown in Figure 25 to 40.

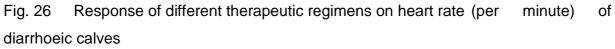
The interpretation of the results in respect to evaluation of five therapeuticregimens is based on analysis of data among different treatment groups using ANOVA.Itwasclearlyindicatedthatrecovery

259









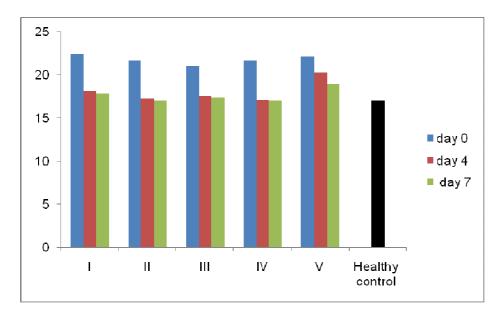
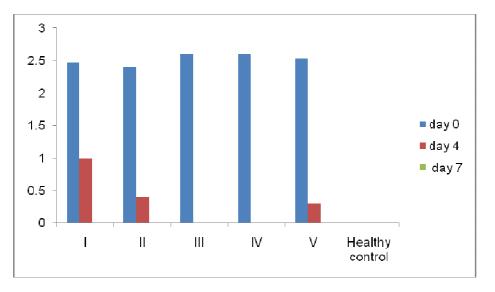
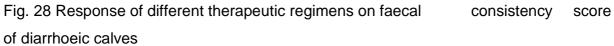


Fig. 27 Response of different therapeutic regimens on respiration rate (per minute) of diarrhoeic calves





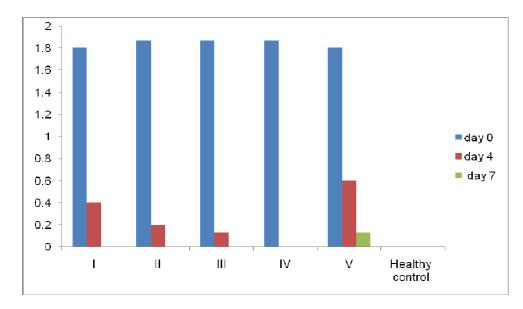
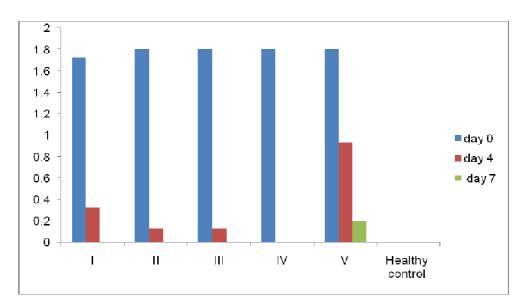
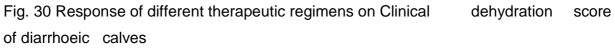


Fig. 29 Response of different therapeutic regimens on Clinical depression score of diarrhoeic calves





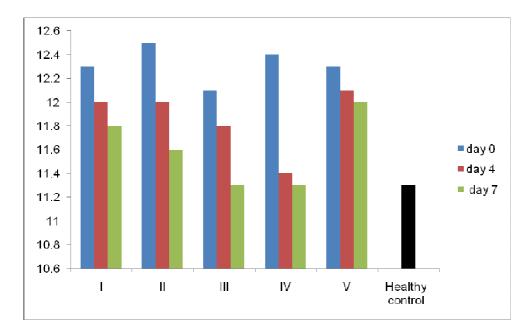


Fig. 31 Response of different therapeutic regimens on Hb (g/dl) values of diarrhoeic calves

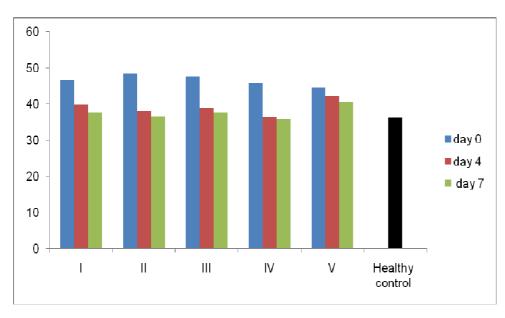
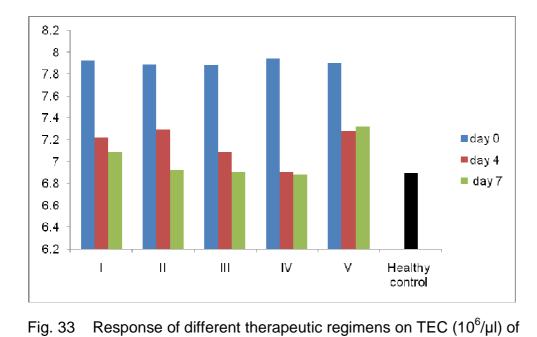


Fig. 32 Response of different therapeutic regimens on PCV (%) of calves

diarrhoeic



calves

diarrhoeic

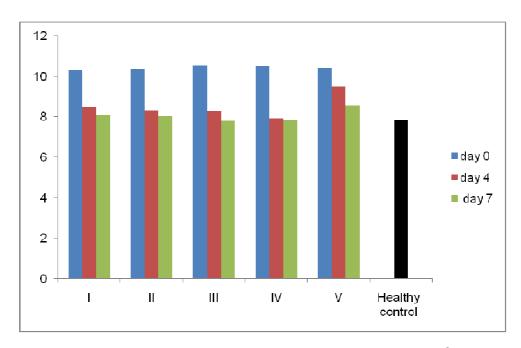


Fig. 34 Response of different therapeutic regimens on TLC (10³/µI) of diarrhoeic calves

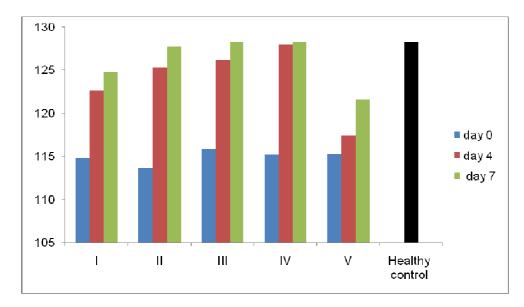


Fig. 35 Response of different therapeutic regimens on serum sodium (mmol/L) values of diarrhoeic calves

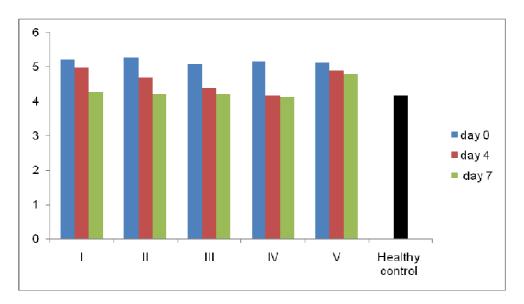


Fig. 36 Response of different therapeutic regimens on serum potassium (mmol/L) values of diarrhoeic calves

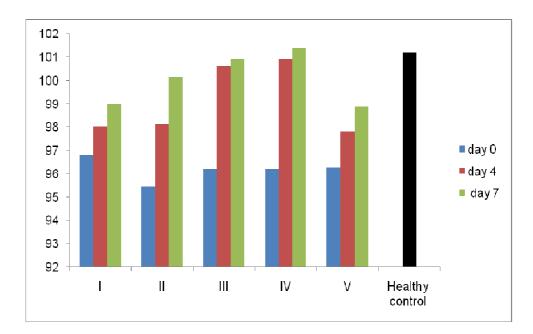


Fig. 37 Response of different therapeutic regimens on serum chloride (mmol/L) values of diarrhoeic calves

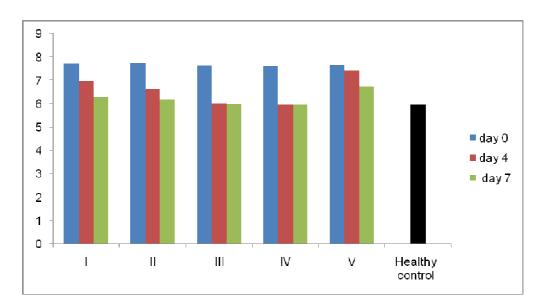


Fig. 38 Response of different therapeutic regimens on total serum protein (g/dl) values of diarrhoeic calves

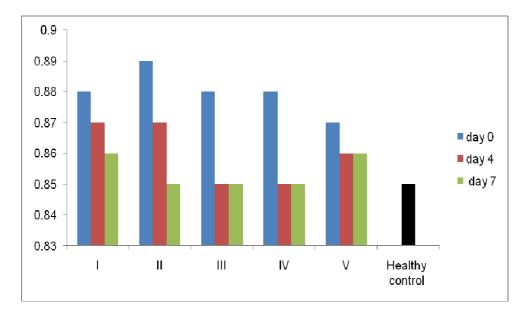


Fig. 39 Response of different therapeutic regimens on A/G ratio of diarrhoeic calves

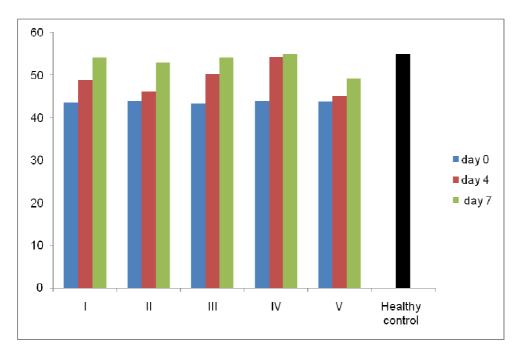


Fig. 40 Response of different therapeutic regimens on serum glucose (mg/dl) values of diarrhoeic calves

from the ailment was observed in all the five treatment groups but there was variation in different groups as far as faster recovery was concern. Out of group I (ofloxacin with ORS) and II (ofloxacin and parenteral fluid with sodium bicarbonate), diarrhoeic calves in group II showed better recovery than group I (Figure 25 to 40). Like-wise in between group III (Ofloxacin, ORS and Shisham leaves powder) and IV (ofloxacin, parenteral fluid with sodium carbonate and shisham leaves powder), diarrhoeic calves in group IV showed complete recovery from the disease on day 4th post treatment than group III (Figure 25 to 40). Better clinical recovery in group II diarrhoeic calves than group I diarrhoeic calves as well as group IV diarrhoeic calves than group III diarrhoeic calves, indicated that parenteral fluid therapy as supporting treatment was superior in terms of faster recovery than oral rehydration fluids. It is attributed due to the fact oral rehydration therapy provided slow rehydration as compared to parenteral fluid therapy.

Like-wise comparing the response of treatment in group II and IV as well as group I and III, faster recovery was observed in group IV and III, respectively (Figure 25 to 40). It was concluded that herbal supportive therapy in the form of shisham leaves powder was responsible for faster recovery in the respective groups.

The comparison of response of therapy in group II and III revealed that the response of treatment was almost similar in both the groups which again highlighted the importance of shisham leaves powder as supportive therapy in the treatment of diarrhoeic calves. The use of shisham leaves powder with the ORS made this therapy almost at par with the parenteral fluid therapy without shisham leaves powder. The group III therapy had the advantage of being easy and convenient in respect of administration. Brooks et al. (1997) also reported that oral rehydration therapy may be very useful in early cases especially of mild to moderate diarrhoea. They also reported it as very convenient for animal patients, owners and clinicians in respect of administration. The importance of correcting dehydration in diarrhoeic calves by oral fluid therapy has been well documented by Michell *et al.* (1992), Brooks *et al.* (1996a), Brooks *et al.* (1996b), Turvill and Farthing (1996), Bouda *et al.* (1997), Alone *et al.* (2000), Kaur *et al.* (2003) and Pal and Pachauri (2009). Brooks *et al.* (1996b) and

Turvill and Farthing (1996) observed that glutamine containing ORS was better at correcting metabolic acidosis in diarrhoeic calves due to its ability to promote Na⁺ - H⁺ exchange on enterocytes. Brooks *et al.* (1997) observed that glutamine had beneficial effect on glucose levels and body weight gains in *E. coli* diarrhoea in calves. In present study, glutamine was one of the ingredient of ORS.

The response of therapeutic regimen in group V diarrhoeic calves (ofloxacin and shisham leaves powder therapy) was found comparatively poor than other groups (Figure 25 to 40).

It was concluded that best therapeutic response was evoked with the use of ofloxacin, parenteral fluid (Ringer's lactate) with sodium carbonate and shisham leaves powder. Complete clinical recovery was observed on day 4th post therapy in this group and all the clinical and haemeto-biochemical parameters retuned to normal on day 4th after treatment. It was revealed that the use of supportive therapy in the form of parenteral fluid (Ringer's lactate) and shisham leaves powder was responsible for better therapeutic response than other groups. The importance of correcting dehydration in diarrhoeic calves by supportive therapies comprising of intravenous fluid has also documented by Verma et al. (1995), Kumar and Mandial (2002), Kumar et al. (2002), Roy and Fernandes (2007), Tikoo and Soodan (2009), Kumar et al. (2010) and Mir et al. (2010). Fluid loss in calf diarrhoea is principally extracellular and sodium is the predominant in extracellular fluid. The fluid replacement as guickly as physiologically possible is required. Further resuscitation by use of oral electrolyte solution alone is slower than that achieved by intravenous administration of fluid because the gastrointestinal tract can accommodate only a limited fluid at a time. It was the reason for better therapeutic response in parenteral therapy than oral fluid therapy. Ringer's lactate solution has been used as a supportive therapy to treat the associated acidosis and electrolyte imbalance in calf diarrhoea (Walker et al., 1998 and Alone et al., 2000). Ringer's lactate produced good effects due to instant replenishment of the electrolytes and energy in the body that has lost due to diarrhoea.

The bicarbonate in ORS as well as with the ringer's lactate has the potential to immediately neutralize the metabolic acidosis associated with the diarrhoea. Booth and

Naylor (1987) observed that electrolyte solution with bicarbonate helped in restoration of acid base balance and corrected depression better than electrolyte solution without bicarbonate. Geishauser and Thunker (1997) concluded that administration of 1.3 % sodium bicarbonate solution was useful to help diarrhoeic calves regain the suckling reflex and ability to stand.

The use of shisham leaves powder as a herbal supportive therapy in calf diarrhoea with good results has been documented previously by Das *et al.* (1992), Kumar *et al.* (2000), Swaroop *et al.* (2004) and Brijesh *et al.* (2006). The mechanism of action of anti-diarrhoeal effect of shisham (*Delbergia sissoo*) leaves is difficult to explain in view of scanty literature. The leaves of shisham have high content of tannin due to which it has astringent property and given in non-specific diarrhoea (Das *et al.*, 1992). The anti-inflammatory activity of shisham leaves in rats has been documented by Hazare *et al.* (2001). The effectiveness of shisham leaves could be due to its non-specific spasmolytic activity (Kumar *et al.*, 2000). The spasmolytic activity of shisham has been supported by work of Sarg *et al.* (1999) indicating that the alcoholic extract of aerial parts of shisham has a dose dependent inhibitory effect on the motility of isolated rabbit duodenum, significant anti-inflammatory, antipyretic and analgesic activity.

5. Summary and Conclusion

The present investigation was undertaken to find out the prevalence of diarrhoea in the calves of 0 to 4 month age group at an organized farm as well as under field condition in the southern part of the Rajasthan. The effect of age, sex, season and parity of dam on prevalence of diarrhoea was also studied. The faecal and blood samples of 100 diarrhoeic calves were collected. The faecal samples were subjected to identification of causative agent(s) in the faeces and blood samples for haemato-biochemical characterization of diarrhoea in calves. Antibiotic sensitivity pattern of *E. coli* isolated from the faecal samples of diarrhoeic calves were evaluated based on improvement in the clinical and haemato-biochemical profile of diarrhoeic calves.

The overall prevalence of diarrhoea in bovine calves (buffalo and cow calves both) was found 41.67 %. The prevalence of diarrhoea at the organized farm was found significantly lower than that of field condition. The overall prevalence of diarrhoea in buffalo and cow calves was 53.92 % and 32.61 %, respectively. The prevalence was significantly higher in buffalo calves than cow calves. The prevalence of diarrhoea in crossbred calves and Gir / local non-descript calves was 42.86 % and 28.12 %, respectively. The higher prevalence has been recorded in crossbred calves than indigenous / local non-descript cow calves. The prevalence of calf diarrhoea was found decreasing with the advancement of the age in the calves. There was highly significant difference in the overall prevalence of diarrhoea in calves in different age groups (P>0.01). There was no association of sex with the development of diarrhoea in calves (P<0.05). Season-wise prevalence of diarrhoea in calves showed highest prevalence during rainy season followed by winter and summer season. There was highly significant difference in the prevalence of diarrhoea in different seasons (P>0.01). The highest prevalence of diarrhoea was also observed in the calves of first parity dams followed by calves of second parity dams.

The faecal samples of the diarrhoeic calves were subjected to identification of causative agents viz. bacteria, rotavirus and parasitic ova or oocyst. Out of 100 diarrhoeic faecal samples, 76 samples were found positive for single isolate i.e. *Escherichia coli*. Remaining 24 faecal samples were positive for mixed infections and infestations. In mixed infections, *E. coli*, *Salmonella* spp., rotavirus, *Cryptosporidium* spp., *Eimeria* spp. and other parasitic ova (Amphistomes, *Toxocara* spp., *Trichuris* spp., Strongyles and *Strongyloides* spp.) were observed.

Occurrence of *E. coli* in diarrhoeic faecal samples of calves was found highest (86.00 %), followed by rotavirus, *Eimeria* spp. and Amphistomes (15.00 % each), *Toxocara* spp. (12.00 %), Strongyles (9.00 %), *Cryptosporidium* spp. (6.00 %), *Trichuris* spp. (5.00 %) and *Salmonella* spp. and *Strongyloides* spp. (3.00 each). *E. coli* was the major organism observed in the faecal samples of the diarrhoeic calves. There was non significant difference in the occurrence of *E. coli* between organized farm and field, buffalo and cow calves and also between crossbred and Gir / local non-descript calves (P<0.05). The occurrence of *Salmonella* spp. was found very low in the faecal samples of diarrhoeic calves. The organism was isolated only from the faecal samples of diarrhoeic crossbred calves.

There was non significant difference in the occurrence of rotavirus and *Cryptosporidium* spp. at the organized farm and field (P<0.05). The occurrence of rotavirus, *Cryptosporidium* spp. and *Eimeria* spp. was found significantly higher in buffalo calves and crossbred calves than cow calves and Gir/local non-descript calves, respectively (P>0.05). The occurrence of coccidiosis at the organized farm was found lower than field condition. The occurrence of *Toxocara* spp. in diarrhoeic buffalo calves was significantly higher than cow calves (P>0.01). Similarly, the occurrence of Amphistomes and Strongyles was significantly higher in diarrhoeic buffalo calves than cow calves (P>0.05). The parasitic infestation was not observed in faeces of diarrhoeic calves of the organized farm except *Toxocara* spp. (3.33 %).

Highest occurrence of *E. coli* and rotavirus was observed in faecal samples of diarrhoeic calves of 0-15 days age group, followed by 16-30 days age group. Rotavirus

was not detected in faecal samples of diarrhoeic calves above 60 days age. The susceptibility of bovine calves for *E. coli* and rotavirus was found decreased with the advancement of the age. There was highly significant difference in the occurrence of *E. coli* and rotavirus in the faecal samples of diarrhoeic calves of different age groups (P>0.01). Highest occurrence of *Salmonella* spp. in diarrhoeic faecal samples of calves was observed in 16-30 days age group, followed by 31-60 days age group. There was no occurrence of *Salmonella* spp. in the faecal samples of diarrhoeic calves of 0-15 days and 61-120 days age group. The occurrence of *Cryptosporidium* spp. in diarrhoeic faecal samples of bovine calves was found only in 0-30 days age group. *Cryptosporidium* spp. oocysts were not observed in the faecal samples of diarrhoeic calves of diarrhoeic calves above 30 days of age. The other parasitic infestation was observed after 30 days of age in calves except *Eimeria* spp. Amphistomes and *Toxocara* spp. which appeared after 15 days of age in diarrhoeic bovine calves. There was significant difference in the occurrence of parasitic infestation in different age groups in diarrhoeic calves (P>0.05).

There was non significant difference in the occurrence of different organisms in male and female diarrhoeic calves (P<0.05). The calves of both sexes were equally susceptible to different causative agents of diarrhoea.

The occurrence of *E. coli* in the faecal samples of diarrhoeic calves was found maximum during rainy season, followed by summer and winter season. There was highly significant difference in the occurrence of *E.coli* in faecal samples of diarrhoeic calves in different seasons (P>0.01). The highest occurrence of the rotavirus was observed in the faecal samples of diarrhoeic calves during winter season, followed by summer and rainy season. The difference in the occurrence of rotavirus in the faecal samples of diarrhoeic calves was found highly significant in different seasons (P>0.01). There was non significant difference in the occurrence of *Salmonella* spp., *Cryptosporidium* spp. and *Eimeria* spp. in the faecal samples of diarrhoeic calves was found highest during rainy season in most of the isolates. There was significant difference in the occurrence of parasitic infestation

(*Toxocara* spp., *Trichuris* spp., Strongyles and *Strongyloides* spp.) in calves in different seasons (P>0.05).

The occurrence of *E. coli, Salmonella* spp., rotavirus and *Cryptosporidium* spp. was found highest in the faecal samples of the diarrhoeic calves of first or second parity dams. There was significant difference in the occurrence of *E. coli* diarrhoea in calves of dams of different parities (P>0.05). The occurrence of other parasites viz. *Eimeria* spp., Amphistomes, *Toxocara* spp., *Trichuris* spp., Strongyles and *Strongyloides* spp. in the faecal samples were not found higher in diarrhoeic calves of first or second parity dams.

As regards clinical and haemato-biochemical characterization of calf diarrhoea, there was slight increase in the mean rectal temperature, heart rate and respiration rate in the diarrhoeic calves affected with colibacillosis but the difference in above parameters was not found significant (p<0.05) whereas the faecal consistence score, clinical depression score and clinical dehydration score in colibacillosis affected diarrhoeic calves was found significantly higher than healthy calves. In colibacillosis affected diarrhoeic calves, the clinical manifestations as semisolid to watery consistency of faeces, mild to moderate depression, weak and disorganized suckling, mild to moderate dehydration, eyes not recess or slightly recess in to orbit and loss of skin elasticity were observed.

There was non significant difference in the mean rectal temperature, heart rate and respiration rate in healthy calves and diarrhoeic calves affected with mixed infections but mean faecal consistency score, clinical depression score and clinical dehydration score was found significantly higher than healthy calves. In diarrhoeic calves affected with mixed infections of bacteria, virus and/or parasites, semisolid to watery faeces, mild to moderate depression, weak and disorganized suckling and moderate dehydration with eyes slightly recess in to orbit were the main clinical findings.

There was significant increase in the mean Hb, PCV, TEC, TLC and neutrophils in diarrhoeic calves affected with colibacillosis than healthy calves whereas the mean MCV, MCH, MCHC and lymphocytes were found significantly lower in calves affected with colibacillosis than healthy calves. There was marked haemo-concentration, erythrocytosis, leucocytosis, neutrophilia and lymphopenia in diarrhoeic calves affected with colibacillosis.

The mean value of Hb, PCV, TEC, MCV, MCHC and neutrophils in diarrhoeic calves, affected with mixed enteric infections was found significantly lower than healthy calves whereas the TLC, lymphocytes and eosinophils were found significantly higher than healthy calves. Marked erythrocytosis, neutropenia, lecocytosis, lymphocytosis and eosinophilia was observed in the calves affected with mixed enteric infections.

There was significant decrease in the mean serum sodium, serum chloride, serum glucose, serum IgG and serum IgM in the colibacillosis affected calves than healthy calves whereas significant increase was observed in the serum potassium, total serum protein and A/G ratio in diarrhoeic calves affected with colibacillosis than healthy calves. Marked hyponatraemia, hypochloraemia, hypoglycemia, decrease in serum immunoglobulins, hyperkalaemia and hyperproteinaemia was observed in colibacillosis affected diarrhoeic calves.

In diarrhoeic calves affected with mixed infection of bacteria, virus and/or parasites, hyponatraemia, hypokalaemia, hypochloraemia, hypoproteinaemia, hypoglycemia and decrease in serum IgG and serum IgM was observed.

The antibiotic sensitivity pattern of *E.coli* isolated from faecal samples of diarrhoeic calves was determined. The *E.coli* isolates were found highly sensitivity to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, enrofloxacin and gentamicin. The *E. coli* isolates were found highly resistance against erythromycin, followed by tetracycline, oxytetracycline, sulphadimidine, streptomycin and cotrimoxazole. Out of 86 *E. coli* isolates, 34.88 % showed multiple antibiotic resistance for 3 to 8 antibiotics.

Five therapeutic regimens were evaluated for their efficacies in calf diarrhoea caused by *E. coli*. The diarrhoeic calves were divided in to five groups, each consisting of 15 calves. Comparative therapeutic efficacies were assessed based on improvement in the clinical and haemato-biochemical profile of diarrhoeic calves. The five therapeutic

regimens comprised of (i) Ofloxacin + ORS; (ii) Ofloxacin + Parenteral fluid (ringer's lactate) with sodium bicarbonate; (iii) Ofloxacin + ORS + Shisham leaves powder; (iv) Ofloxacin + Parenteral fluid (ringer's lactate) with sodium bicarbonate + Shisham leaves powder; and (v) Ofloxacin + Shisham leaves powder.

The recovery from the ailment was observed in all the five treatment groups but there was variation in different groups as far as faster recovery was concern. Ofloxacin, Parenteral fluid (ringer's lactate) with sodium bicarbonate and shisham leaves powder (Group IV) was found to be most effective therapy followed by ofloxacin, ORS and shisham leaves powder (Group III); and ofloxacin and parenteral fluid (ringer's lactate) with sodium bicarbonate (Group II). The response of therapeutic regimen in group V (ofloxacin and shisham leaves powder) was found comparatively poor than other groups. Use of supportive therapy in the form of parenteral fluid (Ringer's lactate) and shisham leaves powder was responsible for better therapeutic response.

It was concluded that there was marked difference in the prevalence of diarrhoea in calves at the organized farm and under field condition. Further, the prevalence of calf diarrhoea was significantly higher in buffalo calves and crossbred cow calves than cow calves and Gir / local non- descript cow calves, respectively. Age, season and parity of dam had significant effect on the prevalence of calf diarrhoea. There was no association of sex with the development of diarrhoea in calves.

E. coli was the major organism observed in the faecal samples of the diarrhoeic calves followed by rotavirus, *Eimeria* spp. and Amphistomes; *Toxocara* spp.; Strongyles; *Cryptosporidium* spp.; *Trichuris* spp.; and *Salmonella* spp. and *Strongyloides* spp. Age had significant effect on the occurrence of different isolates in the faecal samples of diarrhoeic calves. The calves of both sexes were equally susceptible to different causative agents of diarrhoea. The occurrence of *E. coli* and helminth ova in the faecal samples of diarrhoeic calves was found maximum during rainy season whereas the rotavirus was observed maximum in the faecal samples of diarrhoeic calves of *E. coli*, *Salmonella* spp.,

rotavirus and *Cryptosporidium* spp. was found highest in the faecal samples of the diarrhoeic calves of first or second parity dams.

In colibacillosis affected diarrhoeic calves, the clinical manifestations as semisolid to watery consistency of faeces, mild to moderate depression, weak and disorganized suckling, mild to moderate dehydration, eyes not recess or slightly recess in to orbit and loss of skin elasticity were observed. There was marked haemoconcentration, erythrocytosis, leucocytosis, neutrophilia, lymphopenia, hyponatraemia, hypochloraemia, hypoglycemia, decrease in serum immunoglobulins, hyperkalaemia and hyperproteinaemia in diarrhoeic calves affected with colibacillosis.

The *E.coli* isolates were found highly sensitivity to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, enrofloxacin and gentamicin in antibiotic sensitivity test. Ofloxacin, Parenteral fluid (ringer's lactate) with sodium bicarbonate and shisham leaves powder (Group IV) was found to be most effective therapy.

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वत्स अतिसार का जनपादकीय, शयनिक एवं रक्त जैव–रासायनिक निरूपण एवं चिकित्सीय पथ्यों का मूल्यांकन

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अनुक्षेपण

वर्तमान अन्वेक्षण 0 से 4 माह के वत्सों में अतिसार का प्रायोभाव ज्ञात करने हेतु किया गया। अतिसार के प्रायोभाव पर उम्र, लिंग एवं मां के ब्याँत के प्रभाव का भी अध्ययन किया गया। एक सौ अतिसार ग्रस्त वत्सों के मल एवं रक्त के नमूने एकत्रित किये गये। मल के नमूनों को मल में रोग कारकों की पहचान एवं रक्त के नमूनों को वत्सों में अतिसार के रक्त-जैव रासायनिक निरूपण हेतु विश्लेषित किया गया। अतिसार ग्रस्त वत्सों के मल के नमूनों से प्राप्त *ई.* कोलाई की प्रतिजैविक संवेदनशीलता निर्धारित की गई। अतिसार ग्रस्त वत्सों के शयनिक एवं जैव-रासायनिक रचना में सामान्यता की ओर सुधार के आधार पर विभिन्न चिकित्सीय पथ्यों का मूल्यांकन किया गया।

वत्सों (भैंस एवं गौ) में सम्पूर्ण प्रायोभाव *४१.६७* % था। भैंस एवं गौ वत्सों में अतिसार का सम्पूर्ण प्रायोभाव क्रमशः *५३.६२* % एवं *३२.६१* % पाया गया जबकि संकर गौ वत्सों एवं गिर/स्थानीय अपरिभाषित गौ-वत्सों में अतिसार का प्रायोभाव क्रमशः *४२.८६* % एवं *२८.१२* % था। वत्सों में अतिसार के प्रायोभाव में उम्र के बढने के साथ कमी पाई गई। वत्सों में अतिसार के विकास से लिंग का कोई सम्बन्ध नहीं देखा गया। वत्स अतिसार वर्षा ऋतु में एवं प्रथम ब्याँत की मां के वत्सों में सर्वाधिक पाया गया।

अतिसार ग्रस्त वत्सों के मल नमूनों में *ई.कोलाई* जीव प्रमुखता से देखा गया। तदन्तर रोटाविषाणु, आइमिरीया प्रजाति एवं एम्फीस्टोम; टोक्सोकेरा प्रजाति; स्ट्रोंगाइल; क्रिप्टोस्पोरिडियम प्रजाति; ट्राइच्रुरिस प्रजाति; एवं साल्मोनेला प्रजाति तथा स्ट्रोंगाइलोइडिस प्रजाति देखी गई। रोटाविषाणु, क्रिप्टोस्पोरिडियम प्रजाति एवं आइमिरीया प्रजाति की व्यापकता क्रमशः भैस वत्सों एवं संकर गौ वत्सों में, गौ-वत्सों एवं गिर/स्थानीय अपरिभाषित गौ-वत्सों से सार्थक रूप से अधिक पाई गई। टोक्सोकेरा प्रजाति, एम्फीस्टोम एवं स्ट्रोंगाइल की व्यपकता भैस वत्सों में, गौ वत्सों से सार्थक रूप से अधिक पाई गई। संस्थागत फार्म के अतिसार ग्रस्त वत्सों के मल नमूनों में परजीवी संक्रमण सामान्यतया नहीं देखा गया।

ई.कोलाई तथा रोटाविषाणु की सर्वाधिक व्यापकता 0-15 दिन के अतिसार ग्रस्त वत्सों के मल नमूनों में देखी गई। साट दिन से अधिक उम्र के अतिसार ग्रस्त वत्सों के मल नमूनों में रोटा विषाणु की व्यापकता नहीं पाई गई। वत्सों में उम्र वृद्धि के साथ- साथ *ई.कोलाई* एवं रोटा विषाणु के प्रति संवेदनशीलता में कमी पाई गई। अतिसार ग्रस्त वत्सों के मल नमूनों में *साल्मोनेला* प्रजाति की व्यापकता मात्र 16-60 दिन की उम्र में जबकि *क्रिप्टोस्पोरिडयम* प्रजाति की व्यापकता मात्र 0-30 दिन की उम्र के वत्सों में पाई गई। वत्सों में अधिकांश परजीवी संक्रमण 30 दिन की उम्र के पश्चात देखे गये। अतिसार के विभिन्न रोग कारकों के प्रति दोनो लिंगो के वत्सों में समान संवेदनशीलता देखी गई। अतिसार ग्रस्त वत्सों के मल नमूनों में *ई.कोलाई* एवं अधिकांश कृमि अण्डों की व्यापकता वर्षा ऋतु में, जबकि रोटाविषाणु की व्यापकता शीत ऋतु में देखी गई। प्रथम अथवा द्वितीय ब्याँत की माताओं के वत्सों में *ई.कोलाई, साल्मोनेला* प्रजाति, रोटाविषाणु एवं *क्रिप्टोस्पोरिडियम* प्रजाति की व्यापकता सर्वाधिक थी।

कोलीबेसीलोसिस प्रभावित अतिसार ग्रस्त वत्सों में अर्धटोस से जलीय प्रकृति का मल, अल्प से मध्यम सुस्तता, दुर्बल एवं असंगठित दुग्ध भोजन, अल्प से मध्यम निर्जलन, नेत्रों का नेत्रकोष में लेशमात्र अथवा बिल्कुल जुड़ाव नहीं होना तथा त्वचा में खिंचाव का अभाव जैसे शयनिक लक्षण देखे गये। कोलीबेसीलोसिस प्रभावित अतिसार ग्रस्त वत्सों में सार्थक रक्त सान्द्रता, लाल रक्त कोशिकाओं की गणना में वृद्धि, श्वेत रक्त कोशिकाओं की गणना में वृद्धि, न्यूट्रोफिलिया, लिम्फोपिनिया, हाइपोनेट्रीमिया, हाइपोक्लोरिमिया, हाइपोग्लाइसिमिया, सीरम इम्प्यूनोग्लोब्युलिन में कमी, हाइपरकेलिमिया तथा हाइपरप्रोटीनिमिआ देखा गया।

प्रतिजैविक संवेदनशीलता परीक्षण में अतिसार ग्रसित मल नमूनों से प्राप्त *ई.कोलाई* की ओफ्लोक्सासीन, नोरफ्लोक्सासीन, क्लोरमफेनीकोल, सिप्रोफ्लोक्सासीन, नालिडिक्सिक एसीड, एन्रोफ्लोक्सासिन तथा जेंटामाइसीन के प्रति अत्यधिक संवेदनशीलता पाई गई। जबकि एरिथ्रोमाइसिन, टेट्रासाइक्लीन, ऑक्सीटेट्रासाइक्लीन, सल्फाडिमिडिन, स्ट्रेप्टोमाइसिन तथा कोट्रीमोक्सेजोल के प्रति प्रतिरोधकता देखी गई।

ओफ्लोक्सासीन, सोडियम बाई कार्बोनेट युक्त रिंगर लेक्टेट पेरेन्टेरल द्रव्य तथा शीशम की पत्तियों का पाउडर सर्वाधिक प्रभावी उपचार था। रिंगर लेक्टेट पेरेन्टरल द्रव्य तथा शीशम की पत्तियों के पाउडर के रूप में सह-उपचार उच्चतम चिकित्सीय प्रभाव हेतु उत्तरदायी रहा।

Epidemiological, Clinical and Haemato-biochemical Characterization of Calf Diarrhoea and Evaluation of Therapeutic Regimens Ph.D. Thesis Department of Veterinary Clinical Medicine, Ethics and Jurisprudence College of Veterinary and Animal Science, Bikaner (Rajasthan University of Veterinary and Animal Sciences, Bikaner)

Submitted by: Major Advisor:

Shiv Kumar Sharma Dr. R. K. Tanwar

ABSTRACT

The present investigation was undertaken to find out the prevalence of diarrhoea in the bovine calves of 0 to 4 month age group. The effect of age, sex, season and parity of dam on prevalence of diarrhoea was also studied. The faecal and blood samples of 100 diarrhoeic calves were collected. The faecal samples were subjected to identification of causative agent(s) in the faeces and blood samples for haemato-biochemical characterization of diarrhoea in calves. Antibiotic sensitivity pattern of *E. coli* isolated from the faecal samples of diarrhoeic calves was determined. Different therapeutic regimens were evaluated on the basis of improvement in the clinical and haemato-biochemical profile of diarrhoeic calves towards normalcy.

The overall prevalence of diarrhoea in bovine calves (buffalo and cow calves both) was 41.67 %. The overall prevalence of diarrhoea in buffalo and cow calves was 53.92 % and 32.61 %, respectively whereas in crossbred calves and Gir / local nondescript cow calves was 42.86 % and 28.12 %, respectively. The prevalence of calf diarrhoea was found decreasing with the advancement of the age in the calves. There was no association of sex with the development of diarrhoea in calves. The prevalence of diarrhoea in calves was highest during rainy season as well as in the calves of first parity dams.

E. coli was the major organism observed in the faecal samples of the diarrhoeic calves followed by rotavirus, *Eimeria* spp. and Amphistomes; *Toxocara* spp.; Strongyles; *Cryptosporidium* spp.; *Trichuris* spp.; and *Salmonella* spp. and *Strongyloides* spp. The occurrence of rotavirus, *Cryptosporidium* spp. and *Eimeria* spp. was found significantly higher in buffalo calves and crossbred calves than cow calves and Gir/local non-descript calves, respectively. The occurrence of *Toxocara* spp., Amphistomes and Strongyles in diarrhoeic buffalo calves was significantly higher than cow calves. The parasitic infestation was not generally observed in faeces of diarrhoeic calves of the organized farm.

Highest occurrence of *E. coli* and rotavirus was observed in faecal samples of diarrhoeic calves of 0-15 days age group. Rotavirus was not detected in faecal samples of diarrhoeic calves above 60 days age. The susceptibility of bovine calves for *E. coli* and rotavirus was found decreased with the advancement of the age. The occurrence of *Salmonella* spp. in diarrhoeic faecal samples of bovine calves was observed only in 16-60 days age whereas *Cryptosporidium* spp. was found only in 0-30 days age. The most of the parasitic infestations were observed after 30 days of age in calves. The calves of both sexes were equally susceptible to different causative agents of diarrhoea.

The occurrence of *E. coli* and most of the helminth ova in the faecal samples of diarrhoeic calves was found maximum during rainy season whereas the rotavirus was observed mostly during winter season. The occurrence of *E. coli, Salmonella* spp.,

rotavirus and *Cryptosporidium* spp. was found highest in the faecal samples of the diarrhoeic calves of first or second parity dams.

In colibacillosis affected diarrhoeic calves, the clinical manifestations as semisolid to watery consistency of faeces, mild to moderate depression, weak and disorganized suckling, mild to moderate dehydration, eyes not recess or slightly recess in to orbit and loss of skin elasticity were observed. There was marked haemoconcentration, erythrocytosis, leucocytosis, neutrophilia, lymphopenia hyponatraemia, hypochloraemia, hypoglycemia, decrease in serum immunoglobulins, hyperkalaemia and hyperproteinaemia in diarrhoeic calves affected with colibacillosis.

The *E.coli* isolated from diarrhoeic faecal samples was found highly sensitivity to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, enrofloxacin and gentamicin in antibiotic sensitivity test. The *E. coli* isolates were found resistance against erythromycin, tetracycline, oxytetracycline, sulphadimidine, streptomycin and cotrimoxazole.

Ofloxacin, Parenteral fluid (ringer's lactate) with sodium bicarbonate and shisham leaves powder was found to be most effective therapy. Use of supportive therapy in the form of parenteral fluid (Ringer's lactate) and shisham leaves powder was responsible for better therapeutic response.

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The blessings of almighty god made this task successful for me. Place: Bikaner Date: (Shiv Kumar Sharma)

	Appendix I Values of the clinical parameters in diarrhoeic calves						
				-			
S. No.	R.Temp.	H.R.	R.R	FCC	Cl.deh S.	Cl.depS.	
1	100.80	115.00	21.00	2.00	2.00	2.00	
2	100.20	114.00	21.00	3.00	3.00	32.00	
3	100.40	115.00	21.00	2.00	3.00	3.00	
4	103.40	118.00	22.00	3.00	2.00	2.00	
5	102.20	117.00	23.00	2.00	3.00	3.00	
6	100.80	115.00	22.00	3.00	2.00	2.00	
7	101.00	115.00	22.00	2.00	3.00	3.00	
8	104.00	120.00	23.00	1.00	3.00	2.00	
9	101.20	115.00	22.00	3.00	3.00	3.00	
10	100.20	115.00	22.00	2.00	2.00	3.00	
11	100.80	115.00	23.00	2.00	3.00	2.00	
12	100.80	115.00	22.00	2.00	3.00	2.00	
13	101.20	116.00	23.00	3.00	2.00	2.00	
14	101.80	116.00	23.00	3.00	2.00	2.00	
15	101.20	116.00	23.00	2.00	2.00	2.00	
16	103.80	119.00	23.00	3.00	2.00	2.00	
17	104.20	117.00	22.00	2.00	2.00	2.00	
18	103.60	118.00	23.00	3.00	2.00	2.00	
				222			

S. No.	R.Temp.	H.R.	R.R	FCC	Cl.deh S.	CI.depS.
19	103.40	119.00	22.00	2.00	2.00	2.00
20	103.40	119.00	23.00	3.00	2.00	2.00
21	103.40	118.00	23.00	2.00	2.00	2.00
22	103.80	118.00	24.00	1.00	1.00	2.00
23	102.70	115.00	25.00	1.00	1.00	2.00
24	102.40	119.00	24.00	2.00	2.00	3.00
25	101.20	116.00	21.87	1.00	1.00	2.00
26	102.20	117.00	23.00	3.00	3.00	3.00
27	103.40	118.00	23.00	3.00	3.00	3.00
28	103.60	118.00	23.00	2.00	2.00	2.00
29	100.80	115.00	22.00	1.00	1.00	2.00
30	100.40	115.00	22.00	2.00	2.00	2.00
31	100.20	115.00	23.00	2.00	1.00	2.00
32	100.80	115.00	24.00	3.00	3.00	2.00
33	102.40	117.00	22.00	2.00	2.00	2.00
34	101.60	116.00	23.00	3.00	3.00	2.00
35	101.20	116.00	25.00	3.00	3.00	2.00
36	101.60	116.00	21.00	3.00	3.00	2.00
37	102.20	117.00	24.00	3.00	3.00	2.00
38	102.40	117.00	24.00	2.00	2.00	2.00
39	102.20	118.00	23.00	3.00	3.00	2.00
40	103.80	118.00	24.00	2.00	2.00	2.00
41	104.60	119.00	25.00	3.00	2.00	2.00
42	104.50	119.00	21.00	2.00	3.00	2.00
43	104.80	119.00	23.00	3.00	2.00	2.00
44	104.40	119.00	21.00	2.00	3.00	2.00
45	103.80	118.00	23.00	3.00	2.00	2.00
46	102.60	117.00	21.00	2.00	3.00	3.00
47	102.80	117.00	24.00	3.00	3.00	2.00
48	102.20	118.00	25.00	2.00	2.00	2.00
49	103.80	118.00	21.00	3.00	3.00	2.00
50	104.60	119.00	33.00	2.00	2.00	2.00
51	103.20	118.00	22.33	3.00	3.00	2.00
52	103.70	118.00	23.00	2.00	2.00	2.00
				222		

S. No.	R.Temp.	H.R.	R.R	FCC	Cl.deh S.	CI.depS.
53	104.20	119.00	24.00	3.00	3.00	3.00
54	102.60	117.00	21.00	2.00	2.00	3.00
55	102.40	117.00	20.00	3.00	3.00	3.00
56	103.40	119.00	21.00	2.00	2.00	2.00
57	102.60	117.00	23.00	3.00	3.00	3.00
58	100.20	115.00	21.00	2.00	2.00	2.00
59	101.60	115.00	23.00	3.00	3.00	3.00
60	102.80	117.00	24.00	2.00	2.00	2.00
61	100.40	115.00	2.00	3.00	3.00	3.00
62	103.80	119.00	21.00	2.00	2.00	2.00
63	102.60	117.00	24.00	3.00	3.00	3.00
64	102.40	117.00	23.00	3.00	3.00	3.00
65	102.40	117.00	21.00	3.00	3.00	2.00
66	100.20	114.00	23.00	3.00	2.00	2.00
67	102.40	117.00	23.00	3.00	3.00	2.00
68	100.80	115.00	24.00	3.00	3.00	2.00
69	101.60	115.00	21.00	2.00	2.00	2.00
70	101.20	116.00	20.00	2.00	2.00	2.00
71	103.20	118.00	18.00	2.00	2.00	2.00
72	102.40	117.00	19.00	1.00	2.00	2.00
73	102.40	117.00	23.00	1.00	2.00	2.00
74	100.40	115.00	21.00	3.00	3.00	2.00
75	103.60	118.00	24.00	2.00	3.00	3.00
76	102.40	117.00	26.00	2.00	2.00	3.00
77	99.00	108.00	16.00	2.00	1.00	1.00
78	101.00	109.00	16.00	3.00	3.00	3.00
79	100.00	108.00	18.00	2.00	2.00	2.00
80	102.20	109.00	17.00	2.00	3.00	3.00
81	101.40	103.00	16.00	2.00	2.00	2.00
82	99.00	108.00	16.00	3.00	3.00	3.00
83	100.00	108.00	16.00	2.00	2.00	2.00
84	104.00	112.00	17.00	3.00	2.00	2.00
85	100.00	108.00	16.00	2.00	3.00	3.00
86	100.00	108.00	16.00	3.00	3.00	2.00
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S. No.	R.Temp.	H.R.	R.R	FCC	Cl.deh S.	Cl.depS.
87	100.00	108.00	16.00	2.00	2.00	3.00
88	101.00	108.00	16.00	2.00	3.00	2.00
89	101.00	109.00	16.00	3.00	2.00	3.00
90	101.00	109.00	16.00	3.00	2.00	2.00
91	101.00	109.00	17.00	2.00	2.00	2.00
92	103.00	111.00	17.00	3.00	3.00	3.00
93	103.00	111.00	17.00	2.00	1.00	2.00
93	104.00	112.00	18.00	2.00	3.00	3.00
95	103.00	112.00	19.00	3.00	2.00	2.00
96	102.00	111.00	20.00	3.00	3.00	2.00
97	102.00	111.00	17.00	2.00	2.00	2.00
98	103.00	112.00	17.00	2.00	2.00	2.00
99	102.00	110.00	16.00	3.00	3.00	3.00
100	102.00	110.00	16.00	2.00	2.00	2.00

Appendix II										
Values of the haematological parameters in diarrhoeic calves										
S. No.	Hb	PCV	TEC	TLC	Ν	L				
1	12.20	46.00	7.78	10.26	40.00	54.00				
2	12.30	45.70	7.73	10.19	42.00	52.00				
3	12.30	45.80	7.75	10.22	40.00	54.00				
4	12.60	47.20	7.98	10.52	41.00	53.00				
5	12.80	46.70	7.89	10.40	41.00	52.00				
6	13.00	46.00	7.78	10.26	40.00	54.00				
7	12.00	46.10	7.79	10.28	40.00	54.00				
8	12.80	47.90	8.10	10.68	42.00	55.00				
9	12.00	46.20	7.80	10.29	40.00	54.00				
10	13.00	45.80	7.74	10.21	40.00	54.00				
11	12.80	46.00	7.78	10.26	42.00	52.00				
12	12.60	46.00	7.77	10.25	41.00	53.00				
13	13.00	46.20	7.81	10.30	42.00	52.00				

S. No.	Hb	PCV	TEC	TLC	Ν	L
14	13.80	46.30	7.82	10.31	40.00	51.00
15	12.00	46.30	7.82	10.31	40.00	50.00
16	13.60	47.50	8.02	10.58	42.00	54.00
17	12.80	47.60	8.04	10.61	42.00	52.00
18	13.00	47.30	8.00	10.55	42.00	54.00
19	14.00	47.40	8.01	10.56	43.00	53.00
20	12.00	47.40	8.01	10.60	43.00	51.00
21	14.00	47.20	7.98	10.53	42.00	53.00
22	15.00	47.30	8.00	10.55	41.00	55.00
23	13.00	46.90	7.93	10.46	45.00	52.00
24	12.80	46.90	7.93	10.46	42.00	53.00
25	12.40	46.30	7.82	10.31	40.00	52.00
26	12.40	46.70	7.90	10.42	42.00	52.00
27	12.60	47.20	7.98	10.52	42.00	52.00
28	12.80	47.30	7.99	10.53	43.00	54.00
29	13.20	45.90	7.76	10.24	43.00	54.00
30	12.20	46.00	7.77	10.25	43.00	50.00
31	12.20	46.00	7.77	10.25	42.00	52.00
32	12.80	46.00	7.78	10.26	43.00	52.00
33	13.60	46.60	7.88	10.39	45.00	52.00
34	12.60	46.40	7.83	10.33	41.00	54.00
35	12.80	46.40	7.84	10.34	42.00	53.00
36	12.40	46.40	7.84	10.35	42.00	51.00
37	12.80	46.70	7.89	10.41	41.00	51.00
38	13.80	46.70	7.89	10.40	43.00	51.00
39	13.00	47.00	7.94	10.47	42.00	52.00
40	14.00	47.30	7.98	10.53	40.00	54.00
41	12.00	47.80	8.07	10.64	42.00	51.00
42	12.60	47.70	8.06	10.63	41.00	54.00
43	12.80	47.70	8.06	10.63	42.00	52.00
44	12.60	47.70	8.06	10.63	43.00	51.00
45	12.60	47.10	7.96	10.50	43.00	51.00
46	12.80	46.90	7.92	10.45	43.00	50.00
47	13.00	46.90	7.92	10.45	43.00	50.00

S. No.	Hb	PCV	TEC	TLC	Ν	L
48	13.20	47.00	7.94	10.47	43.00	50.00
49	14.00	47.10	7.95	10.49	42.00	50.00
50	12.60	47.50	8.03	10.59	43.00	51.00
51	12.80	47.20	7.98	10.53	43.00	51.00
52	12.60	47.30	8.00	10.55	41.00	53.00
53	12.80	47.70	8.06	10.63	42.00	52.00
54	13.00	46.80	7.92	10.44	42.00	51.00
55	12.40	46.80	7.90	10.42	41.00	53.00
56	12.60	47.40	8.01	10.56	41.00	53.00
57	12.40	46.80	7.91	10.44	41.00	53.00
58	13.00	45.90	7.76	10.23	42.00	52.00
59	13.20	46.10	7.79	10.28	42.00	52.00
60	13.40	47.00	7.94	10.47	42.00	53.00
61	13.60	46.10	7.79	10.27	41.00	51.00
62	12.80	47.40	8.01	10.57	41.00	53.00
63	12.60	46.90	7.92	10.45	41.00	52.00
64	12.40	46.80	7.90	10.42	43.00	49.00
65	12.40	46.80	7.90	10.42	42.00	50.00
66	12.80	45.70	7.73	10.20	42.00	52.00
67	13.80	46.90	7.93	10.46	42.00	54.00
68	14.20	46.00	7.78	10.26	41.00	54.00
69	12.00	46.10	7.79	10.28	41.00	50.00
70	13.00	46.30	7.82	10.31	41.00	50.00
71	14.20	47.10	7.96	10.50	41.00	50.00
72	12.40	46.90	7.93	10.46	42.00	51.00
73	12.60	46.90	7.93	10.46	42.00	52.00
74	13.40	45.80	7.75	10.22	41.00	54.00
75	14.00	47.40	8.01	10.56	41.00	53.00
76	12.40	46.90	7.93	10.46	42.00	52.00
77	12.80	32.00	5.79	9.96	31.00	61.00
78	11.00	32.40	5.85	10.06	32.00	62.00
79	11.00	31.90	5.77	9.92	31.00	63.00
80	10.00	32.90	5.94	10.22	32.00	63.00
81	10.30	32.50	5.87	10.10	33.00	63.00

S. No.	Hb	PCV	TEC	TLC	N	L
82	1.40	32.00	5.79	9.96	31.00	64.00
83	10.40	32.10	5.80	9.98	31.00	63.00
84	10.60	33.40	6.03	10.37	32.00	62.00
85	10.60	32.10	5.81	9.99	31.00	61.00
86	10.30	32.00	5.78	9.94	31.00	62.00
87	10.20	32.00	5.79	9.96	31.00	65.00
88	10.20	32.00	5.78	9.95	31.00	61.00
89	10.40	32.20	5.81	10.01	32.00	62.00
90	10.50	32.20	5.82	10.01	33.00	63.00
91	10.80	32.20	5.82	10.02	32.00	63.00
92	10.10	33.30	6.01	10.34	32.00	62.00
93	10.20	32.90	5.95	10.24	32.00	63.00
93	10.40	33.50	6.06	10.43	31.00	62.00
95	10.60	33.30	6.02	10.36	32.00	63.00
96	10.60	33.00	5.96	10.26	32.00	62.00
97	10.80	32.90	5.94	10.23	32.00	63.00
98	10.40	33.00	5.95	10.25	34.00	63.00
99	10.20	32.70	5.90	10.16	33.00	64.00
100	10.60	32.70	5.90	10.16	31.00	63.00

Appendix III

	Values	s of the bi	ological p	aramete	ers in diar	rhoeic c	alves	
S.No.	Serum Sod.	S. Pot.	S. Chl.	TSP	A/G ratio	S. Glu.	S. IgG	S.IgM
1	113.61	5.10	94.71	7.56	0.87	43.12	14.57	0.69
2	112.88	5.07	94.10	7.51	0.86	42.85	14.48	0.68
3	113.15	5.08	94.32	7.53	0.86	42.95	14.51	0.69
4	116.53	5.23	97.14	7.76	0.89	44.23	14.95	0.71
5	115.23	5.17	96.06	7.67	0.88	43.73	14.78	0.70
6	113.61	5.10	94.71	7.56	0.87	43.12	14.57	0.69
7	113.84	5.11	94.90	7.58	0.87	43.21	14.60	0.69
8	118.29	5.31	98.61	7.87	0.90	44.90	15.17	0.72
9	113.96	5.12	95.00	7.58	0.87	43.25	14.62	0.69
10	113.09	5.08	94.28	7.53	0.86	42.92	14.50	0.69
11	113.61	5.10	94.71	7.56	0.87	43.12	14.57	0.69
12	113.50	5.09	94.61	7.55	0.87	43.08	14.56	0.69

S.No.	Serum Sod.	S. Pot.	S. Chl.	TSP	A/G ratio	S. Glu.	S. IgG	S.IgM
13	114.11	5.12	95.12	7.59	0.87	43.31	14.63	0.69
14	114.19	5.13	95.19	7.60	0.87	43.34	14.64	0.69
15	114.22	5.13	95.22	7.60	0.87	43.35	14.65	0.69
16	117.13	5.26	97.64	7.80	0.89	44.46	15.02	0.71
17	117.48	5.27	97.93	7.82	0.90	44.59	15.07	0.71
18	116.82	5.24	97.38	7.77	0.89	44.34	14.98	0.71
19	116.96	5.25	97.50	7.78	0.89	44.39	15.00	0.71
20	116.96	5.25	97.50	7.78	0.89	44.39	15.00	0.71
21	116.61	5.23	97.21	7.76	0.89	44.26	14.96	0.71
22	116.84	5.24	97.40	7.78	0.89	44.35	14.99	0.71
23	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
24	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
25	114.22	5.13	95.22	7.60	0.87	43.35	14.65	0.69
26	115.38	5.18	96.18	7.68	0.88	43.79	14.80	0.70
27	116.55	5.23	97.16	7.76	0.89	44.24	14.95	0.71
28	116.67	5.24	97.26	7.76	0.89	44.28	14.96	0.71
29	113.38	5.09	94.52	7.55	0.86	43.03	14.54	0.69
30	113.50	5.09	94.61	7.55	0.87	43.08	14.56	0.69
31	113.53	5.10	94.64	7.56	0.87	43.09	14.56	0.69
32	113.61	5.10	94.71	7.56	0.87	43.12	14.57	0.69
33	115.11	5.17	95.96	7.66	0.88	43.69	14.76	0.70
34	114.42	5.14	95.38	7.61	0.87	43.43	14.67	0.69
35	114.53	5.14	95.48	7.62	0.87	43.47	14.69	0.69
36	114.59	5.14	95.53	7.63	0.87	43.49	14.70	0.70
37	115.26	5.17	96.08	7.67	0.88	43.75	14.78	0.70
38	115.23	5.17	96.06	7.67	0.88	43.73	14.78	0.70
39	115.98	5.21	96.68	7.72	0.88	44.02	14.87	0.70
40	116.63	5.24	97.23	7.76	0.89	44.27	14.96	0.71
41	117.88	5.29	98.27	7.85	0.90	44.74	15.12	0.72
42	117.77	5.29	98.17	7.84	0.90	44.70	15.10	0.71
43	117.71	5.28	98.12	7.83	0.90	44.68	15.10	0.71
44	117.77	5.29	98.17	7.84	0.90	44.70	15.10	0.71
45	116.27	5.22	96.92	7.74	0.89	44.13	14.91	0.71
46	115.69	5.19	96.44	7.70	0.88	43.91	14.84	0.70
47	115.71	5.19	96.46	7.70	0.88	43.92	14.84	0.70
48	115.98	5.21	96.68	7.72	0.88	44.02	14.87	0.70
49	116.17	5.21	96.84	7.73	0.89	44.09	14.90	0.70
50	117.30	5.27	97.79	7.81	0.89	44.52	15.04	0.71
51	116.59	5.23	97.19	7.76	0.89	44.25	14.95	0.71

S.No.	Serum Sod.	S. Pot.	S. Chl.	TSP	A/G ratio	S. Glu.	S. IgG	S.IgM
52	116.87	5.25	97.42	7.78	0.89	44.36	14.99	0.71
53	117.77	5.29	98.17	7.84	0.90	44.70	15.10	0.71
54	115.63	5.19	96.39	7.70	0.88	43.89	14.83	0.70
55	115.40	5.18	96.20	7.68	0.88	43.80	14.80	0.70
56	116.96	5.25	97.50	7.78	0.89	44.39	15.00	0.71
57	115.57	5.19	96.34	7.69	0.88	43.87	14.82	0.70
58	113.30	5.09	94.45	7.54	0.86	43.00	14.53	0.69
59	113.84	5.11	94.90	7.58	0.87	43.21	14.60	0.69
60	115.92	5.20	96.63	7.71	0.88	44.00	14.87	0.70
61	113.74	5.11	94.81	7.57	0.87	43.17	14.59	0.69
62	117.07	5.26	97.59	7.79	0.89	44.44	15.01	0.71
63	115.69	5.19	96.44	7.70	0.88	43.91	14.84	0.70
64	115.40	5.18	96.20	7.68	0.88	43.80	14.80	0.70
65	115.40	5.18	96.20	7.68	0.88	43.80	14.80	0.70
66	112.92	5.07	94.13	7.51	0.86	42.86	14.48	0.68
67	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
68	113.61	5.10	94.71	7.56	0.87	43.12	14.57	0.69
69	113.84	5.11	94.90	7.58	0.87	43.21	14.60	0.69
70	114.22	5.13	95.22	7.60	0.87	43.35	14.65	0.69
71	116.30	5.22	96.95	7.74	0.89	44.14	14.92	0.71
72	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
73	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
74	113.15	5.08	94.32	7.53	0.86	42.95	14.51	0.69
75	116.93	5.25	97.48	7.78	0.89	44.38	15.00	0.71
76	115.80	5.20	96.54	7.71	0.88	43.95	14.85	0.70
77	114.60	3.98	96.11	4.94	0.82	45.56	16.38	1.22
78	115.76	4.02	97.08	4.99	0.83	46.03	16.55	1.23
79	114.13	3.96	95.72	4.92	0.81	45.38	16.32	1.22
80	117.54	4.08	98.58	5.07	0.84	46.73	16.80	1.25
81	116.23	4.03	97.47	5.01	0.83	46.21	16.62	1.24
82	114.60	3.98	96.11	4.94	0.82	45.56	16.38	1.22
83	114.83	3.99	96.30	4.95	0.82	45.66	16.42	1.22
84	119.31	4.14	100.06	5.15	0.85	47.44	17.06	1.27
85	114.95	3.99	96.40	4.96	0.82	45.70	16.43	1.22
86	114.36	3.97	95.91	4.93	0.82	45.47	16.35	1.22
87	114.55	3.98	96.07	4.94	0.82	45.54	16.38	1.22
88	114.48	3.97	96.01	4.94	0.82	45.52	16.37	1.22
89	115.10	3.99	96.53	4.96	0.82	45.76	16.45	1.23
90	115.18	4.00	96.59	4.97	0.82	45.79	16.47	1.23

S.No.	Serum Sod.	S. Pot.	S. Chl.	TSP	A/G ratio	S. Glu.	S. IgG	S.IgM
91	115.21	4.00	96.62	4.97	0.82	45.81	16.47	1.23
92	118.90	4.13	99.72	5.13	0.85	47.28	17.00	1.27
93	117.74	4.09	98.74	5.08	0.84	46.81	16.83	1.25
94	119.95	4.16	100.60	5.17	0.86	47.69	17.15	1.28
95	119.14	4.13	99.91	5.14	0.85	47.37	17.03	1.27
96	117.97	4.09	98.94	5.09	0.84	46.90	16.86	1.26
97	117.62	4.08	98.65	5.07	0.84	46.77	16.81	1.25
98	117.86	4.09	98.84	5.08	0.84	46.86	16.85	1.26
99	116.81	4.05	97.96	5.04	0.83	46.44	16.70	1.24
100	116.81	4.05	97.96	5.04	0.83	46.44	16.70	1.24

INTRODUCTION

REVIEW OF LITERATURE

MATERIALS AND METHODS

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SUMMARY AND CONCLUSION

LITERATURE CITED

APPENDICES

ANTIBIOTIC SENSITIVITY PATTERN OF *E. COLI* ISOLATED FROM THE FAECAL SAMPLES OF THE DIARRHOEIC CALVES^{*}

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ABSTRACT

Antibiotic sensitivity pattern of the E. coli isolated from the faecal samples of the diarrhoeic calves was investigated. *E.coli* isolates were found highly sensitive to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, kanamycin, enrofloxacin, gentamicin and doxycycline. The *E. coli* isolates were resistance against erythromycin, tetracycline, oxytetracycline, sulphadimidine and streptomycin.

Key words: Antibiotic, Sensitivity, E. coli, diarrhoeic calves

Introduction:

Antibiotic resistance of *E.coli* is one of the major challenges being faced in the treatment of calves suffering with colibacillosis. *E. coli* can acquire high level of resistant to some of the antibiotics either by spontaneous genetic mutation or by transfer of drug resistant plasmid to the recipient cells. This generally occurs due to indiscriminate use of antibiotics as chemotherapeutic agent or feed additives (Cruickshank *et al.*, 1975). The emergence and dissemination of antimicrobial resistance in bacteria has been well documented as a serious problem worldwide (Cohen, 2000). The results of faecal antimicrobial susceptibility testing have traditionally been used to guide treatment decisions. Antimicrobial sensitivity tests are needed to be carried out repeatedly in different populations and regions. Antibiotic sensitivity pattern of bacterial isolates pave way in suggesting the treatment and control of the disease. It also helps in preventing development of resistant strains against the drugs which may be either intermediate or

*Part of the Ph.D. thesis submitted by the first author to the Rajasthan University of Veterinary and Animal Sciences, Bikaner

resistant to the bacteria in antibiotic sensitivity test. It aids the clinician to go for a direct approach of treatment with drugs to which bacteria are highly susceptible.

Materials and Methods:

Total 86 *E. coli* strains isolated from the faecal samples of the bovine calves up to 4 months of age were subjected to antibiotic sensitivity test by disc diffusion method as per technique of Bauer *et al.* (1966) against the commonly used antibiotics for the treatment of calf diarrhoea.

Results and Discussion:

The percent antibiotic sensitivity pattern of *E.coli* isolates of diarrhoeic calves is presented in Table 1. The antibiotic sensitivity pattern of *E.coli* isolated from the faecal samples of the diarrhoeic calves showed 100.00 % sensitivity to ofloxacin, norfloxacin and chloramphenicol followed by ciprofloxacin and nalidixic acid (98.84 % each), kanamycin (94.19 %), enrofloxacin (90.70%), gentamicin (81.39 %), doxycycline (79.07 %), streptomycin (65.12 %), trimethoprim (58.14 %), sulphadimidine (48.84 %), cotrimoxazole (46.51 %), oxytetracycline (41.86 %), tetracycline (38.37 %) and erythromycin (6.98 %).

It was concluded that *E.coli* was highly sensitivity to ofloxacin, norfloxacin, chloramphenicol, ciprofloxacin, nalidixic acid, enrofloxacin and gentamicin. This might be attributed to the fact that these antibiotics were seldom used in treatment of enteric infections in bovine calves. High degree of sensitivity of *E.coli* to these antibiotics have also been reported by Hussain and Saikia (2000), Sharma *et al.* (2004), Bandyopadhyay *et al.* (2008), Dubal *et al.* (2009), Devkate *et al.* (2010), Kumar *et al.* (2010), Pan and Bhatia (2010) and Gupta *et al.* (2011).

The *E. coli* isolates showed maximum resistance against erythromycin (93.02 %), followed by tetracycline (40.70 %), oxytetracycline (34.88 %), sulphadimidine (33.72 %), streptomycin (32.56 %), cotrimoxazole (30.23 %), trimethoprim (20.93 %), doxycycline (9.30 %), enrofloxacin (6.98 %) and ciprofloxacin (1.16 %). Similar findings have been reported by Kaura *et al.*(1991), Chattopadhyay *et al.* (2003), Hariharan *et al.* (2004), Tikoo and Soodan (2009), Sharma *et al.* (2009) and Pan and Bhatia (2010).

Multiple antibiotic resistance of diarrhoeic *E. coli* isolates is depicted in Table 2. Out of 86 *E. coli* isolates, 30 (34.88 %) showed multiple antibiotic resistance for 3 to 8 antibiotics. Multiple resistance pattern has been recorded among *E.coli* isolates from diarrhoeic calves by Shah and Jhala (1990), Kaura *et al.* (1991) and Sharma *et al.* (2009) also. Multiple resistant pattern is possibly due to indiscriminate use of antibiotic therapy which exerts a selection pressure and leads to the development of multiple drug resistance among different strains of bacteria.

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Table 21Percent antibiotic sensitivity pattern of diarrhoeic calvesEscherichia coli isolates

S. No.	Name of antibiotic disc	Escherichia coli isolates (n=86)					
		Resistant	Intermediate	Sensitive			
1	Ofloxacin	0.00	0.00	100.0			
		(0)	(0)	(86)			
2	Ciprofloxacin	1.16	0.00	98.84			
		(1)	(0)	(85)			
3	Enrofloxacin	6.98	2.32	90.70			
		(6)	(2)	(78)			
4	Norfloxacin	0.00	0.00	100.			
		(0)	(0)	(86)			

5	Oxytetracycline	34.88	23.25	41.86
		(30)	(20)	(36)
6	Sulphadimidine	33.72	17.44	48.84
		(29)	(15)	(42)
7	Cotrimoxazole	30.23	23.25	46.51
		(26)	(20)	(40)
8	Erythromycin	93.02	0.00	6.98
		(80)	(0)	(6)
9	Gentamicin	0.00	18.60	81.39
		(0)	(16)	(70)
10	Trimethoprim	20.93	20.93	58.14
		(18)	(18)	(50)
11	Doxycycline	9.30	11.63	79.07
		(8)	(10)	(68)
12	Chloramphenicol	0.00	0.00	100.0
		(0)	(0)	(86)
13	Nalidixic acid	0.00	1.16	98.84
		(0)	(1)	(85)
14	Kanamycin	0.00	5.81	94.19
		(0)	(5)	(81)
15	Streptomycin	32.56	2.32	65.12
		(28)	(2)	(56)
16	Tetracycline	40.70	20.93	38.37
		(35)	(18)	(33)

Figures in parenthesis indicate number of E. coli isolates

Particulars	Multiple resistance (No. antibiotics)									
	0	1	2	3	4	5	6	7	8	
Resistance	0	44	12	1	4	8	9	7	1	
Intermediate	9	55	7	9	5	1	0	0	0	

 Table 2
 Multiple antibiotic resistance of diarrhoeic Escherichia coli isolates