CHAPTER 1

INTRODUCTION

Parasitism is one of the major problem of different mammalian hosts, particularly cattle, buffaloes, sheep, goats and humans. Parasitic problems caused by ectoparasites or endoparasites, are the major obstacles to the production of livestock throughout the world (Ijaz et al. 2009, Bilal et al. 2009). The major observed economic losses are “low production, poor weight gain, high mortality, treatment expenses, cost of drugs, veterinary care and highly sensitivity to bacterial, viral and other protozoan diseases due to poor immune status (Gupta et al.1995). In Pakistan, the problem of parasitic infestation is common amounting to annual losses of 26.5 million rupees to the industry of livestock (Anwar et al.1995).

Cryptosporidiosis is caused by intracellular, obligate and enteric protozoan parasite belonging to the genus *Cryptosporidium* and phylum *Apicomplexa*. This parasite is worldwide in distribution. Cryptosporidiosis has been documented in more than 22 domestic and wild species such as mammals, birds, fish and reptiles (Fayer and Ungar, 1986).

Cryptosporidiosis is the common parasitic infection carrying veterinary and medical importance affecting human beings and numerous vertebrate species including small and large mammals (cattle, sheep, goats, dogs and cats), fish, reptiles and poultry (Arrowood et al. 2009).The *Cryptosporidium* parasite is mainly found in wet and warm seasons of the year (Jafari et al. 2013) Cryptosporidiosis occurs in many different agro-ecological areas of the world and major restrain to the production of livestock at world level (Paul et al. 2014).

Cryptosporidiosis is an emerging enteric protozoan disease caused by various *Cryptosporidium* species, which causes gastrointestinal infection in different species of mammals such as humans, cattle’s, sheep’s, goats, pigs and horses at global level(Nasir et al. 2009).

The most common enteric infection of goat kids, lambs and cow calves mainly occurs in the early month of life is cryptosporidial infection and caused by *Cryptosporidium parvum* (Delafosse et al. 2006; Sanz et al. 2009; Nasir et al. 2009). Cryptosporidiosis mainly occurs in goat kids at the age of 4-15 days old and caused by *Cryptosporidium* parvum. Goat kids show various clinical signs such as severe watery diarrhea, emaciation and loss of electrolytes which may result in dehydration as well as electrolytes imbalance. Heavy economic losses occur due to high morbidity and mortality (Giadinis et al. 2008; Smith and Sherman, 2009; Cacciet et al. 2012; Giadinis, 2012).

In ruminants, the most obvious clinical sign is watery diarrhoea that has been mostly observed by veterinarians during the physical examination of the animals suffering from cryptosporidiosis(De Graaf et al. 1999).

Cryptosporidiosis is also an important zoonotic disease. All the infected animals are the big source of infection to human beings and healthy animals. It can cause a serious life-threatening infection in those patients suffering from Acquired Immuno deficiency Syndrome (AIDS). Hence cryptosporidiosis causes health risk to human beings because it is one of the big sources of zoonotic potential and there is no discovery of proper effective treatment to achieve 100% results (Tzipori, 1998).

Cryptosporidiosis appears in different agro-ecological zones as a serious threat to small ruminants as well as large ruminants which result in heavy economic losses. Cryptosporidiosis has become a serious threat to the livestock economy worldwide reported by different researchers (Ayinmode and Fagbemi, 2011; Paul et al. 2014). Cryptosporidiosis seriously affects the production of livestock sector which result in lowering of production and poor weight gain (Akinkuotu and Fagbemi, 2014). During the period of four weeks of Cryptosporidial infection, there was difference of two kg of body weight between sick (natural infection) and healthy goat kids of the same age (de Graaf et al. 1999).

Cryptosporidiosis causes high morbidity has been recorded in various domestic animals leading to heavy mortalityin young animals such as lambs, kids, foals and calves. As a result of heavy mortality during *Cryptosporidium* infection, huge economic losses were recorded by different researchers (; Degerli S, 2005; Nasir, 2009; Prakash et al. 2009; Potter and Esbroeck, 2010; Ayinmode and Fagbemi, 2011).

During the *Cryptosporidium* infection, the major intestinal disorders were seen and observed in form of enteritis when caused by the *Cryptosporidium parvum* in various species of mammals such as Kids, Lambs, goats, Sheep, foals, cattle and pigs. The cryptosporidiosis can be diagnosed by various clinical signs under field conditions such as watery diarrhoea, emaciation, dehydration, loss of appetite, loss of weight and weakness which result in high mortality and poor weight gain (Wang et al. 2010; Maurya, 2013).

Cryptosporidiosis was first reported by Barker and Carbonell in the year1974, in lambs showing the clinical sign of diarrahoea.The causative organism was not explained in detail because this organism was found by chance when studying other pathogenic bacteria. A research was conducted in the year 1980, where two different groups of lambs were studied .One group was naturally infected while other group was experimentally infected. As a result, it was concluded that *Cryptosporidium* infection is responsible for the primary cause of diarrhea in lambs (Angus et al. 1982; Snodgrass et al. 1984). The *Cryptosporidium* is an important enteric pathogen causing early diarrhea in small ruminants. When observed the diarrhoea in the early life of young ruminants such as lambs, kids and cow calves etc., then *Cryptosporidium* infection should keep in mind while diagnosing the disease. In Australia, cryptosporidiosis was first recorded in 1981, in a goat kid at the age of 2 weeks, showing the clinical sign of diarrhoea (Mason et al. 1981).

The Cryptosporidiosis has been also mainly observed in neonatal calves at an early age of life while in other small ruminants (lambs and kids) has been also recorded at an early age of life (Radostits et al.2008).

The Cryptosporidiosis is an important enteric apicomplexan protozoan, also known as *Cryptosporidia*. All vertebrates suffer from *Cryptosporidium* infection. Due to its Zoonotic nature, several times waterborne transmission was also recorded world widely. As a result, life threatening infections were recorded in those patients suffering from AIDS such as children, elders and pregnant women. Small number of *Cryptosporidium* oocyst such as 1-10 can cause a disease in all susceptible hosts (Pereira et al. 2000). There is no need of intermediate host for development of oocysts after excretion in feces. Cryptosporidiosis is also an important disease in goats where heavy economical losses as well as health hazards were also reported on the basis of the causative agent known as *Cryptosporidium parvum* (zoonotic in nature). Kids and lambs are highly susceptible to the *Cryptosporidium* infection as reported in many countries of the world. Cryptosporidiosis was recorded in kids and lambs showing clinical signs in form of diarrhoea while some were without diarrhoea (De Graaf et al. 1999).

The life cycle of *Cryptosporidium* occurs in two phases, sexual phase and an asexual phase.

In sexual phase, fusion of male and female gametes occurs while in asexual phase multiplication occurs on the mucosal surface. The infective stages are encysted in both parasites when released in faeces.The encysted oocysts are highly resistive to high temperature and can live for long time in various environmental conditions. Sometimes re-infection also occurs when there is ingestion of contaminated food, water or arthropods with oocysts or cysts occur (Chappell et al. 2006).

There are different methods used for the detection of *Cryptosporidium* oocysts in fecal and stool samples. Generally, microscopic examination is used for detection of *Cryptosporidium* oocysts in fecal samples. For microscopic examination, proper centrifugations, preparation of fecal smear from sediments and finally proper staining with Modified Ziehl- Nielsen (MZN) acid fast stain is necessary. Different antigen detection methods such as ELISA (enzyme-linked immunosorbent assay), immunofluorescence and genome detection methods such as PCR (amplification of 18SS rRNA genes) are used for detection of the *Cryptosporidium*. Each method is different from other in sensitivity and specificity. Among all diagnostic procedures, no one is accepted as the "best method" world widely (Areeshi et al. 2007).

To control cryptosporidiosis, is the big global challenge for veterinary as well as for human medicine. Different therapeutic agents up to thousands had been tested in vivo as well as in vitro conditions to treat cryptosporidiosis. Some agents were active against *Cryptosporidium* infection in vitro conditions but showed poor efficacy or no efficacy during treatment therapy under field conditions (Shahiduzzaman and Daugschies, 2012). The efficacy of different plant extracts such as garlic has been studied to treatment cryptosporidiosis (Wahba and A.A. 2003; Toulah et al. 2012) While in some experiments, the efficacy of different plant extracts were also studied to treat cryptosporidiosis such as pine-bark extract (Kim and Healey, 2001; blueberry extracts (Anthony et al. 2007) and onion and cinnamon (Abu El Ezz et al. 2011). Garlic (*Allium sativum*) was used as a convenient prophylactic and a promising therapeutic agent for Cryptosporidial infection. The efficacy of garlic as a prophylaxis measures and for the treatment of experimental cryptosporidiosis was explained by different mechanisms (Gaafar, 2012). El Shenaway et al. 2008 reported the enhancement of phagocytosis and an increase in natural killer cell activity, promoted the functions of immune system and strengthened the body's defense mechanism observed during the treatment period with garlic.

Pakistan has a large population of livestock and facing a large number of threats in form of infectious and non- infectious diseases. As a result of large number of threats the producing ability has been seriously affected. Different parasitic infections are responsible for poor performance in form of production and weight gain. These parasitic infections are causing a huge economic loss to our livestock industry. The *Cryptosporidium* infection is highly endemic in small and large ruminants as reported by different researchers in Pakistan.

CHAPTER 2
REVIEW OF LITERATURE

Enteric parasitic infections are the most common problem in humans, domestic and wild animal's world widely but are often reported in less developed countries and poor societies of the world (Saneian et al. 2010).The *Cryptosporidium* is an important zoonotic enteric protozoan parasite belonging to the phylum Apicomplexa and the family *Cryptosporidiida*e (Romero et al. 2001). There are currently 16 recognized species of *Cryptosporidium*, which have been isolated from a large variety of hosts including five classes of vertebrates such as amphibians, fishes, reptiles, birds and mammals (Xiao et al. 2004). Cryptosporidiosis was first described in diarrhoeic lamb in Australia. *Cryptosporidium* is as a primary etiological agent of diarrhea in lamb as it was confirmed in the early 1980 (Angus et al. 1982; Snodgrass et al. 1984).The *Cryptosporidium* affects both cattle's and humans (Bouzid et al. 2013). This parasite causes a big economic loss in cattle which result in heavy morbidity and mortality (Casemore et al. 1997; Lendner, 2011).The *Cryptosporidium parvum* can cause infection in small ruminants and also carries public health significance due to its zoonotic nature (Robertson et al. 2010; Wang et al. 2014). Cryptosporidiosis is a new emerging disease caused by the genus *Cryptosporidium*, and clinically characterized by watery diarrhea, dehydration, and loss of weight (Panciera et al. 1971; Fayer et al. 2000; Santin et al. 2004). The *Cryptosporidium* mainly affects the epithelial surface of the small intestine and rarely affect the liver, stomach, gall bladder and lungs in large number of mammals including human beings (Pilar-Izquierdo et al. 1993; Hunter and Thompson, 2005). The *Cryptosporidium* is a widely distributed opportunistic coccidian parasite and this organism has infected more than 170 species of vertebrates (Romero et al. 2001). Cryptosporidiosis is an important disease in sheep and goats which causes enteric problems and clinically characterized by watery diarrhea, dehydration and poor weight gain (Foreyt, 1990) while occurrence of the disease is more severe in kids and lambs than adult (de Graaf et al. 1999; Chalmers et al. 2002). During cryptosporidiosis, the infected animal shed a large number of oocysts such as (108-------109/gram). The major sources of infection are the *Cryptosporidium* oocysts which are responsible for causing cryptosporidiosis in animals as well as in humans. A single oocyst is adequate to cause an infection in all susceptible hosts. Oocysts are mainly transmitted through fecal-oral route and directly from host-to-host contact.Indirect transmission also occurs via contaminated food or water and though aerosol transmission of oocysts has been also reported (Ramirez et al. 2004). Zoonotic transmission was also documented via epidemiological survey such as farm animals, pets, and veterinary workers (Jafari et al. 2013).

Prevalence of the *Cryptosporidium* infection in small ruminants

A study was conducted by Dora Romero-Salas et al. 2016, to find out the percent prevalence of the *Cryptosporidium* infection in small ruminants and collected 160 fecal samples from sheep and goats and analyzed. As a result, 72.5% prevalence was recorded in goats while in sheep, 67.5% prevalence was observed. The highest percent prevalence was recorded in small ruminants at the age of 1 month where highest percent (88.2%) prevalence of the *Cryptosporidium* infection was recorded. .Overall prevalence was 60-85% among all herds. As result it was concluded that different risk factors such as animal species, sex, breed, town, farms and contact with cattle did not affect the prevalence of the infection.

MAB Shafiq et al. 2015, conducted a study to find out the percent prevalence of the *Cryptosporidium* infection in small ruminants (Sheep, Goats) with correlation to different risk factors such as age, diarrhoeic conditions and water born transmission.

The *Cryptosporidium parvum* is the most important zoonotic specie of the genus *Cryptosporidium*, responsible for causing heavy economic losses in small ruminants. Transmission mainly occurs when contacted with such animals infected with *Cryptosporidium* infection and contaminated water or vegetables or food. To record percent prevalence, a total of 300 small ruminants (n= 150 goats, n=150 sheep's) were studied. As a result, 18.66 percent prevalence was observed in goats while 21.33% prevalence was recorded in sheep. In lambs, 40% prevalence was recorded. As a result of the study, it was concluded that the highest percent prevalence was recorded in diarrhoeic goats and sheep.

During the study, two antiparasitic drugs such as Metronidazole and Paromomycine were used to evaluate their percent efficacy against *Cryptosporidium* infection through various therapeutic trials. As a result of therapeutic trials both drugs showed significant reduction in oocyst count but Metronidazole showed higher percent efficacy than other.

To find out waterborne transmission, 200 samples of water were collected from different sources such as canal water (n=50), tape water (n=50), tube well (n=50) and mineral water (n= 50). *Cryptosporidium parvum (C. parvum)* was recognized by measuring the size with the help of stage micrometry. As a result, an overall percent prevalence of *C. parvum* was 10.5% recorded in all water samples whereas, 28%, 8% and 4% was recorded in canal water, tap water and underground water respectively while there was no oocyst observed in minerals water bottles.

Danladi et al. 2015, conducted a cross sectional study in North Nigeria where percent prevalence of the *Cryptosporidium* infection along with the associated risk factors were studied. Fecal samples were collected directly from the rectum through disposable gloves. All the fecal samples were examined by concentration with formal-ether and stained all the samples by using

Ziehl-Neelsen acid fast staining technique. During the total study period, 900 animals (cattle =300, goats=300, sheep=300) were tested and found 178 positive for *Cryptosporidium* infection. As a result, 19.8% overall prevalence was recorded in cattle, goats and sheep. Overall percent prevalence was 28% (98/350), 17.1% (46/420) and 11.7% (34/290) in cattle, goats and sheep respectively.

According to the age based analysis, significant difference ($P<0.05$) was observed in sheep, goats and cattle.

On the basis of statistical analysis, non-significant ($P>0.05$) difference was observed in percent prevalence of the cryptosporidiosis in sheep, goats and cattle. It was also recorded that cattle were three times more sensitive to the infection than sheep and goats. Furthermore it was also observed that small ruminants with diarrhea were more prone to *Cryptosporidium* infection than healthy animals.

Dinka Ayana and Berhanu Alemu (2015) conducted a cross sectional study to find out the percent prevalence of the cryptosporidiosis in cow calves, lambs and kids and to evaluate different risk factors in Ethiopia. Fecal samples were collected from 364 animals including calves (n=214), lambs (n= 89), kids (n= 61).All the samples were concentrated by Sheather's flotation technique and stained by Modified Ziehl-Neelsen acid fast technique. They found an overall prevalence of 14%, 13.6% (29/214), 16.9% (15/89), and 11.5% (7/61) prevalence was recorded in calves, lambs and kids respectively. On the basis of statistical analysis, significant difference ($P < 0.05$) was observed between diarrheic and non-diarrheic animals whereas non significant differences were observed among the study areas, animal species and different age groups.

A study was conducted by Nektarios et al. 2015 to find out percent prevalence of *Cryptosporidium* infection in goat kids suffering from diarrhea and other risk factors responsible for occurrence of cryptosporidiosis were studied. During the study period, 292 goat kids were studied having age of 4-15 days from 54 different dairy goat herds in Greece. As a result, observed 76.4% prevalence of *Cryptosporidium* species and classify the intensity of the infection was marked as high, moderate and low. As a result, 142, 45, and 36 numbers of samples were ranked in high, moderate and low respectively. Finally it was concluded that prevalence was higher in herds with late kidding season (January-April) than those with was early kidding (September-December).

A study was designed and conducted by Gharekhani et al. (2014), to determine the percent prevalence of the *Cryptosporidium* infection in sheep without clinical signs in Iran where 1749 fecal samples were collected randomly. All the samples were collected from different rural parts of the Iran in the year 2011-2012. All the samples were stained with Modified Ziehl-Neelsen acid fast staining technique. As a result, out of 1749 samples, 198 were found positive and 11.3% prevalence was found positive.

A study conducted by Pam et al. (2013) recorded the highest percent prevalence of the *Cryptosporidium* infection in females in different species of animals in Nigeria. According to the sex based analysis, the highest percent prevalence of the *Cryptosporidium* infection was recorded in female’s cattle and small ruminants. As a result, it was concluded that certain risk factors such as lactation, pregnancy and hormonal imbalances recorded during the pregnancy period and lactation period are responsible for high prevalence of the *Cryptosporidium* infection in female.

2.1 : Historical perspective

Tyzzer was the first researcher who described the *Cryptosporidium* in 1907, where it was found in the laboratory mouse (Tyzzer, 1907) although the veterinary and medical significance of the *Cryptosporidium* infection was not fully studied for another 70 years. The maximum interest in the study and research of the *Cryptosporidium* was seen over the last two decades as the data was published in various journals and the maximum publications were published in 1980 - 1983.The primary history of the *Cryptosporidium* was documented in some book chapters and few review articles were published recently (O'Donoghue, 1995; Fayer et al. 1997; Tzipori et al. 1998).

Taxonomy:

Phylum: *Apicomplexa*: Cryptosporidium has an apical complex.

Class: *Sporozoasida*: Both sexual and asexual reproductive cycle occurs with oocysts formation.

Subclass: *Coccidiasina*: Life cycle consists of gametogeny, merogony and sporogeny.

Order: *Eucoccidiida*: Development of microgamy and macrogamy occurs independently.

Family: *Cryptosporidae*

Oocysts were containing four sporozoites which were visible when examined under microscope but there was no development of sporocyst (Levine, 1985).

Monoxenous life cycle:

The *Cryptosporidium* species requires a single host to complete its early life within gastrointestinal tract and there is no need of intermediate host. Salient features of the *Cryptosporidium* that can easily differentiate them from other coccidia are lack of proper host, lack of organ specificity, development of resistance to various antibiotics and can cause autoinfection (Tzipori , 1983).The following three broad features about cryptosporidiosis were recognized between 1980 and 1993(Tzipori and Griffiths ,1998).

The first feature of the *Cryptosporidium* infection was reported in 1980 that it is the most common, serious, and primary cause of an outbreak of diarrhea in mammals as well as also responsible for sporadic causes of diarrhea in some ruminants (Tzipori, 1983). Cryptosporidiosis was emerged as the most severe life-threatening disease in the year 1983 and onwards when the AIDS appeared as epidemic in nature in human population (Anonymous, 1982; Ma P and Soave R. 1983; Current et al. 1983; Forgacs et al. 1983) .In 1993, cryptosporidiosis became more popular when it was recognized the most important serious disease, difficult to control by using various drugs and are responsible for causing waterborne related diarrhea in humans and in mammals (MacKenzie et al.1994). The first seriousness and significance of the *Cryptosporidium* infection was reported in late 1970, when the cow calves were mainly affected by the *Cryptosporidium parvum* infection (Morin et al. 1976; Pohlenz et al. 1978) .It was also reported that the *Cryptosporidium* is an opportunistic enteric protozoan that is responsible for a few clinical signs or without any clinical signs. The *Cryptosporidium* infection has been documented as one of the major enteric protozoan linked with enteritis in goats causing diarrhea (Diaz et al. 2010).

2.2 : Morphology

The different species of the *Cryptosporidium* can be differentiated from each other on the basis of oocysts morphology and the site/ area of the infection. The length of the *Cryptosporidium* oocysts is varying from species to species, ranging from 4.5 um to 7.9 um. The width of oocysts ranges from 4.5 um to 6.5um. The oocysts of *C. parvum* are mainly ovoid to elliptical in shape which mainly depends upon the host of parasite (O` Donoghue, 1995).

2.3 : Life cycle

The life cycle of *Cryptosporidium* begins when the susceptible hosts such as cow calves, sheep, goats, kids, lambs, foals and humans ingest oocysts. After ingestion, following excystation occurs within the gastrointestinal tract and there is release of four sporozoites. The sporozoites then infect the intestinal epithelial cells and initiation of asexual development occurs. Asexual life results in production of eight and four merozoites respectively which further infect the epithelial cells of intestine when released in the lumen of intestine. The release of four merozoites from second merogony may result in development of sexual stage, the microgametes and macrogametes. The union of microgametes and macrogametes may result in zygote, which forms the highly resistant oocysts after two asexual divisions. Two types of oocysts are produced as a result of completion of sexual phase, thick wall oocysts and thin wall oocysts. Thick wall oocysts are excreted with feces to the environment and act as a source of infection when ingested by susceptible hosts while thin wall oocyst cause autoinfection, when the wall of oocyst rupture inside the host body. The resistive oocysts contain four sporozoites and remains still within a membrane known as parasitophorous membrane (O'Donoghue, 1995; Tzipori and Griffiths, 1999).

Figure: 2.3 Life cycle of the *Cryptosporidium parvum*

The life cycle of the *Cryptosporidium* is represented in Fig. 2.3, and all major phases are mentioned briefly here. The life cycle of the *Cryptosporidium* is completed within a single host (monoxenous cycle) without need of intermediate host and both sexual and asexual replication occurs.

2.4: Incubation Period

It has been estimated that the incubation period of the *Cryptosporidium* lies between 5-7 days as confirmed in various experimental infections (Chappel et al. 2006). The average reported incubation period ranged from 3-7 days as reported in Milwaukee during the large water born outbreak of cryptosporidiosis, but it was shorter in elders (5-6 days) when compared with children where average range was 7 days while in adults, average range was 5-6 days (Naumova et al. 2003).

2.5 : Mode of transmission and source of infection

Humans, cow calves, lambs and goat kids can release oocysts in large quantities when clinically are positive for the *Cryptosporidium* infection. During an acute infection or chronic infection, >10^{10} oocysts can released. The main transmission occurs through fecal- oral route, either directly or indirectly. Indirect transmission mainly occurs through contaminated water or food when washed with contaminated water and ingested. Infected humans, cattle and other domestic animals are the most important sources of contamination of surface water and environment. The *Cryptosporidium parvum* is mainly responsible to cause water born diarrhea. The waterborne diarrhea mainly occurs through the ingestion of oocysts (< 6-10 oocysts) in small numbers. Oocysts are highly resistant to various common disinfectants. Infected humans and animals discharged oocysts in large quantity and finally contaminate the surface water. Small ruminants (Sheep and goats) act as source of reservoirs for the *Cryptosporidium* infection in humans (Koinari et al. 2014). An outbreak of the *Cryptosporidium* infection was reported in school children where contact with lambs and kids were recorded (Lange et al. 2014). Furthermore, an association was also reported about prevalence of the *Cryptosporidium* infection in children and drinking of unpasteurized milk of goat (Rosenthal et al. 2015). It has been reported recently that the mammalian species known as *Cryptosporidium parvum*, is mainly responsible for causing infection in humans (O'Donoghue, 1995; Fayer at al. 1997; Tzipori et al. 1998).

A study was conducted in Turkey where an infection was reported in humans caused by *C. meleagridis* which is a turkey respiratory *Cryptosporidium* (Slavin D, 1955).The significance of the *Cryptosporidium parvum* infection was became more evident in the early 1980s, when reported in new born cow calves. The incubation period of *C. parvum* infection and its clinical signs in small ruminants were experimentally reproduced and reported extensively in small ruminants (O'Donoghue, 1995; Fayer at al. 1997; Tzipori et al. 1998).

The *Cryptosporidium parvum* mainly occurs in every herd of domestic cattle, world widely. There are two major reasons for continuous contamination of environment, asymptomatic infections and prolonged excretion of oocysts. The *C. parvum* infection is also common in goat, sheep and swine herds but prevalence is poorly documented (O'Donoghue, 1995; Fayer et al. 1997; Tzipori et al. 1998).

Cryptosporidial infection has been also reported in cats, dogs (Pohjola, 1984) and horses which are considered as the potential source of infection for humans. However it is not clear that *C. parvum* causes a diarrhea in dogs, cats and horses (O'Donoghue, 1995; Fayer at al. 1997; Tzipori et al. 1998.) and prevalence of cryptosporidiosis is not well documented in these species of animals. The wild animals suffering from the *Cryptosporidium* infection has also play an important role in contamination of environment and transmission of the disease.

There is also evidence that the *Cryptosporidium* transmission may also occurs by inhalation route in addition to the oral-fecal route. Pulmonary cryptosporidiosis has been reported in a patient suffering from AIDS (Ma P et al. 1984) and also in children, suffering from laryngotracheaitis, confirmed by examination of tracheal aspirates (Harari et al. 1986). The prevalence of laryngotracheal infection in immunocompetent humans is still unknown. It has been also reported that the children when suffering from diarrhea due to cryptosporidiosis, may also show mild type of respiratory signs (42%). The transient RTI (respiratory tract infection) is mostly common in healthy children and act as a source of transmission from person to person (Egger et al. 1990), So we should be aware about the risk involved in laboratory while working with the *Cryptosporidium parvum* and should adopt all the necessary precautions to avoid any accidental infections by inhalation of aerosolized oocysts (Tzipori, 1988).

2.6 : Economic significance

Cryptosporidiosis is one of the most important disease in various agro-ecological zones causing an enormous economic loss in livestock sector. Cryptosporidiosis is a big threat to the economy of livestock worldwide (Ayinmode and Fagbemi, 2011; Paul et al. 2014).It has been also documented as a main constraint to the production of livestock worldwide and causing high morbidity and occasionally high mortality rates among different domestic animals such as cattle, sheep, goat, horses and pig which result in big economic losses (Degerli et al. 2005; Nasir et al. 2009; Prakash et al. 2009; Potter and Esbroeck. 2010; Ayinmode and Fagbemi, 2011). Hence cryptosporidiosis is zoonotic in nature so infected animals can cause the health risk to the humans especially in those individuals suffering from AIDS/HIV (Tzipori and Griffiths, 1998).

The *Cryptosporidium* has been considered as one of the most important enteric pathogen causing neonatal diarrhea in goats, sheep and cattle (Wang et al. 2010; Maurya et al. 2013). The primary infection mainly occurs through fecal- oral route and less than 50 oocysts can infect the healthy animal (Fayer et al. 2000). Infection takes place, when the microscopic oocysts are ingested with feed or water. Infected animals, discharge oocysts in the feces, which spread rapidly and can survive in any harsh environment (Bowman, 2003).There will be rapid spread of oocysts especially when the animals are housed overcrowd or when the udders are contaminated with infected feces (Nasir et al. 2009). Oocysts are highly resistant to various harsh environmental factors such as hot or cool or dry environments and act as a source of infection for months in cold water (Olson et al. 1997). Cryptosporidiosis causes heavy economic losses due to retarded growth, high mortality, cost of drugs, veterinary assistance and labour cast (De Graaf et al. 1999). During cryptosporidiosis, there is damage of small intestine microvilli which mainly predisposes the combined infection of Rotaviruses, coronaviruses, E. coli and rarely with salmonella species (Rebhun, 1995). Due to the mix enteric infection, the prognosis became unfavorable, on the basis of complicated treatment and clinical signs which may result in high mortality. When the mixed infections become more severe then death of the lambs occur within 2-3 days after the onset of diarrhoea (Coop and Wright, 2000)

2.7 : Zoonotic significance

Cryptosporidiosis is the main enteric problem in children and adults in less developed countries, characterized by causing a significant level of morbidity and mortality in those individuals suffering from AIDS (Saneian et al. 2010).The *Cryptosporidium* causes a severe enteric infection in humans, known as cryptosporidiosis and reported in developed as well as in less developed countries. The *Cryptosporidium* is zoonotic and ubiquitous in nature and can cause infection with a few numbers of oocysts when ingested with a food or water. Upto10 oocysts or less than 10 oocysts are enough to cause a disease if ingested (Okhuysen et al. 1999). However there is also evidence of transmission of Cryptosporidial infection to humans from birds (*Cryptosporidium meleagridis*), Cats (*C. felis) and* dogs (*C. canis*) because these species have been detected in human patients suffering from cryptosporidiosis (Pedraza-Diaz et al. 2001; Xiao et al. 2001; Fayer et al. 2001).

Children are mostly suffering from the Cryptosporidial infection at the time of weaning or after the weaning period and the infection remain in episodic form throughout the life.

When exposure occurs to the *Cryptosporidium* oocysts either directly or indirectly, may result in acute diarrhea. The *Cryptosporidium parvum* causes an acute diarrhea, abdominal pain and self- limiting infection in those patients suffering from AIDS/HIV and the onset of the disease may be fast and extend from 3 to 7 days depending upon a combination of host (age, presence of maternal antibodies or previous exposure, and infectious dose) and parasite (origin and age of oocysts, and species/genotype) factors.

Initially, the infection occurs in the small intestine when the emerging sporozoites attack on enterocytes where amplification of sporozoites occurs in the epithelial surface of crypts and villi. The *Cryptosporidium* infection occurs throughout the GIT or sometime the infection remains localized in various segments of small and large intestine.

Cryptosporidiosis causes a serious infection in patients suffering from AIDS and clinically characterized by persistent life-threatening diarrhea (Blanshard et al. 1992). Prolonged infections remain for long time from months to years in those peoples suffering from immunodeficiency which may either be acquired (Blanshard et al. 1992) or congenital (Hayward et al. 1997). There is spread of immunodeficiency from the gut to the pancreatic ducts causing cholecystitis, cholangiohepatitis, pancreatitis or choledochitis. When the gut is chronically infected, there is disruption of epithelial surface, cellular infiltration, fibrosis and abscessation of crypt (Tzipori et al. 1995). In developed countries, the prevalence of cryptosporidiosis is not high (5-15%) in those patients suffering from AIDS. Due to lack of proper effective treatment of cryptosporidiosis is very difficult to control in those patients suffering from HIV/AIDS.

Goats are an important source of potential zoonosis for cryptosporidiosis. Infected domestic animals and wild animals contaminate the water and the outbreak of water born cryptosporidiosis has been documented 19 times in various research papers affecting more than 427100 individuals (Smith and Rose, 1998).It has been also estimated that infection rate with crypto was ranging from 250 to 500 million peoples in Africa, Asia and Latin America (Current and Garcia, 1991).

The first case of human cryptosporidiosis was reported in animal handlers. In the year 1983, an outbreak of human cryptosporidiosis was recorded in human beings. Similarly an outbreak of cryptosporidiosis was recorded in Georgia in the year 1987 where 13000 peoples, were infected. The first information about the spread of the *Cryptosporidium* infection was recorded through drinking water where 400000 peoples were infected and death was recorded in those patients suffering from AIDS/ HIV.

Nemes also reported high mortality rate in humans suffering from cryptosporidiosis and 5 to 10 million deaths were recorded internationally each year (Nemes, 2009). In such situation, the *Cryptosporidium* is considered as one of the major cause of diarrheal disease, globally (Shirley et al. 2012).

2.8 : Diagnosis of cryptosporidiosis

Detection of cryptosporidial infection depends on the presence of intact oocysts in stool or feces because the infected individual excrete large number of oocysts so the diagnosis is an easy task and not difficult. Cryptosporidiosis can be diagnosed in the laboratory by one of the following methods;

A. Using simple microscopic examination of slides, stained with Modified Ziehl Neelsen acid fast staining technique.

B. Collection of biopsy specimens from small intestine.

C. Detection of the *Cryptosporidium* antigen in fecal materials or stool specimen through ELISA.

D. Molecular detection methods (PCR) through extraction of DNA.

A. Detection of the *Cryptosporidium* oocysts in stool or fecal material.

The *Cryptosporidium* infection is commonly detected through conventional microscopic examination of fecal smears, stained with Modified Ziehl Neelsen acid fast staining technique under 100X (Ayinmode and Fagbemi, 2010)**.** Mature oocysts of the *Cryptosporidium*, can be recovered from fecal material or stool examination. The oocysts are rounded and 4-6 um in size. The Thick wall oocysts contain four sporozoites. The oocysts can be easily identified by using various staining methods such as acid fast stain, safranin stain and certain dyes such as fluorescent dyes such as carbol fuchsin method. Different diagnostic methods have been discovered for diagnosis of the *Cryptosporidium*. The most general and easily accessible method for finding of oocyst by making fecal smears stained with Modified Ziehl- Neelsen acid fast staining technique (Ramirez et al. 2004; Fresan et al. 2004).

Proper concentration and centrifugation of the fecal or stool sample is necessary before microscopic examination to recover maximum oocysts. During diagnosis, if the result is negative then at least three times fecal or stool sample should be tested. However it has also been reported in certain studies that the first sample is usually enough to provide 90% accurate result for diagnosis of the *Cryptosporidium oocysts* in fecal or stool sample if the satins are fresh.

B. Detection of *Cryptosporidium* oocysts in intestinal fluids or in biopsy specimens

Nowadays biopsy technique is not commonly used. This method was used in very early time when antigen detection methods and various staining chemicals were not available. False negative results were commonly faced during this method due to irregular nature of the enteric protozoan infection (Flanigan and Soave, 1993)

C: Detection of the *Cryptosporidium* antigen in Fecal or stool sample

(i): ELISA (Enzyme Linked Immunosorbent Essay)

Immunological detection techniques are highly sensitive and specific techniques and were used in clinical and environmental monitoring of the cryptosporidial infection. There was no need of concentration of specimens before testing. ELISA was used to screen large number of specimens in a very short time. This technique did not depend on skills as in microscopy. However, it was necessary to maintain the proper quality of the reagents commercially available.

(ii): Immunofluorescence assay

This technique has proved the maximum good results due to highest level of sensitivity and specificity and has been considered the standard test by maximum laboratories.

This technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories. On the other hand, this test is unable to provide permanent record information such as stained slides which can be preserved for a long time. Proper concentration of fecal or stool sample is necessary before performing this test.

The sensitivity and specificity of the commercially available kit for detection of antigen may range from 66 to 100 percent and 93 to 100 percent respectively.

D: Detection of the *Cryptosporidium* oocysts by using Molecular Techniques

PCR is an important diagnostic tool on the basis of highly sensitivity and specificity to detect Cryptosporidial infection in clinical and environmental specimens and to explain the classification and genotypes within *Cryptosporidium* species. (Higgins et al. 2001). Detection of the *Cryptosporidium* oocysts in fecal samples of sheep has been documented in certain countries(Quilez et al. 2008, Silva-Fiuza et al. 2011).Molecular technique (PCR) is used for the detection of *Cryptosporidium* infection in fecal or in stool specimens. Fecal material must be frozen or must be stored in potassium dichromate for detection of the DNA through PCR. Finally, it is concluded that acid fast staining methods are mainly used in various clinical laboratories for diagnosis of cryptosporidiosis. Immunofluorescence microscopy is the best method due to highly sensitivity and specificity. Molecular techniques are mainly used for research tool. The sensitivity and specificity of PCR ranging from 97-100% and 100% respectively for detection of the *Cryptosporidium* infection (Bialek et al. 2002). PCR is highly specific and sensitive technique for exact diagnosis, proper identification and classification of various species of the *Cryptosporidium* (McGlade et al, 2003).

2.9 : Examination of oocysts in feces

Oocysts can be detected by making a thin smear of fecal materials, stained with the help of Modified Ziehl-Nielsen (MZN) acid fast staining technique. The oocysts appeared bright red granules after proper staining. (Taylor et al. 2000). The severity of the infection can be classified as 0-5 classes on the basis of total number of oocysts/slide/sample. At the end of first and second weeks of post infection, the numbers of oocysts were at peak (Enemark et al. 2003)

2.10 : Clinical Findings of cryptosporidiosis

Young animals are highly sensitive to the *Cryptosporidium* infection, whereas clinical signs in adult animals are usually asymptomatic or may not be appearing (Ulutus and Voyvoda, 2004; Ramirez et al. 2004; Ozdal et al. 2009). In small ruminants (sheep), Cryptosporidiosis is characterized by mild to severe type liquid and yellowish diarrhea with a strong dirty smell, depression, weight loss, colic, abdominal cramps, flatulence and usually death occurs up to the age of one month (Ramirez et al. 2004; Castro- Hermida et al. 2007; Silva-Fiuza et al. 2011)

Similar clinical signs were also documented by other researchers and scholars such as severe diarrhea, emaciation, loss of body fluids result in dehydration and loss of body weight (Panciera et al.1971; Fayer et al. 2000; Santin et al. 2004). In small ruminants, cryptosporidiosis is caused by an important enteric protozoan known as *Cryptosporidium parvum* and clinically characterized by diarrhea, dehydration, anorexia, intermittent discharge and poor weight gains (Foreyt, 1990). Cryptosporidiosis is more severe in lambs and kids than adult small ruminants (de Graaf et al. 1999; Chalmers et al. 2002).The severity of the disease may be connected with the nutritional status and immune status of the animal (Olson et al. 1997). There was loss of 2 kg body weight in goat kids suffering from natural infection with *C. parvum* when it was compared with the same age healthy goat kids as reported by de Graaf et al. 1999.

2.11 : Symptomatic Infection in Humans

In humans, the symptomatic period of cryptosporidiosis is clinically characterized by abdominal pain, diarrhoea, low grade mild fever, vomiting or nausea, malaise, weight loss, anorexia and fatigue (Fayer and Ungar 1986; Casemore, 1990).There is sudden onset of watery diarrhoea and about 3-6 number of stools are passed per day but some times the number may increase which may contain mucus and sometimes offensive. Symptoms usually remain for 3 weeks but in some patient's chronic diarrhoea may also develop which last up to the month or longer. Oocysts are continuously shed from the body with an average period of 7 days and the range may be extended from 1-15 days even after the disappearance of symptoms while the shedding of oocysts occurs exceptionally up to 2 months in some cases (Jokipii and Jokipii, 1986).

2.12 : Pathological Examination

Gastrointestinal tract of the animal should be selected for postmortem examination within 15 minutes following death or euthanasia. For histological examination, samples should be taken from various parts of small and large intestine such as duodenum, jejunum, ileum (small intestine parts), caecum, colon and rectum (large intestine). During cryptosporidiosis histological changes were observed in the mucosal layer of the affected part of the gastrointestinal tract (Tzipori et al. 1981).

2.13 : Immunohistochemistery:

Direct immunofluorescent-monoclonal antibody staining technique, has been shown to find the highest detection rate in comparison to acid-fast staining (Allies et al. 1995) although the later showed 83.7% sensitivity and 98.9% specificity as compared to PCR. Whenever there is low grade infection due to the *Cryptosporidium*, usually "immunohistochemistery" is the only tool used for proper diagnosis of the *Cryptosporidium* infection in a fecal specimen, mainly as an immunofluorescence with FITC conjugated monoclonal antibody directed against the wall of oocysts. The *Cryptosporidium* infection was diagnosed by Xiao and Herd, 1993; with the help of immunofluorescence technique in those animals showing diarrhea. "Cellulose acetate membrane filter dissolution method" was reported by (McCuin et al. 2001) to recover the oocysts of the *Cryptosporidium*.

2.14 : Polymerase Chain Reaction (PCR)

At molecular level, percent prevalence of the *Cryptosporidium* infection was studied by Agnieszka Kaupke1 et al 2017 to identify the *Cryptosporidium* species in different breeds of sheep and goats reared under different managemental conditions in Poland. Different risk factors were studied such as breeds, sex and age to find out percent prevalence of the *Cryptosporidium* infection.

Fecal samples were collected from 234 lambs and 105 goat kids up to 9 weeks of age from 24 breeds. In lambs, the molecular percent prevalence was 19.2% (45/234) detected through PCR whereas 37.1% (39/105) was detected in goat kids. The 18SSU rRNA was the common locus for identification of the *Cryptosporidium*. All *Cryptosporidium* species were identified on the basis of 18SSU ribosomal RNA (rRNA).On the basis of molecular detection, we identified the following species of the *Cryptosporidium* such as *C. bovis*, *C. parvum*, *C. xiaoi*, **C.** *hominis* and *C. ubiquitum.* In sheep, GP60 subtype was identified which revealed the occurrence of the *Cryptosporidium parvum*. In sheep, subtype *C. parvum* IIaA17G1R1 was identified while in goats IIdA23G1 subtype was identified. According to the study, there was non-significant difference was observed between different two age groups. In sheep, there was no correlation between the breeds and occurrence of different species of the *Cryptosporidium* while in goats, breed wise differences was correlated with prevalence of the *Cryptosporidium* infection in Poland.

Microscopy, immunoassay and PCR diagnostic techniques are the most commonly used in routine clinical diagnosis for presence or absence of the genus *Cryptosporidium* in various diagnostic laboratories. Polymerase chain reaction is followed by RFLP is widely employed for the detection, identification and sequencing of the *Cryptosporidium* species which are needed for epidemiological investigations. The genome is targeted by these assays at different regions which include the small subunit rRNA, thrombospondin related adhesive protein, *Cryptosporidium* oocyst wall protein, 70-kDa Heat Shock Protein and actin gene (Rochelle et al. 1996 ; Peng et al. 1997; Spano et al. 1997; Elwin et al. 2001; Sulaiman et al. 2002; Jiang et al. 2005).

On the basis of zoonotic nature of *Cryptosporidium,* the interest was grown in the study of cryptosporidiosis in the last few years, especially when the molecular techniques were used to describe different spp., genotypes and subtypes (Plutzer and Karanis, 2009). At present there are more than 22 species of the *Cryptosporidium* has been reported by using various molecular techniques. Out of 22 species, 12 species are present in mammals. By using PCR technique, more than 61 genotypes of the *Cryptosporidium* were identified on the basis of host and genetic analysis (Xiao, 2010). PCR technology is one of the most important alternative techniques for detection of the *Cryptosporidium parvum* in both clinical and environmental samples (Chung et al. 1998). PCR is an effective technique to identify even a single *Cryptosporidium* oocysts in clinical and environmental samples (Wagner- Wiening and Kimming,1995).In spite of the variability in DNA extraction for each sample, PCR is one of the best diagnostics technique for the detection of the *Cryptosporidium*. Above 50% correlation has been recorded between PCR results and various tests results. In a previous study, it was reported that 62.4% correlation is present between nested PCR and IFA results while exceptional correlation was 100% between IFA and nested PCR results in detection of the *Cryptosporidium* from fecal samples (Kostrzynska et al. 1999).

Microscopic examination can expose the presence of blank oocysts in fecal samples and similar results were declared by IFA but negative results were declared by PCR (Kostrzynska et al. 1999). Developments of specific antibodies are necessary for detection of the *Cryptosporidium* species by immunofluorescence method (Kostrzynska et al. 1999).

2.15 : Rapid immune- chromatographic assays

Cryptosporidium oocysts can be easily detected in animal feces with the help of Rapid immune- chromatographic assays and the performance of this assay can be evaluated by employing a reverse transcriptase PCR and a golden standard (Klein et al. 2009). The sensitivity and specificity of this assay is highly correlated with sedimentation and flotation technique for detection of *C. parvum.* Such assays are very fast and sensitive and can overcome all the deficiencies face in conventional diagnostic methods. These assays are also practicable for veterinarians, which require sensitive, reproducible, fasts and simple diagnostic techniques for fast therapeutic and prophylactic procedures (Luginbiihl et al. 2005).

2.16 : Source of infection

The *Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia lamblia* and *Cyclospora* species are highly prevalent in the environment and are responsible for causing water born and food borne diseases (Ayeh et al. 2009). *Cryptosporidium* is worldwide in distribution and its infection has been reported in more than forty domestic and wild species including mammals, reptiles, birds and fish (Fayer and Ungar, 1986).The *Cryptosporidium* species are the big source and threat of contamination to water supplies because they are highly resistant to chlorine disinfection, low and high temperature, can cause the infection with low dose and are transmitted by many animal species (Xiao and Fayer, 2008). As a result of big threat to water supplies, abundant waterborne outbreaks of Cryptosporidiosis have been observed in various parts of the world (Jiang et al. 2005). So, *Cryptosporidium* is one of the main pathogen occurring in drinking water, identified by drinking water regulatory authority in different industrialized countries (Gostin et al. 2000). There are few factors which are responsible for contamination of water by *Cryptosporidium* oocysts such as farm animals, wild animals and humans (Jiang et al. 2005).

There are few other factors such as age and season, which can also influence the prevalence (%) of the *Cryptosporidium* infection. The percent prevalence of the cryptosporidiosis on the basis of age and season has been reviewed as under:

2.17(a): Age related prevalence of cryptosporidiosis

A highly significant percent prevalence of the *Cryptosporidium* infection was 55.07% observed in diarrhoeic lambs having age less than 14 days while low prevalence up to 15.18% was recorded in healthy lambs having age ranging from 15-30 days old with the history of diarrhea. A study was conducted in Tobago and Trinidad, where the percent prevalence of the *Cryptosporidium* infection was 25.5% in diarrhoeic lambs and their ages was ranged from 1-90 days while on the other hand the percent prevalence was 11.4%, in non diarrhoeic lambs (Kaminjolo et al. 1993).

A study was conducted in Poland, where 60 healthy lambs having age less than 3 months and 90 adult sheep's, were selected randomly for percent prevalence of the *Cryptosporidium* infection on the basis of fecal examination. As a result, the highest percent prevalence of the *Cryptosporidium* infection was reported in lambs as compared to adult sheep (Majewska et al. 2000). Causape et al.(1999), reported that 1-14 days old lambs were 2-3 times more infected with *Cryptosporidium* infection than thos having age more than 15 days old while prevalence(%) rate was 7.8% in ewes excreting oocysts of the *Cryptosporidium* in a very small number. Furthermore, it was also reported in the same study that the highest percent prevalence was 83% in lambs with diarrhoea.

A similar study with the same findings was also conducted by Alonsofresan et al. 2005, in Mexico in lambs without diarrhea and in healthy sheep, though the exact age of the lambs was not mentioned. A small percentage of the *Cryptosporidium* infection was reported by Sevinc et al. 2005 in lambs having age about 2 months and it was detected through either presence of oocyst in fecal material or with fecal antibody detection (ELISA test).

In Switzerland, a study was conducted where small numbers of lambs with diarrhoea were examined by Ozmen et al. 2006 for detection of percent prevalence of the *Cryptosporidium* infection in fecal samples who documented that the *Cryptosporidium* infection was common.

In Serbia, a study was reported by Misic Z et al. 2006 and observed that the *Cryptosporidium* infection is the most common problem in diarrhoeic lambs. It has been also proved and acknowledged by different researchers that an age related resistance to the *Cryptosporidium* infections are exists in small ruminants. During the first two weeks of life, the *Cryptosporidium* infection is more common in young lambs while visible decrease has been reported in lambs, positive for *Cryptosporidium* infection with the passage of time as the immunity develops (Ortega-Mora et al. 1999; Causape et al. 2002; Giadinis et al. 2007).

The present study reveals that high prevalence of *Cryptosporidium* infection is responsible for causing diarrhea in new born lambs in the Greece. These findings provide useful information to the clinicians who can consider cryptosporidiosis while making differential diagnosis for the treatment of lamb diarrhea. The present study also illustrate that high prevalence of diarrhea mainly occur at the age of 15 days in neonatal lambs which is highly associated with the cryptosporidiosis. Cryptosporidiosis is one of the most common cause of diarrhoea in lams at the early age of 4-15 days and many experiments have been conducted for its treatment, prevention and control (Naciri and Yvore 1989; Causape et al. 1999; Viu et al. 2000; Castro-Hermida et al. 2001; Naciri et al. 2005).

The *Cryptosporidium* infection also occurs in cow calves suffering from calf scour alone or with other enteric pathogens such as bacteria or viruses (Bulgin et al. 1982; Reynolds et al. 1986). Deficient data is available on the epidemiology and various risk factors of lamb diarrhoea and its association with Cryptosporidiosis worldwide (Berg et al .1978; Tzipori 1983; Ozmen et al. 2006).

A study was conducted by Ortega- Mora et al. (1999) in Spain and it was estimated that an asymptomatic ewe can expel about 20,000 to 444000 oocysts / day.

It was also reported that the minimum infective dose is 1 oocyst/lamb while the average infective dose is 5 oocysts/lamb so it was recorded that 4000-110000 infective doses/ day can shed by an asymptomatic ewe(Ortega-Mora et al. 1999).If we use highly sensitive diagnostic technique such as an immunologic technique(ELISA, IFAT) or PCR technique, the number of infected ewes could be decreases because these diagnostic techniques are more sensitive and accurate than simple microscopic examination and an early diagnosis is possible with a single oocyst (Ortega-Mora et al. 1999; Ryan et al. 2005; Sevinc et al. 2005). The shedding of increased number of oocysts also depends upon the stage of production of ewes and it was recorded at the stage of periparturient period in ewes (Ortega-Mora et al. 1999). Higher prevalence of *Cryptosporidium* infection was also reported in healthy dairy ewes that can serve as a source of reservoirs for the transmission of the infection.

2.18(b): Season related prevalence of Cryptosporidiosis

The cryptosporidiosis is worldwide in distribution and commonly found in all wet and warm seasons of the year as described by Jafari et al. 2013 and Green et al. 2004.

The effect of season has been also reported on the percent prevalence of the cryptosporidiosis in various cattle farms while seasonal cryptosporidiosis mainly related with the calving season so the highest number of calves are at high risk at the age of 1-3 weeks (Lefay et al. 2000). In US, a study was conducted by Garber et al. 1994; with the results that slightly high percent prevalence of the *Cryptosporidium* infection was reported in the summer season while high prevalence was reported by Mohammed et al. (1999) in winter season and no seasonal effect on prevalence of cryptosporidiosis was reported by Ongerth and Stibbs, 1989. There is limited data, available about the seasonal prevalence (%) of cryptosporidiosis in lambs. In NW Spain, a study was conducted about seasonal percent prevalence of the *Cryptosporidium* infection and as a result, 90% farms of small ruminants were found positive and 24% lambs were found positive in spring while in autumn, 40% farms were infected where 8% lambs were positive for Cryptosporidiosis (Matos-Fernández et al. 1994). The highest percent prevalence was documented in summer and spring than in winter and autumn.

2.19 : Country wise prevalence of the *Cryptosporidium* infection in small ruminants

Different studies were conducted to find out the percent prevalence of the *Cryptosporidium* infection in small ruminants in different countries of the world is illustrated in the table. (2.1)**:**

Table 2.1: Country wise prevalence (%) of the Cryptosporidium infection in small ruminants.

Sr.	**Species**	**Animal**	**Country**	**Prevalence**	**Reference**
1	*C. parvum*	Sheep	Spain	31-59%	Causape et al. 2002
2	*C. parvum*	Sheep	Poland	10.1%	Majewska et al. 2000
3	*C. parvum*	Sheep	Egypt	2.5%	Mahfouz et al. 2014
4	*C. parvum*	Sheep	Iran	11.3%	Gharekhani et al. 2014
6	*C.parvum*	Sheep	Iraq	13.3%	Mahdi and Ali, 2002
7	*C. parvum*	Sheep	Tunisia	11.2%	Soltane et al. 2007
8	*C. parvum*	Sheep	India	1.8%	Maurya et al. 2013
9	*C. parvum*	Sheep	Papua New Guinea	2.2%	Koinari et al. 2014
10	*C. parvum*	Goat	Spain	30%	Castro-Hermida et al. 2007
11	*C. parvum*	Goat	Poland	0%	Majewska et al.2000
12	*C. parvum*	Goat	Iraq	17.7%	Mahdi and Ali, 2002
13	*C. parvum*	Goat	Papua New Guinea	4.4%	Koinari et al. 2014

2.20 : Management

Overcrowded or intensive management system could be attributed to higher percent prevalence of cryptosporidiosis in domestic animals where animals of different ages are placed together or when infected animals are housed together with healthy animals. When animals are raised under such management system, will be highly susceptible to the infection because the oocyst can easily contaminate the living area and infect the animals by ingestion (Castro-Hermida et al. 2002; Ayinmode and Fagbemi, 2010; Alemayehu Regassa and OdaGizaw, 2013). Similarly small ruminants such as sheep and goats should not be raised together because it can transmit the infection from infected animals to the healthy animals. We can also minimize the chance of infection in small ruminants when the adequate space is available at night.

2.21 : Chemotherapy

There are two major problems regarding proper control of cryptosporidiosis (a): Lack of effective remedy to control the disease (b): To protect the environment from contamination of the *Cryptosporidium* oocyst. The oocysts are highly resistant to different disinfectants and various stressful environmental conditions such as high temperature or low temperature so it is the source of contamination for long time (O'Donoghue, 1995).As a prophylactic agent different vaccines and drugs have been used against Cryptosporidiosis in humans and animals but low efficacy have been observed (Santin and Trout , 2008).

In south Europe, several experiments have been conducted during the last few years on the prevention and treatment of the cryptosporidiosis in lambs, kids and cow calves. Different drugs have been used and their efficacy was recorded. Halofuginone lactate was reported as the most effective drug and authorized drug for the treatment as well for the prevention of the cryptosporidiosis as reported in Greece where used as a best therapy for treatment of the cryptosporidiosis in cow calves, lambs and goat kids. The selected Halofuginone dose rate was

100 μg per kg bodyweight (1mL per 5 kg body weight) with no side-effects. This dose rate was generally accepted for the treatment and prevention of the cryptosporidial infection in cow calves (Jarvie et al. 2005) and the same dose rate has been used in lambs during experimental infection in France (Naciri et al. 2005). Two studies were conducted in France where halofuginone was diluted with 20 ml reconstituted milk powder before administration while in other study it was administered undiluted. Two other experiments were conducted in lambs, where halofuginone was used at the dose rate of 500 μg per kg body weight. One experiment was conducted in France (Naciri & Yvore, 1989) while other was in Spain (Causape et al. 1999). During these two experiments, no side-effects were recorded when halofuginone was used at the dose rate of 500 μg per kg body weight for three days consecutively but weight gain and feed consumption was reduced in treated lambs where halofuginone was used for five days consecutively (Naciri & Yvore, 1989). Major side-effects were observed when halofuginone was used at the dose rate of 300 and 500 μg/kg body weight (Jarvie et al. 2005). Halofuginone has shown ineffective results when used at the dose rate of 30 μg/kg and satisfactory results have shown when used at the dose rate of 60 μg/kg. Excellent results have been reported when halofuginone was used at the dose rate of 100 μg/kg reported by Naciri et al. 1993. Halofuginone has been used in low doses in calves. The drug was shown to be ineffective at the dose rate of30 μg/kg, but produced satisfactory results when used at the dose rate of 60 μg/kg. However, the best results have been achieved at the dose rate of 100 μg/kg (Naciri et al., 1993).Therefore halofuginone, has not been used in lower doses than 100 μg/kg in lambs as reported by (Naciri et al. 2005).

When other drugs such as paromomycin (Viu et al. 2000) and cyclodextrin (Castro-Hermida et al. 2002), were tested against cryptosporidiosis in lamb, the highest therapeutic results were observed.

Duration of administration was one of the most important aspects of halofuginone for the prevention of the natural cryptosporidiosis (Naciri and Yvore 1989) and the best results were achieved when halofuginone was administered for 3 days at the dose rate of 500 μg per kg body weight in lambs experimentally infected. When Causape et al. 1999, administered halofuginone at the dose rate of 100 μg/kg to lambs naturally infected with cryptosporidial infection, oocyst shedding and clinical signs disappeared in a short time while similar findings were also observed by Naciri et al. 2005.

NTZ (nitazoxanide) is one of the new chemotherapeutic agent, highly effective against cryptosporidiosis. NTZ has been shown a broad spectrum parasiticidal activity against nematodes, cestodes, trematodes and protozoa (Rossignol and Maisonneuve, 1984; Romero Cabello et al. 1997). NTZ is the drug of trials for cryptosporidiosis on the basis of its reported efficacy against various protozoans and parasites. A trial was conducted in Mali to find out the efficacy of NTZ in 12 patients suffering from AIDS and Cryptosporidiosis. In seven patients there was more than 95% reduction in oocyst excretion and four of seven were completely free of diarrhea (Doumbo et al.1997).

Table 2.2: The efficacy of different drugs used for treatment of cryptosporidiosis in small and large ruminants.

Efficacy of different drugs used against cryptosporidiosis in small and large ruminants.						
Drug	Animal species	Dose Rate	Period of administration	Shedding of oocyst	Occurrence of diarrhoea	References
Halofuginone lactate	Lamb	500 μg	1–5 days	Prevented	Prevented	Naciri and Yvoré, 1989
Halofuginone lactate	Cow calf	30 to 500 μg	3–14 days	Prevented	Prevented	Harp et al. 1990; Naciri et al. 1993; Peeters et al. 1993
Paromomycin	Cow calf	25 to 100 mg	11 days	Prevented	Reduced and improved	Fayer and Ellis, 1993
Paromomycin	goat kid	100 mg	12 days	Prevented	Reduced and Improved	Mancassola et al. 1995
Decoquinate	Cow calf	2.5 to 10 mg	8 weeks	Decreased	Reduced and Improved	Redman and Fox, 1994
Decoquinate	goat kid	2.5 mg	21 days	Decreased	Reduced and Prevented	Mancassola et al. 1997

A study was conducted to evaluate the alcoholic and watery effect of some medicinal plants against cryptosporidiosis in small and large ruminants. The watery and alcoholic extracts of three medicinal plants were used. The name of three plants used as medicinal plants we*re* *Curcuma longa, Viscum album* and *Corindrumn sativum.* The same dose was used for all three plants. It was found that *Curcuma longa* had the highest efficacy against cryptosporidiosis and it was confirmed through laboratory tests on the basis of reduction in oocysts in infected mice. The efficacy was 100% at the dose rate of 750 mg/Kg body weight at the 7^{th} day of post treatment and the plant was used at the dose rate of 1000mg/Kg body weight at day 5^{th} in the form of watery extracts. The drug was used at the dose rate of 1000mg/Kg body weight in the form of alcoholic extracts on the *4^{th} day. Then Viscum album* was used at the day 7^{th} with the dose rate of

750 mg/Kg body weight in form of watery extract and 1000 mg/Kg body weight in form of alcoholic extract with concentration 48% and 54% respectively. The lowest efficacy of *Coriondrum sativum* was recorded at all concentrations when used in form of watery extracts and alcoholic extracts. When folic acid and potassium chloride were added to the watery and alcoholic extracts of *Coriondrum sativum, no* major differences were recorded. The efficacy of *Coriondrum sativum* was increased up to 100% when it was used in combination with the azithromycin at the 4th day of treatment while its efficacy was 68% when it was used without azithromycin (Hiro et al. 2012).

Statement of problem

Pakistan has a large population of livestock and facing a large number of threats in form of parasitic infestations. The producing ability has been seriously affected. Different parasitic infections are responsible for poor performance in form of production and weight gain. These parasitic infections are causing a huge economic loss to our livestock industry. Animals are screened for presence of various parasites by using simple conventional diagnostic tools. The *Cryptosporidium infection* is highly endemic in small ruminants in our country. There was need of proper diagnosis of cryptosporidiosis to find out the percent prevalence in sheep, goats, lambs and goat kids in small ruminants and children. It was also needed to determine the efficacy of various herbal and allopathic drugs against cryptosporidiosis. On the basis of the highest prevalence of the disease in small ruminants and children, the current study was designed and conducted to find out the percent prevalence of the Cryptosporidial infection in southern areas of KPK, Pakistan to achieve the following objectives.

1. To determine the prevalence of cryptosporidiosis in small ruminants.

2. To identify the various risk factors responsible for contributing the occurrence of the disease.
3. To find out the zoonotic aspect of the *Cryptosporidium* infection.
4. To develop PCR based techniques for detection of the *Cryptosporidium infection*.
5. To study and compare the hematological parameters and biochemical analysis of serum collected from the *Cryptosporidium* infected animals and non-infected animals (Healthy animals).
6. To conduct and compare the percent efficacy of different herbal (Indigenous) and allopathic drugs through various therapeutic trials against cryptosporidiosis.

CHAPTER 3
MATERIALS AND METHODS

Cryptosporidium is the most common enteric pathogen in cattle, buffaloes, sheep, goats and humans throughout the year. The *Cryptosporidium* parvum causing infection in sheep and goats also carries public health significance. The study was conducted to find out the percent prevalence of the *Cryptosporidium* infection in small ruminants (Sheep, goats, lambs and kids) of different age groups, sex, areas and seasons of the year under various managemental conditions in three selected districts of Khyber Pakhtunkhwa(KP or KPK) Pakistan. Identification of *Cryptosporidium* oocysts was made on the basis of microscopic morphological features after proper centrifugation and staining. In sheep fecal samples were first tested through simple microscopic examination followed by confirmation with molecular technique known as polymerase chain reaction (PCR).

3.1: Study Area / Sample collection Area

The source of the samples was sheep, goat, kids and lambs reared by different farmers in three selected districts of the southern Khyber Pakhtunkhwa (KP) namely District Bannu, District Lakki Marwat and District Kohat.

District Bannu is located at 32.99° North latitude, 70.61° East longitude and 371 meters elevation above the sea level while the District Lakki Marwat is located next to the District Bannu, KPK Pakistan, Asia and its geographical coordinates are 32° 36' 27" North, 70° 54' 45" East. The Kohat district is the southern districts of the KPK Pakistan and lies between north latitude 32° 47' and 33° 53' and east longitude 70° 34' and 72° 17.

3.2: Experimental Site

All samples were processed in Medicine Laboratory, Department of Clinical Medicine and in the Laboratory of the Department of Parasitology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan.

3.3: Sampling strategy

Fecal samples were collected from sheep, goats, lambs and kids reared in the three selected areas for one year (January, 2016 to December, 2016). Observational study was conducted on the basis of related information recorded during each visiting day on a questionnaire. The data regarding sheep, goat, lambs and kids was recorded at the time of sampling and entered on questionnaire for gathering of useful indistinguishable data. The dichotomous pre tested questionnaire is hereby attached as annexure (3) within last chapter of thesis. The different entries and informations collected at the time of sampling included in proforma were "Name of the owner, address and mobile contact if any, characterization of animal (sex, age), Animal species (Ovines or Caprines), Management history such as feeding (grazing or stall feeding), Water source (Deep well or Stream or Pond), Colostrum intake (Fed or Deprived), Deworming (Yes or No),Environmental conditions(temperature, humidity, average rainfall), Season (Spring, Summer, Autumn, Winter), present history including (body temperature F^0), diarrhea (Present or Absent) and physical condition of the body (emaciated, weak, healthy)

All animals included in study plan, were selected from three selected districts using convenient sampling method randomly, from asymptomatic small ruminants (Sheep, Goats, Kids and Lambs) for detection of percent prevalence of the cryptosporidiosis. During the total study period, 1440 samples were collected for the period of one year. Out of 1440 samples, 360 samples were collected per sheep, goats, lambs and kids for the period of one year in three selected districts. Ten samples per month per district were collected from sheep, goats, lambs and kids. Thus 40 samples were collected per district per month per animal group. As there were three districts (n= 3) so 120 samples were collected randomly for the period of one year so collected 1440 samples (n=360 per animal category) for the period of one year. Experimental sampling plan has been presented in table (3.1).Similarly, fecal samples were also collected from 360 goats (n=30/ month/ 3 districts, 10/ each district/ month) and 360 goat kids per year per three districts (n=30/ month/ 3 districts, 10/ each district/ month). All the samples were collected from asymptomatic adult sheep, goat, lambs (age from day old - 6 months) and kids (age from day old-6 days) randomly on monthly basis.

Human stool samples (n=360) were collected from three selected Head Quarter Hospitals of District Bannu, Lakki Marwat and Kohat. All the samples were collected from those patients suffering from abdominal disturbance such as acute or chronic diarrhea. All the samples collected from adults and children were preserved in 10% formalin with the ratio of 2:3. The samples were collected into sterile wide mouthed plastic bottles properly labeled and transported to the refrigerator till analysis within one or two weeks. All the samples were analyzed in the Medicine laboratory, Department of Clinical Medicine & Surgery, University of Veterinary and Animal Sciences, Lahore. All the collected samples were examined microscopically and screened out for the presence of the Cryptosporidium oocysts.

Table 3.1: Experimental convenient sampling technique for collection of fecal samples from January to December, 2016 from small ruminants reared under different managemental conditions in three selected Districts of southern KPK, Pakistan.

Sampling District	Type and designation of animals	Month wise Sampling											
		1	2	3	4	5	6	7	8	9	10	11	12
Bannu	Sheep (A)	10	10	10	10	10	10	10	10	10	10	10	10
	Goats (B)	10	10	10	10	10	10	10	10	10	10	10	10
	Lambs (C)	10	10	10	10	10	10	10	10	10	10	10	10
	Kids (D)	10	10	10	10	10	10	10	10	10	10	10	10
Lakki Marwat	Sheep (A)	10	10	10	10	10	10	10	10	10	10	10	10
	Goats (B)	10	10	10	10	10	10	10	10	10	10	10	10
	Lambs (C)	10	10	10	10	10	10	10	10	10	10	10	10
	Kids (D)	10	10	10	10	10	10	10	10	10	10	10	10
Kohat	Sheep (A)	10	10	10	10	10	10	10	10	10	10	10	10
	Goats (B)	10	10	10	10	10	10	10	10	10	10	10	10
	Lambs (C)	10	10	10	10	10	10	10	10	10	10	10	10
	Kids (D)	10	10	10	10	10	10	10	10	10	10	10	10

Study Protocol

3.4: Ethics Statements

All the fecal and blood samples were collected with the permission of owners at the time of visiting to small ruminants. Sufficient veterinary care was taken at the time of sampling and free veterinary service was provided to all animals without any fee or medicine charges. Informed the farmers about proper vaccination and deworming programs for the betterment of the herd and uplifting the life standards of the poor farmers.

3.5: Microscopic identification of the *Cryptosporidium* oocysts

3.5.1: Collection of Fecal Samples:

Each fecal sample (about 5 gram) was collected with the help of rectal swabs from each lamb and kid only while fecal samples were directly collected from the rectum of adult sheep and goats after wearing with the help of the plastic disposable gloves. In children, the stool samples (5-10 gram) were collected from the freshly passed diarrheic stool. All the collected samples from small ruminants and children were preserved in 10% formalin at the ratio of 1:3 into sterile, clean, moisture resistant, labeled and disposable wide- mouthed plastic bottles free of urine contamination. All the samples were transported and refrigerated at $4C^0$ till analysis within one or two weeks.

Precautions: Following precautions were adopted during collection of fecal samples.

- Did not freeze any fecal sample during storage period.
- Fecal samples and preservatives were used at correct proportion.
- All the vials were tightly sealed to avoid any leakage.
- All the vials were properly labeled at the time of fecal collection.
- Fecal material and fixatives were properly mixed at the ratio of 1:3.

3.5.2 : Laboratory Analysis of Fecal Samples

All the fecal samples were properly analyzed by using a Faust modified centrifuge-flotation technique (Leventhal and Cheadle, 1992). By using electric balance, 3 grams of fecal materials were weighed. After weighing, fecal materials were dissolved in distilled water to make homogenized solution. After homogenization, the solution was centrifuged at 1500 rpm for 1-2 min. As a result of centrifugation, the supernatants were removed from the solution and the sediments were resuspended in the flotation solution known as ZnSO4 (44%). The solution was again centrifuged at 1500rpm for 1 mint. Finally the sediments were examined under microscope and the supernatants were removed from the solution. The *Cryptosporidium* oocysts were stained by modified Ziehl- Neelsen (MZN) staining technique (Casemore DP et al. 1985). In the present study, after proper centrifugation, the slides were properly stained by modified Ziehl- Neelsen (MZN) acid fast staining technique and were confirmed through simple microscopic examination.

3.5.2.1 : Modified Ziehl-Neelsen acid fast staining technique

The following procedure was applied to stain the *Cryptosporidium* oocyst in fecal Smear;

I. Air dry thin fecal smear was prepared and fixed in methanol for 2-3 minutes.

II. Stained with strong Carbol fuchsin for 15-20 minutes.

III. Then slides were washed with tape water.

IV. Now the decolorized agent (1% acid alcohol) was applied for 2 minutes.

V. After decolorization, all the slides were rinsed with tape water.

VI. Methylene blue or Malachite green was used as a counter stain for 1 minute.

VII. Finally slides were rinsed again with tape water and air dried.

All the stained fecal slides were examined with the help of calibrated light microscope for detection of the *Cryptosporidium* oocysts at 100 x magnification using oil immersion as reported by Bakiret et al. 2003.The *Cryptosporidium* oocysts appeared as bright red granules on a blue-green background in MZN stained fecal smears. A fecal sample was considered positive if at least one, clearly identifiable the *Cryptosporidium* oocyst was identified. The total number of oocysts per gram (OPG) of feces was obtained by multiplying the total number of oocysts on the slide by 50.

3.5.2.2 Identification of Cryptosporidium oocysts/ Eggs

The *Cryptosporidium* oocysts/ eggs were identified on the basis of morphology, size and the key as reported by Wantanbe et al. 2005. Then infection rate using the following equatiion;

$$\text{Prevalence (\%) rate} = \frac{\text{Total No. of Sheep or Goats infected}}{\text{Total No of Sheep or Goats Examined}} \times 100$$

Prepared a single slide from each fecal sample and examined under microscope. If at least single oocyst was seen per slide then such animal was considered a positive case for the cryptosporidiosis.

3.5.2.3 : Counting of oocysts

Oocyst per gram (OPG) was calculated on the basis of formula reported by Handley et al. 1999. Known W/Volume suspension was prepared for each fecal sample. On the basis of following formula, OPG was calculated for each fecal sample before and after treatment.

$$N = \frac{S}{\text{Vol.} \times \text{Wt} \times \text{PV}}$$

Where,

N = Total number Oocysts / gram of feces

S = Total number of counted Oocysts on the surface of slide

Vol = Total volume of the sample examined e 20 µL and the volume was taken with the help of P- 20 micropipette which can pick 20 µL volume accurately.

Wt = Stands for weight of fecal sample (20 gram).

PV = Stands for volume of pellet (1mL)

3.6 : Molecular Detection of the *Cryptosporidium* oocyst

Johnson et al. (1995), described a technique for identification and detection of the *Cryptosporidium* oocysts at molecular level by using Polymerase Chain Reaction (PCR)**.**Before processing, all the samples were stored at -60°C and PCR (Polymerase Chain Reaction) was performed after isolation or extraction of DNA from the *Cryptosporidium* oocyst by using the following technique.

3.6.1 : Extraction of De-oxy Ribose Nucleic Acid (DNA)

DNA (Deoxy ribose nucleic acid) was extracted according to the method as described by Da Silva et al. 1999; after making some minor modifications. DNA extraction kit (Made in USA GFC vivantis) was used for disruption of the Crypto oocysts tissue. Weighed 1.32 gram of fecal material and mixed with proteinase K (5µl), TL buffer (200 µl) and cell lysis enhancer from DNA extraction kit and the mixture was incubated at 37C°/22-24hrs. After incubation period, 200 µl TB buffer was poured in the mixture and placed at 64- 65°C in water bath for a period of 20 minutes. The sample solution was centrifuged at the rate of 13200 rpm for a period of 30 minutes. To collect supernatants, Eppendorf tubes were used. Mixed properly 200 µl chilled absolute ethanol with the supernatants immediately and the samples were centrifuged at the rate of 13200 rpm for a period of 35 minutes. After proper centrifugation, the pellets were obtained and resuspended in 750 µl washing buffer and then centrifuged at 13200 rpm/30 minutes. After centrifugation, the supernatants were discarded and the pellets were washed several times until

DNA was extracted. Then TBE buffer was used to dissolve the pellets and stored at -80°C for further use. By using 0.8% Agarose gel, the extracted DNA was detected with the help of agarose gel electrophoresis. All the samples were weighed down in the wells with the following ratio= DNA Sample of 10µL+ A Loading dye of 2 µL6X.

PCR product was stained by Ethidium bromide after gel electrophoresis at the rate of 50 volts/ 40 minutes and photograph was taken by Gel Doc.

3.6.2 : Amplification of DNA

Amplification of gene (Cryptosporidium oocyst DNA) was obtained according to the procedure as reported by Da Silva et al. 1999. The different reagents that were used for the preparation of reaction mixture for the process of the amplification were prepared according to the following instructions and information as provided by the manufacture of kit. De-ionized water was added to the dNTPs stocks for dilution purpose to reach the strength of 2.5mM.

Johnson DW et al. 1995, documented the primer sequences and these primers were used for Polymerase Chain Reaction (PCR) by Da Silva et al. 1999. According to the instructions and directions of bioinformatics programme, the reference primers were blasted and the results were similar to the sequences of the *Cryptosporidium*. The targeted gene of parasite was 18s rRNA which result in amplification of a segment of genomic DNA at 435 bp (base pair). The following sequence of primers was used as a Forward primer: (5-AAGCTCGTAGTTGGATTTCTG-and the sequence of reverse primers was used as (5-TAAGGTGCTGAAGGAGTAAGG-3. (Gen Bank accession number L16996).

TE buffer, Stock primers and TBE buffer were numerically converted into the concentrations as required according to the instructions, protocol and directions mentioned by

Johnson et al. 1995. The composition of the PCR reaction mixture used in the experiment, was prepared according to the instructions as mentioned in the annexure= 1.

In the laboratory, the reaction mixture was properly mixed in ice tar safety cabinet and prepared the mixture up to five reactions of the PCR. When PCR started, then maintained at initial temperature at 94°C and programmed at 65°C. PCR was run for 35 cycles and completed the procedure in two hrs. Then the temperature was lowered up to 4°C. Properly labeled all the amplified DNA samples and stored at -20°C for further use.

3.6.3 : Agarose gel electrophoresis

The fragments of amplified DNA were extracted from Cryptosporidium oocyst and were detected and confirmed with the help of agarose gel electrophoresis. Then weighed 1 gram of molecular grade agarose with the help of electric balance and added distilled water to prepare a solution of 100 ml. The mixture was properly incubated at 100°C/ 2 minutes in the oven by using the non insulating gloves. The flask was cooled in an open air and added 5 µl Ethidium bromides at temperature of 40-45°C in the mixture. Gently poured the solution in the gel apparatus and the comb was fixed in the agarose gel to make solidify. Then with the help of gentle motion, comb was picked up and positioned in the electrophoresis tank. Tank buffer composition has been shown in annexure 2. All the independent wells were properly loaded with amplified DNA samples along with DNA ladder. A dye was used in ladder known as Bromophenol blue dye and the samples of DNA were used as an indicator to confirm the movement of samples in the gels. During electrophoresis, 60 volts of current was constantly supplied to carry out the process in an horizontal fashion. When all the samples were run through electrophoresis, then the standard system was properly stopped and gently removed the gel. Now the gel was placed at gel documentation apparatus and photographed the nucleic acid bands to visualize properly for recording the results.

3.7 : Determination of percent prevalence

The rate of prevalence (%) of cryptosporidiosis was calculated as per the formula,

Percent prevalence = d/n× 100

Where "d" stands for the total number of infected animals or diseased animals at particular point at a time while

"n" stands for total number of animals examined for presence of cryptosporidiosis in that particular point.

3.8 : Meteorological informations

All the information about environmental factors such as temperature, average rain fall and the relative humidity were collected at daily basis from the meteorological department of Peshawar, KP, Pakistan. All meteorological parameters such as ambient temperature, average rain fall and relative (%) humidity was recorded to find out correlation between high or low load of the *Cryptosporidium* infection and environmental factors in different months of the year.

3.9 : Chemotherapeutic trials

A total of 50 goats were randomly selected of the same age and weight of either sex, naturally infected with the *Cryptosporidium* infection under field conditions. All the animals were kept under same management and feeding pattern. The group "E" was held as a positive control group. The different chemicals were purchased from the local medical stores. The different drugs such as Azithromycin, Metronidazole, *Allium sativum* (Garlic) and Paromomycin were selected for therapeutic trials against cryptosporidiosis in small ruminants.

3.9.1 : Grouping of animals

A total of “50” goats, that were naturally infected with the *Cryptosporidium* infection selected randomly and divided into five groups named as A, B, C, D and E. Placed 10 heads in each group from A-E.

Group A

All the animals in group A, were treated with Azithromycin at the dose rate of 500 mg per day per animal per oral (PO) for a period of five consecutive days.

Group B

The animals in group B, were treated with Metronidazole at the dose rate of 50 mg/ Kg body weight per oral (PO) for a period of five consecutive days.

Group C

Allium sativum (garlic) was administrated in form of crude juice for a period of five consecutive days to the experimental animals in group C that were naturally infected with cryptosporidiosis.

Preparation of Crude extract

First separated the bulb of fresh garlic and washed with clean water. Dried in sun light and crushed 500 gram of garlic bulb in a blender until uniform soft mass was achieved. Then paste was diluted in distilled water to achieve 1 gram per ml aqueous solution.Aliquoted raw garlic juice and stored at -20C until use (Riad et al. 2009; Burke et al. 2009, Masamha et al. 2010). Stock solution was diluted with distilled water and working solution was made from stock solution. The selected dose for present experiment was 50 mg/kg body wt.

The animals of Group C, were treated with *Allium sativum* (garlic) at the dose rate of 50 mg/ kg body weight per day per oral (PO) for 5 consecutive days.

Group D

The group D was treated with paromomycin at the dose rate of 100 mg/kg body weight per day per oral (PO) for a period of five consecutive days.

Group E

The animals of group E, served as a control positive (+) group and no treatment was provided during the trial period and placed in a hygienic environment with great care to avoid further spread of the infection in experimental site.

3.9.2 : Assessment Criteria

The efficacy of different drugs was evaluated on the basis of reduction in counting of oocysts per gram (OPG) before and after treatments. Oocyst per gram (OPG) was counted at day "0" before provision of any treatment (Pre-medication) whereas at day 7, 14, 21 and 28 oocysts were counted after treatment and were compared with infested control group E.

3.9.3 : Detection and Counting of Oocysts /Oocyst Enumeration

Thin fecal smears were fixed with methanol spirit and stained with MZN stain for detection of the *Cryptosporidium* infection as mentioned by Henriksen et al. 1981.

All the preparations were properly stained and examined under compound microscope and oocysts were counted according to the protocol as described by Soulsby, 1982.

A single stained slide was properly prepared for each fecal sample and examined under the compound microscope and counted oocysts in 20 microscopic fields/ slide for detection of the *Cryptosporidium* oocysts. For oocyst counting, slides were examined within 30 minutes after preparation under compound microscope at different level of magnifications such as 100X, 200X, 400X and 1000X. All those animals were declared positive if even at least one or two oocysts/microscopic field were detected under microscope.

3.9.4 : Calculation of percent efficacy of the selected drugs

Efficacy of different selected drugs was calculated according to the formula stated below;

$$\text{Efficacy (\%)} = \frac{\text{Total oocysts counted before medication} - \text{Total oocysts counted after medication}}{\text{Total number of oocysts counted before medication}} \times 100$$

During the therapeutic trials, different side effects of the selected drugs were also recorded.

3.10 : Haematobiochemical Profile

3.10.1: Collection of Blood

At the time of fecal collection, blood samples about 5-10 ml was taken from jugular vein with the help of disposable syringe and transferred into two tubes; the first tube was heparinized and was used for detection of hematological parameters whereas the second tube was placed in centrifuge for separation of serum and was used for biochemical analysis. Blood samples were analyzed for “50 goats” that were positive for cryptosporidiosis and “50 goats” that were negative for cryptosporidiosis after confirmation through coprological examination.

3.10.2 : Analysis of blood samples

Hematology analyzer was used for processing of blood samples to obtain complete hemogram. Hemoglobin, Total leukocyte counts (TLC), Total erythrocyte count (TEC) and Packed Cell Volume (PCV) values for healthy and non-healthy (infected animals with cryptosporidiosis) were compared with each other to determine the effect of the *Cryptosporidium* on the blood parameters in goats.

3.10.3 : Separation of serum

Blood samples were also collected at the time of fecal collection. After screening, the fecal samples with the help of coprological examination, blood samples were divided into two groups, healthy and non healthy. Non healthy group was suffering from the *Cryptosporidium* infection whereas healthy group was free of *Cryptosporidium* infection. Blood samples were analyzed through centrifugation at 1500 rpm for 20 minutes. The sera were separated and kept in a sterile labeled test tube at -20 C^0 until further processed.

Biochemical Analysis

Total serum protein was determined with the help of Biuret Method while albumin was detected by using special reagent kits (Gornal et al. 1949). The concentration of serum globulin was determined when subtracted the albumin value from the total proteins. Copper level was determined by using calorimetric methods with the help of reagent kits (Dreux, 1977). Serum sodium, Chloride and Potassium levels were determined with the help of atomic absorption spectrophotometer. The following parameters were also studied during biochemical analysis such as total protein, Albumin, ALT, AST, ALP, Ca, Mg, Na, and K, chloride, Zn. Cu, urea and Creatinine.

3.11 : Statistical analysis

At the end of study, the collected data was subjected to the statistical analysis using the version 20 Statistical package for Social Sciences (SPSS). Prevalence rates were calculated and presented in form of percentages (%). Data regarding with comparison of differences in prevalence for different variables and associated risk factors were analyzed using chi-square test (X2). Data regarding blood parameters between the two groups were analyzed using Z -test while data regarding the therapeutic trials, were assessed by two way ANOVA using Statistical Package for Social Sciences (SPSS) version 20(Zar, 2004, Steel et al., 1997). All values at ($p<0.05$) were considered as significant.

CHAPTER 4
RESULTS

The *Cryptosporidium* infection was diagnosed on the basis of simple microscopic examination of stained thin fecal smear. All samples were identified by morphological characteristics of the *Cryptosporidium* oocyst and molecular detection through PCR (Polymerase chain reaction).Different risk factors such as species, sex, age, area and season were studied to find out percent prevalence of the *Cryptosporidium* infection. Finally the efficacies of different herbal and allopathic drugs were evaluated against *Cryptosporidium* infection on the basis of reduction in oocysts per gram under different trials to find out the percent efficacy in reduction of OPG.

4. 1: Overall percent prevalence of cryptosporidiosis in small ruminants

During the present study, the highest percent prevalence of cryptosporidiosis was, recorded in lambs (27.22 %) followed by kids (20.56%), Sheep (18.33%) and adult goats (12.22%). In sheep, the highest percent prevalence of the Cryptosporidial infection was recorded in Kohat (21.66%) followed by Bannu (18.33%) while the lowest percent prevalence was observed in District Lakki Marwat (15%). In District Kohat, the highest percent was recorded in goats (18.3%) followed by District Lakki Marwat (11.66%) while the lowest percent prevalence was recorded in District Bannu (6.66%). In lambs, the highest percent prevalence of the Cryptosporidial infection was recorded in Kohat (33.33%) followed by Lakki Marwat (25%) while the lowest in Bannu (23.33%). On the basis statistical analysis, significant difference ($P<0.02$) was observed in percent prevalence of *Cryptosporidium* infection in sheep, goat, lambs and kids.

4.2 : Prevalence of cryptosporidiosis in Sheep

An overall percent prevalence of cryptosporidiosis was 18.33%, in three selected districts of KPK. The highest percent prevalence of the *Cryptosporidium* infection was recorded in District Kohat (21.66%) followed by District Bannu (18.33%) while the lowest percent prevalence was observed in District Lakki Marwat (15%).

4.2.1 : Month wise percent prevalence of cryptosporidiosis in Sheep

The data was analyzed on monthly basis to find out which month of the year having the highest percent prevalence of *Cryptosporidium* infection in sheep. The highest overall percent prevalence was recorded in the month of August (36.66%) followed by April and July (26.66%), May and September (23.33%), June (20%), October (16.66%), February (10.66%), March and November (10%) while the lowest percent prevalence was recorded in the month of December and January (6.66%).

On the basis of statistical analysis, overall significant difference ($P<0.05$) was observed in different months of the year. Statistically significant difference ($P<0.05$) was observed in percent prevalence of the *Cryptosporidium* infection in August and with other months of the year while non significant ($P>0.05$) difference was observed in April and July while significant difference ($P<0.05$) was recorded with other months of the year.

Similarly non-significant difference ($P>0.05$) was observed in several months of the year such as May and September, February, March and November, January and December while significant difference ($P<0.05$) was recorded with other months of the year. All results showing month wise percent prevalence in sheep have been shown in table (4.2) while curve shows the higher or lower monthly percent prevalence as figure (4.1).

4.2.2 : Season wise percent prevalence of cryptosporidiosis in Sheep

In the present study, season wise percent prevalence of the *Cryptosporidium* infection was also studied in sheep where, the highest percent prevalence was recorded in the month of summer (27.5%) followed by autumn (20%), spring (18.33%) while the lowest percent prevalence was recorded in the winter season (8.33%). On the basis of statistical analysis, significant difference ($P<0.004$) was recorded in percent prevalence of the *Cryptosporidium* infection in different seasons of the year. Results for seasonal prevalence have been presented in table (4.3) while the difference has been also presented by figure (4.2).

4.2.3 : Age wise percent prevalence of cryptosporidiosis in Sheep

On the basis of age wise percent prevalence, all the results have been presented in table (4.4). In the current study, the highest percent prevalence was recorded at the age of one year (23.13%), followed by 1-2 yrs (18.85%) while the lowest percent prevalence was recorded at the age of ≥2-3 years and above (11.53%). On the basis of statistical analysis, significant difference ($P<0.05$) was reported in different three age groups of the sheep. At the age of "≤ 1 year" and 1-2 years, statistically non- significant difference ($P>0.05$) was observed while significant difference ($P<0.05$) was recorded with the age group of ≥2-3 years .The difference at age wise percent prevalence in three groups have been presented in (Figure: 4.3).

4.2.4 : Sex wise percent prevalence of cryptosporidiosis in Sheep

During the current study overall percent prevalence of the *Cryptosporidium* infection was higher in female (18.80) % while lower in male (17.02%). In the present study, the male and female ratio was 18.75%:19.04%; 14.28%:15.21% and 20%:22.22% in District Bannu, Lakki Marwat and Kohat respectively. On the basis of Statistical analysis, non-significance difference ($P >0.689$) was recorded in percent prevalence of the cryptosporidiosis in both genders (male and female) in sheep in three selected zones of southern KPK. Percent (%) prevalence of the cryptosporidiosis in both sexes (Male and Female) has been shown in table (4.5) while the difference has been also presented by figure (4.4).

Table 4.1: Overall percent prevalence of cryptosporidiosis in different selected small ruminants in three selected districts of Southern KPK

Factor	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/Total	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
Sheep	22/120	18.33	18/120	15	26/120	21.66	66/360	18.33^{b}	0.02
Goats	8/120	6.66	14/120	11.66	22/120	18.3	44/360	12.22^{c}	
Lambs	28/120	23.3	30/120	25	40/120	33.33	98/360	27.22^{a}	
Kids	24/120	20	22/120	18.33	28/120	23.33	74/360	20.11^{b}	

^{a,b} mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.2: Month wise (Jan-Dec. 2016) percent prevalence of cryptosporidiosis in Sheep in three districts of Southern KPK

Factors	**District Bannu**		**District Lakki Marwat**		**District Kohat**		**Overall percent prevalence**		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
January	0/10	00	1/10	10	1/10	10	2/30	6.66^{d}	0.054
February	2/10	20	0/10	00	1/10	10	3/30	10.66^{c}	
March	1/10	10	1/10	10	1/10	10	3/30	10^{c}	
April	2/10	20	3/10	30	3/10	30	8/30	26.66^{b}	
May	2/10	20	3/10	30	2/10	20	7/30	23.33^{d}	
June	3/10	30	1/10	10	4/10	40	8/30	20^{f}	
July	2/10	20	2/20	20	3/10	30	7/30	26.66^{d}	
August	4/10	40	2/10	20	5/10	50	11/30	36.66^{a}	
September	1/10	10	3/10	30	3/10	20	7/30	23.33^{d}	
October	3/10	30	1/10	10	1/10	10	5/30	16.66^{n}	
November	1/10	10	1/10	10	1/10	10	3/30	10^{c}	
December	1/10	10	0/10	00	1/10	10	2/30	6.66^{d}	
Total	22/120	18.33%	18/120	15%	26/120	21.66%	66/360	18.33%	

$^{a,b\ c,\ d,\ f,\ n}$ mean values carrying same superscript within the row are differ non-significantly ($P>0.05$) whereas with different superscripts are differ significantly at ($P< 0.05$).

Table 4.3: Season wise percent prevalence of cryptosporidiosis in Sheep in three selected districts of Southern KPK.

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall Prevalence		
	Infected / Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P- value
Winter	4/40	10	2/40	5	4/40	10	10/120	8.33^{c}	0.004
Spring	3/20	15	4/20	20	4/20	20	11/60	18.33^{b}	
Summer	11/40	27.5	8/40	20	14/40	35	33/120	27.5^{a}	
Autumn	4/20	20	4/20	20	4/20	20	12/60	20^{ab}	
Total	22/120		18/120		26/120		66/360		

$^{a, b, c, ab}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.4: Age wise percent prevalence of cryptosporidiosis in Sheep in three districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P- value
≤1 year age	11/44	25	8/38	21.05	12/52	23.07	31/134	23.13^{a}	0.05
1-2 years age	9/44	20.45	6/42	14.28	8/36	22.22	23/122	18.85^{ab}	
≥2-3years age	2/32	6.25	4/20	10	6/32	18.75	12/104	11.53^{b}	

$^{b, c, ab}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.5: Sex wise percent prevalence of cryptosporidiosis in Sheep in three selected districts of Southern KPK

Factors	**District Bannu**		**District Lakki Marwat**		**District Kohat**		**Overall**		
	Infected / Total Examined	Prevalence (%)	Infected /Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Male	6/36	18.75	4/28	14.28	6/30	20	16//94	17.02[a]	0.689
Female	16/84	19.04	14/92	15.21	20/90	22.22	50/266	18.80[a]	

[a, b] mean values carrying same superscript such as (a, b) within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

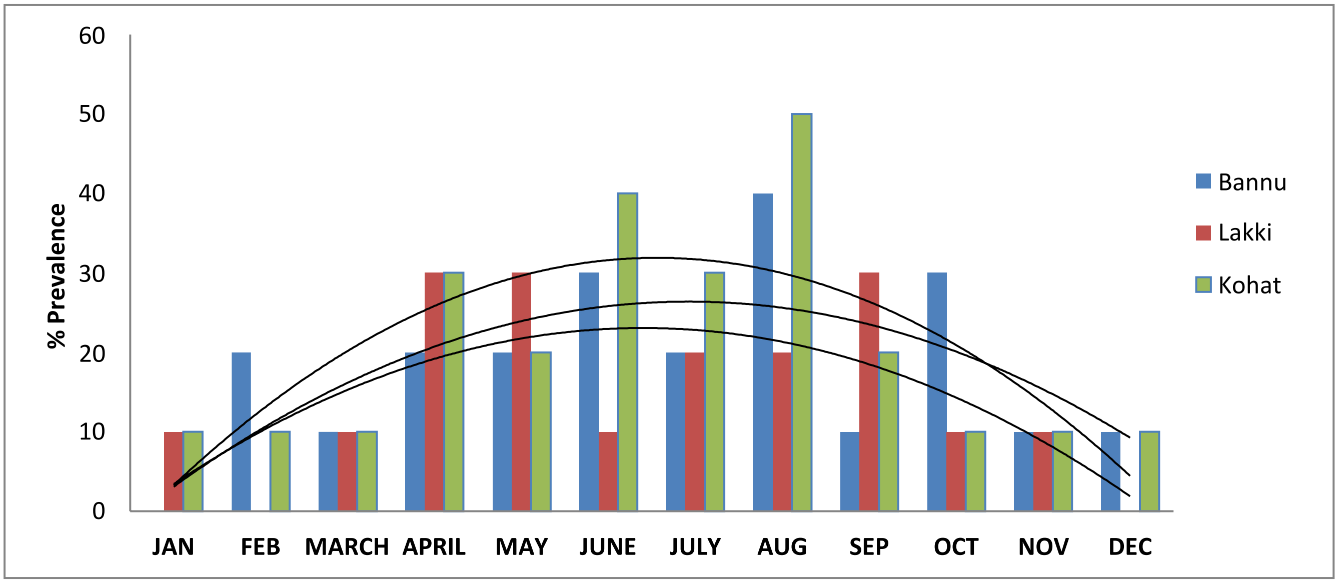

Figure 4.1: Month wise percent prevalence of cryptosporidiosis in sheep in three selected districts of southern KPK. (Kohat: $y = -0.926x^2 + 12.15x - 7.954$, $R^2 = 0.548$; Lakki Marwat: $y = -0.674x^2 + 8.626x - 4.545$, $R^2 = 0.469$; Bannu: $y = -0.664x^2 + 9.195x - 5.454$, $R^2 = 0.463$)

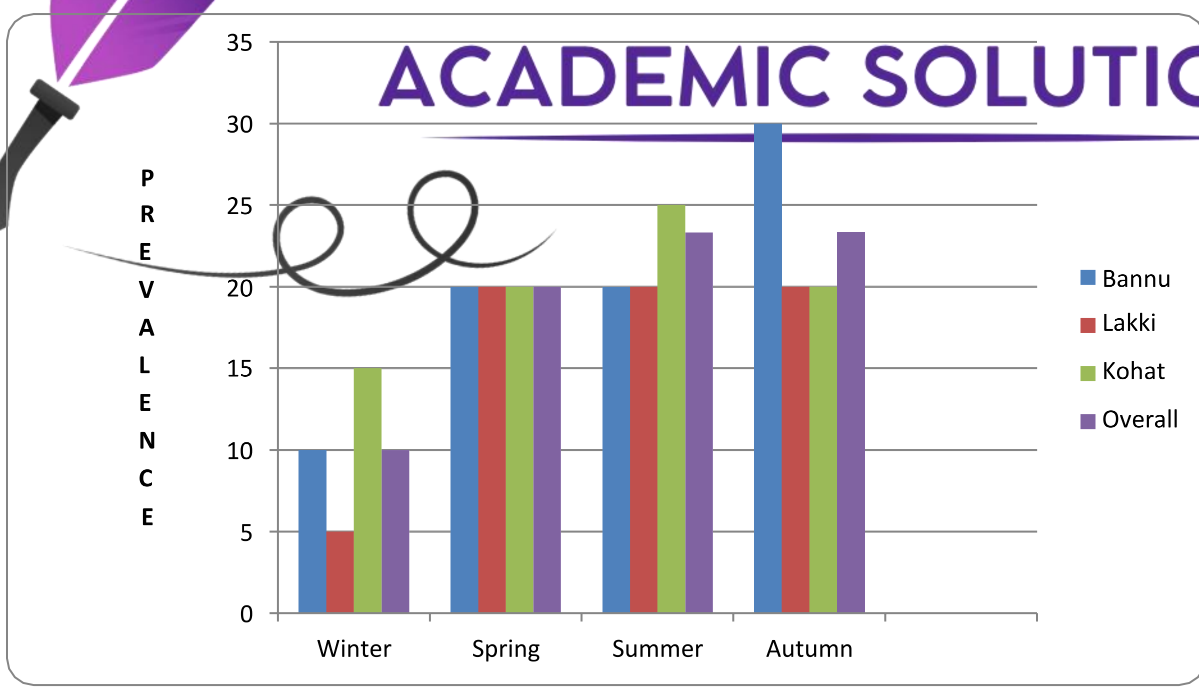

Figure 4.2: Season wise percent prevalence of cryptosporidiosis in Sheep in three selected districts of southern KPK.

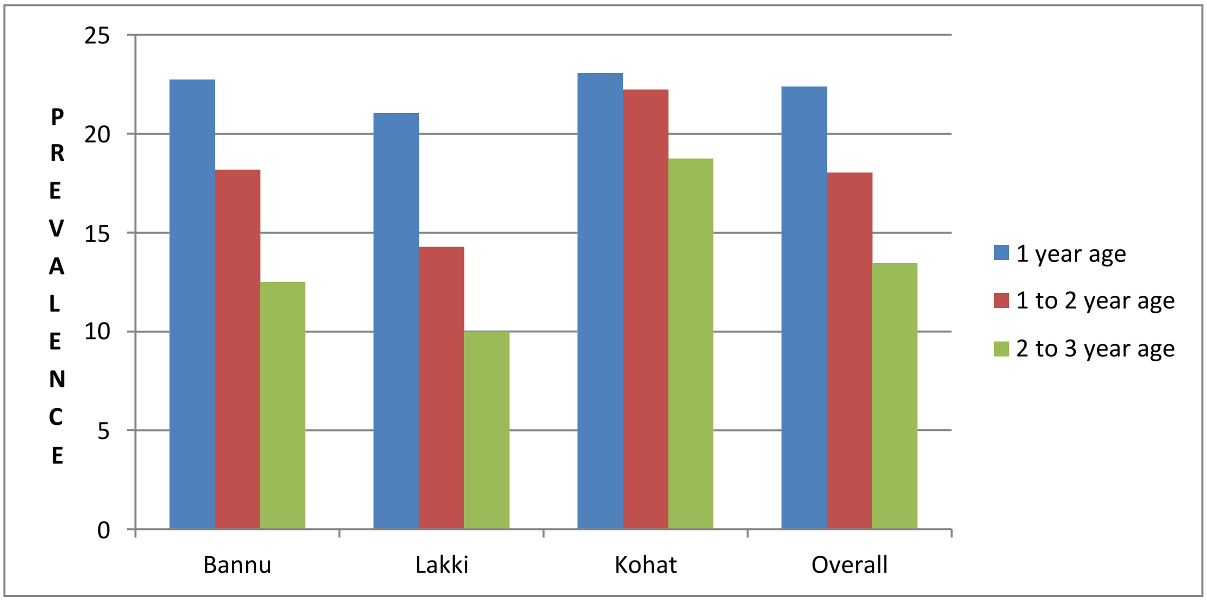

Figure 4.3: Age wise percent prevalence of cryptosporidiosis in Sheep in three selected districts of Southern KPK

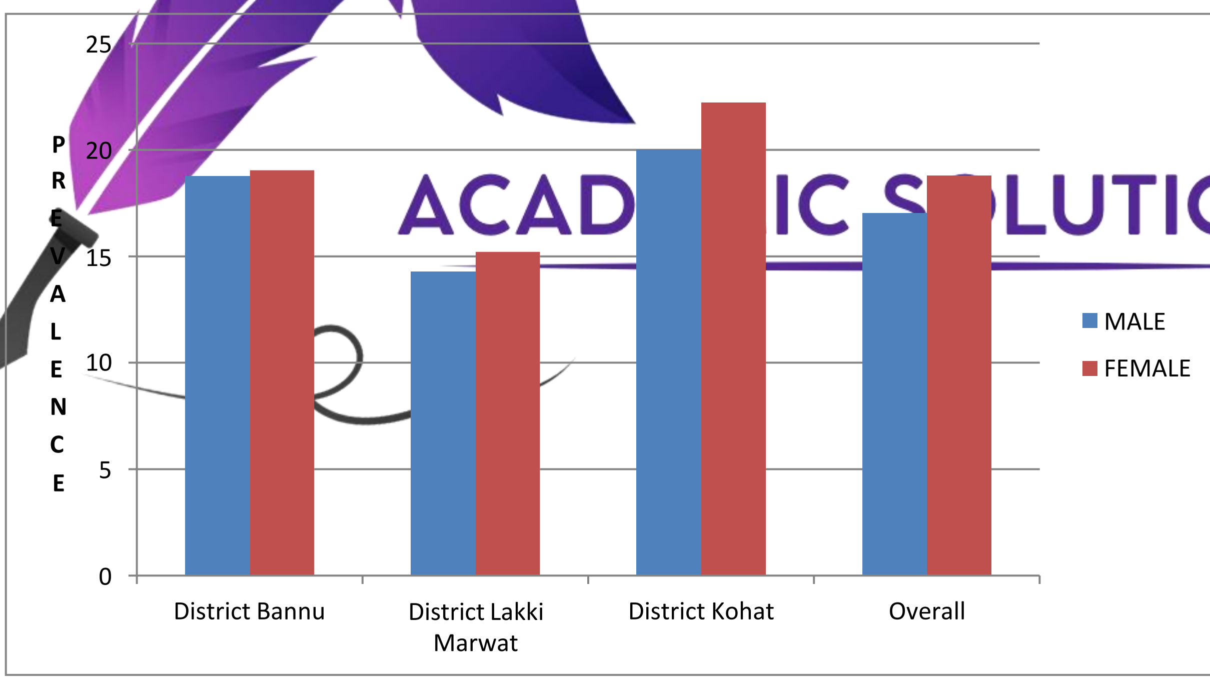

Figure 4.4: Sex wise percent prevalence of cryptosporidiosis in Sheep in three selected districts of Southern KPK

4.3 : Prevalence of cryptosporidiosis in Goats

The *Cryptosporidium* oocysts were identified on the basis of microscopic characters for the purpose to find out the percent prevalence of the Cryptosporidial infection in goats for a period of one year in three selected zones of the southern KPK. As a result, the highest overall percent prevalence was 12.22%, recorded in three selected zones. The highest percent prevalence was recorded in District Kohat (18.33%) followed by District Lakki Marwat (11.66%) while the lowest prevalence was observed in District Bannu (6.66%). Results of percent prevalence in Goats are presented in table (4.5). On the basis of statistical analysis, significant difference ($P<0.05$) was observed in percent prevalence in three selected areas.

4.3.1 : Month wise percent prevalence of cryptosporidiosis in Goats

The highest month wise percent prevalence of cryptosporidiosis was recorded in the month of August (30%), followed by July (23.33%), June (20%), May (16.66%), March and September (13.33%), April and November (10%), January, February and October (3.33%) while the lowest zero percent prevalence was recorded in the month of December.

The highest district wise percent prevalence was recorded in the area of District Kohat (18.33%), followed by Lakki Marwat (11.66%) while the lowest percent prevalence was recorded in Bannu (6.66%). On the basis of statistical analysis, overall non- significant ($P>0.056$) difference was recorded in twelve months of the year.

Results of percent prevalence in goats during various months of the year have been presented in table (4.6) and the difference between different months has been shown by figure (4.5).

4.3.2 : Season wise percent prevalence of cryptosporidiosis in Goats

In the present study, the Overall highest percent prevalence of cryptosporidiosis was recorded in the summer season (20.83%), followed by spring (13.33%), autumn (11.66%) while the lowest prevalence percentage was recorded in winter season (3.33%). In the winter season, the highest percent prevalence of cryptosporidiosis was observed in District Lakki Marwat and Kohat (5%) while the lowest (0%) prevalence was observed in District Bannu.

In the spring season, the highest percent prevalence was recorded in Kohat (20%) followed by Bannu and Lakki Marwat (10%). In summer season, the highest percent prevalence was observed in District Kohat (30%) followed by Lakki Marwat (17.5%) and District Bannu(15%).In the autumn the highest percent prevalence was recorded in District Kohat(20%) , followed by Lakki Marwat(15%) and Bannu (0%)

Statistically, overall non-significant difference (P>0.102) was recorded in percent prevalence of the *Cryptosporidium* infection in four seasons of the year. On the basis of statistical analysis, Significant difference (P<0.05) was recorded in percent prevalence of the *Cryptosporidium* infection in winter and summer season while non significant difference (P>0.05) was recorded in spring and autumn season. Results of seasonal percent prevalence in goats have been presented in table (4.7) while the difference has been shown by figure (4.6).

4.3.3 : Age wise percent prevalence of cryptosporidiosis in Goats

Overall, the highest percent prevalence of cryptosporidiosis was recorded at the age of ≤1 year (18.58%) followed by 1-2 years (10.20%) while the lowest at the age of ≥2-3 years (5.95%). At the age of ≤1year, the highest percent prevalence was observed in District Kohat (22.22%), followed by Lakki Marwat (16.07%) while the lowest in District Bannu ((14.28%). Similarly at the age of ≤1 and ≥2 years, the highest percent prevalence was recorded in District Kohat

(14.28%), followed by District Lakki Marwat (7.89%) while the lowest (6.25%) in District Bannu. At the age of $\geq$2-3 years, the highest percent prevalence was recorded in District Kohat (10%), followed by Lakki Marwat (7.69%) while the lowest (2.5%) in District Bannu.

On the basis of statistical analysis by using chi square test, overall significance difference ($P<0.041$) was recorded in different age groups of the goats. Significant difference ($P<0.05$) was recorded between $\leq$ 1 year and 1-2 years of age while nonsignificant difference ($P>0.05$) was recorded at the age of 1-2 years. All the results calculated on the basis of age factor have been presented in table (4.8) while the difference between different age groups of the goats has been presented by figure (4.7).

4.3.4 : Sex wise percent prevalence of cryptosporidiosis in goats

In the present study, overall the highest percent prevalence was recorded in male (12.30%) followed by female (12.17%). On the basis of statistical analysis by using chi square test, non-significant difference ($P>0.944$) was recorded in percent prevalence of cryptosporidiosis in male and female as presented in table (4.9) and figure (4.8).

Table 4.6: Month wise (January-December, 2016) percent prevalence of cryptosporidiosis in Goats in three selected districts of southern KPK

Factor	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
January	0/10	00	1/10	10	0/10	00	1/30	3.33^{cd}	0.056
February	0/10	00	1/10	10	0/10	00	1/30	3.33^{cd}	
March	1/10	10	2/10	20	0/10	00	3/30	13.33^{bcd}	
April	1/10	10	1/10	10	1/10	10	3/30	10^{bcd}	
May	2/10	20	1/10	10	2/10	20	5/30	16.66^{abcd}	
June	0/10	00	2/10	20	4/10	40	6/30	20^{abc}	
July	2/10	20	1/10	10	4/10	40	7/30	23.33^{ab}	
August	2/10	20	3/10	30	4/10	40	9/30	30^{a}	
September	0/10	00	2/10	20	2/10	20	4/30	13.33^{bcd}	
October	0/10	00	0/10	00	1/10	10	1/30	3.33^{cd}	
November	0/10	00	0/10	00	3/10	30	3/30	10^{bcd}	
December	0/10	00	0/10	00	0/10	00	0/30	00^{d}	
Total	8/120	6.66%	14/120	11.66%	22/120	18.3%	44//360	12.22%	

$^{a,b,d,,ab,abc,abcd,\ bcd,cd}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.7: Season wise percent prevalence of cryptosporidiosis in Goats in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall Prevalence		
	Infected / Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
Winter	0/40	00	2/40	5	2/40	5	4/120	3.33^{b}	0.102
Spring	2/20	10	2/20	10	4/20	20	8/60	13.33^{ab}	
Summer	6/40	15	7/40	17.5	12/40	30	25/120	20.83^{a}	
Autumn	0/20	00	3/20	15	4/20	20	7/60	11.66^{ab}	
Total	8/120		14/120		22/120		44/360		

$^{b,\ c,\ ab}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.8: Age wise percent prevalence of cryptosporidiosis in goats reared in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall Percent Prevalence		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
≤1 year age	4/28	14.28	9/56	16.07	16/72	22.22	29/156	18.58^{a}	0.041
1-2 years age	3/32	6.25	3/38	7.89	4/28	14.28	10/98	10.20^{b}	
≥2-3 years	1/40	2.5	2/26	7.69	2/20	10	5/84	5.95^{b}	

$^{a,\ b}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.9: Sex wise percent prevalence of cryptosporidiosis in Goats in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total	Prevalence (%)	P-Value
Male	5/40	12.5	4/40	10	7/50	14	16/130	12.30[a]	0.944
Female	3/80	3.75	10/80	12.5	15/70	21.42	28/230	12.17[a]	

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

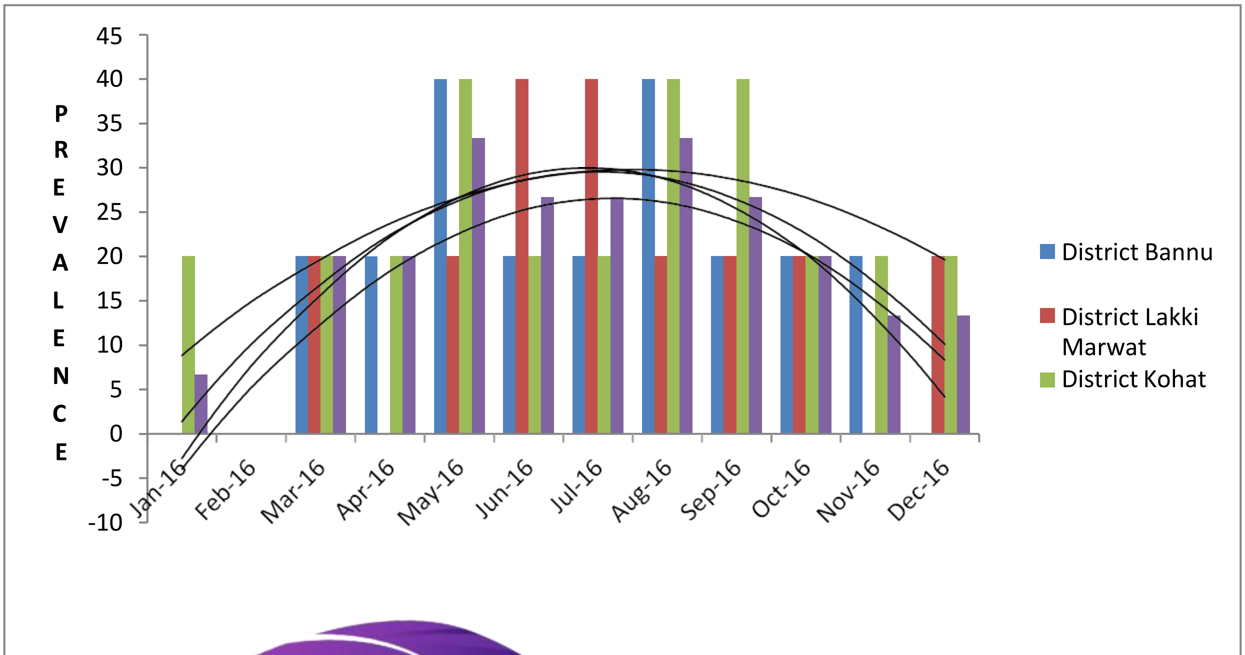

Figure 4.5: Month wise percent prevalence of cryptosporidiosis in Goats (Jan-Dec. 2016) in three selected districts of Southern KPK. (Kohat: $y = -0.000x^2 + 45.70x - 97280$ $R^2 = 0.317$; Bannu: $y = -0.001x^2 + 88.32x - 2E+06$ $R^2 = 0.655$; Lakki Marwat: $y = -0.000x^2 + 72.65x - 2E+06$ $R^2 = 0.445$; Overall: $y = -0.000x^2 + 71.46x - 2E+06$, $R^2 = 0.775$).

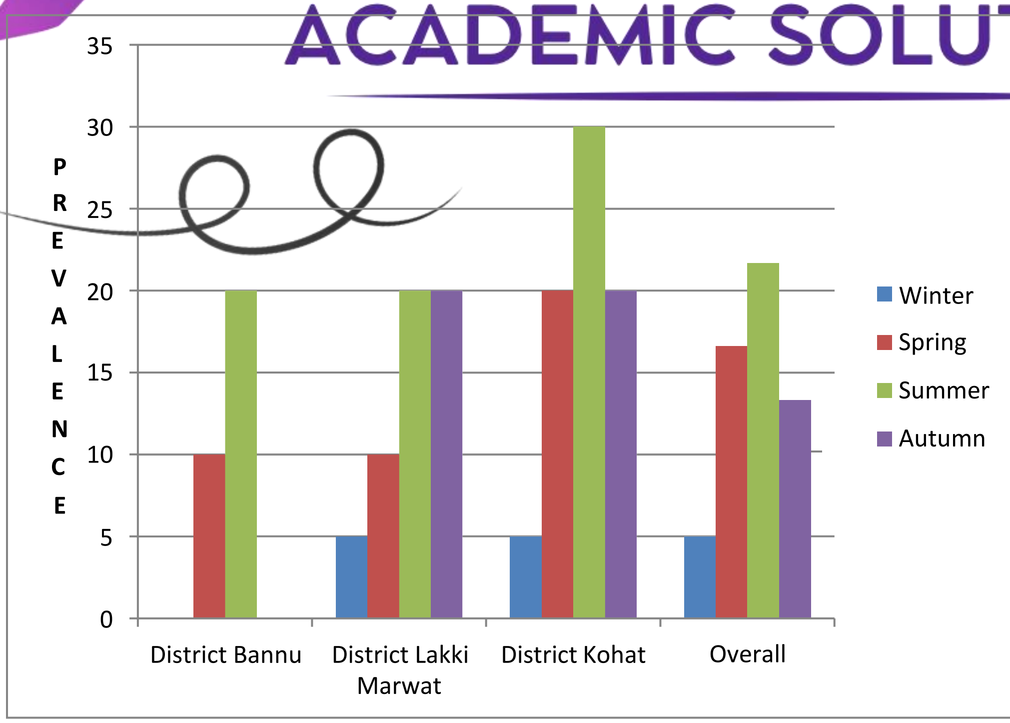

Figure 4.6: **Season wise percent prevalence of cryptosporidiosis in Goats in three selected districts of Southern KPK.**

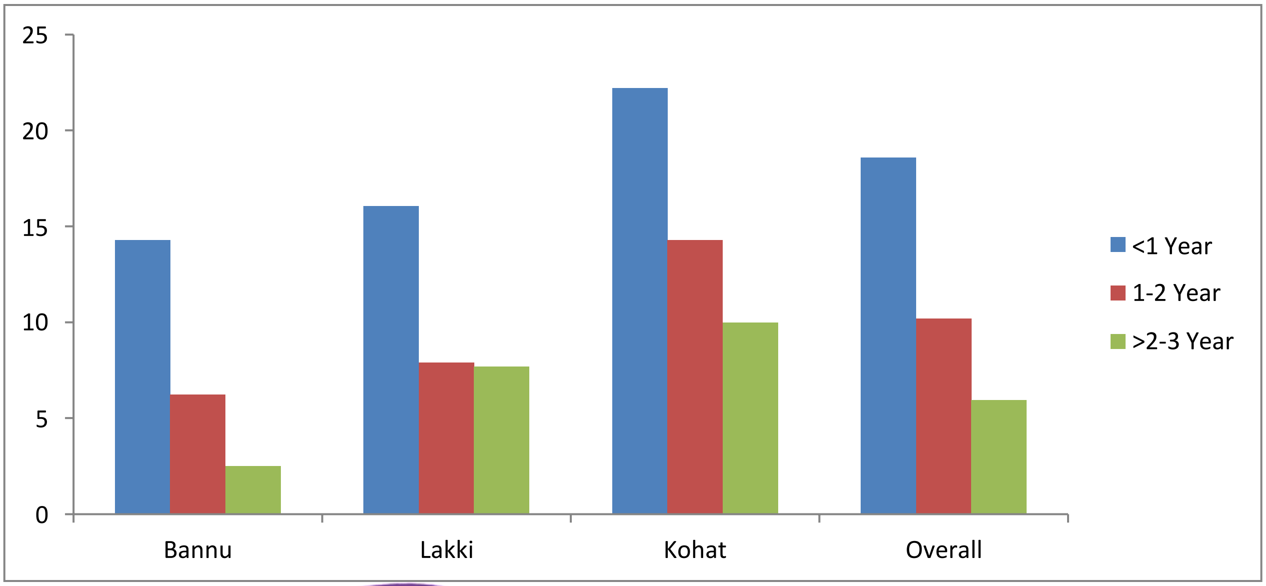

Figure 4.7: **Age wise percent prevalence of cryptosporidiosis in Goats reared in three selected districts of Southern KPK**

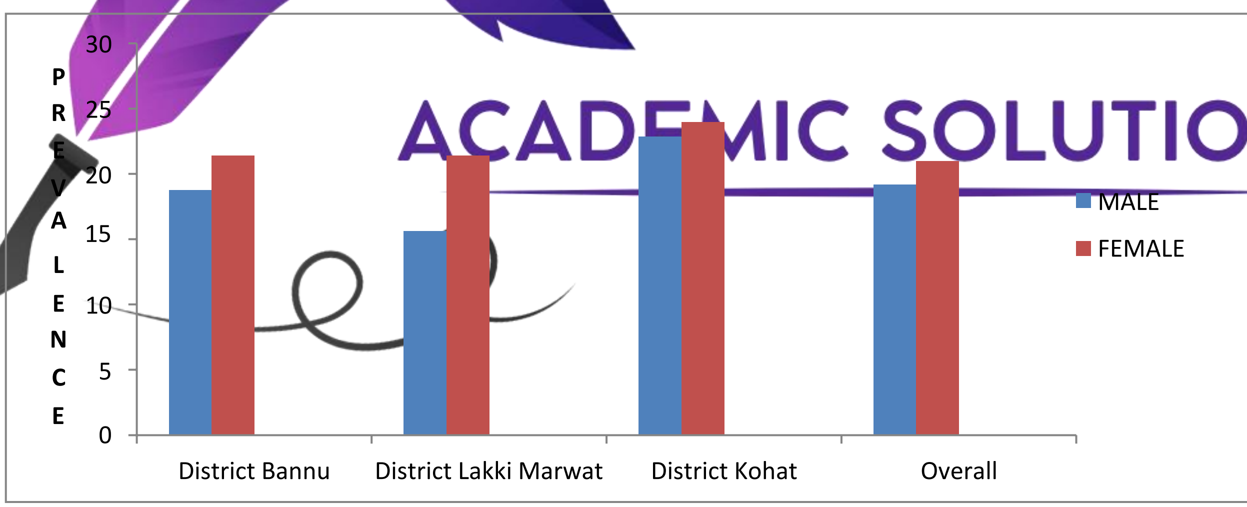

Figure 4.8: **Sex wise percent prevalence of cryptosporidiosis in Goats in three selected districts of Southern KPK**

4.4: Prevalence of cryptosporidiosis in Lambs

In the present study, the overall highest percent prevalence of *Cryptosporidium* infection was 27.22% observed in lambs in three selected zones of Southern KPK. The highest percent prevalence was recorded in District Kohat(33.33%), followed by District Lakki Marwat(25%) while the lowest percent prevalence was recorded in District Bannu(23.3%). On the basis of statistical analysis of the data, overall there was non-significant difference (P>0.057) was recorded in three selected zones for study.

4.4.1 : Month wise percent prevalence of cryptosporidiosis in Lambs

In the current study, the overall highest percent prevalence was recorded in the month of August (46.6%), followed by other months of the year such as July (40%), April, May and June (30%), September and October (26.66%), March (23.33%), November and January (20%) while the lowest percent prevalence was recorded in the month of February and December (16.66%).

On the basis of statistical analysis, there was highly significance difference (P<0.05) was observed in August with other months (January, February, September, October, November and December) of the year while non-significant difference was recorded with March, April, May, June and July. Statistically, non- significance difference (P>0.05) was recorded in the month of January, February, November and December. All results for monthly percent prevalence of cryptosporidiosis have been presented in table (4.10) and figure (4.9).

4.4.2 : Season wise percent prevalence of cryptosporidiosis in Lambs

In the present study, the overall highest percent prevalence of Cryptosporidial infection was recorded in the summer season (36.66%), followed by spring/autumn (26.66%) while the lowest percent prevalence was recorded in the month of winter season(18.33%). In the winter season, the highest percent prevalence was recorded in District Lakki Marwat and Kohat ((20%) while the lowest percent prevalence (15%) was recorded in District Bannu.

On the basis of statistical analysis, overall there was non-significant difference (P>0.102) was observed in season wise percent prevalence of cryptosporidiosis in three selected zones. Statistically, significance difference (P<0.05) was reported in percent prevalence of the *Cryptosporidium* infection in summer and winter season while non-significance difference (P>0.05) was recorded in spring and autumn season.

Results for difference in seasonal percent prevalence are presented in table (4.11) and figure (4.10).

4.4.3 : Age wise percent prevalence of cryptosporidiosis in Lambs

In the current study, the overall highest percent prevalence was recorded at the age of ≤1-15 days (38.09%), followed at the age of 16-30 days (29.41%) while the lowest percent prevalence(15.15%) was recorded at the age of ≥ 31-60 days.

On the basis of statistical analysis, overall significant difference (P<0.011) was recorded in percent prevalence of Cryptosporidiosis in different age groups of lambs in three selected areas. Statistically, significance difference (P<0.05) was observed at the age of (≤1-15 days) and (≥ 31-60 days) while non-significant difference was observed between the two age group (≤1-15 days and 16-30 days age).

Results of percent prevalence of Cryptosporidiosis in relation to age of the lambs have been presented in table (4.12) while the difference among different age groups of lambs is shown in a figure (4.11).

4.4.4 : Sex wise percent prevalence of cryptosporidiosis in lambs

In the present study, the percent prevalence of *Cryptosporidium* infection was also studied at gender level in lambs and as a result the highest percent prevalence was recorded in female (31.18%) while the lowest percent prevalence was recorded in male (22.98%). On the basis of district wise percent prevalence ratio, in male and female, it was 21.42%(M):25%(F), 24.13%(M):25.80%(F) and 28.33%(M):38.33%(F) recorded in District Bannu, Lakki Marwat and Kohat respectively.

On the basis of statistical analysis, non- significant difference (P>0.249) was recorded in both sexes. All results about percent prevalence of cryptosporidiosis in relation to sex of the lambs are presented in table (4.13) and figure (4.12).

Table.4.10: **Month wise (Jan- Dec. 2016) percent prevalence of cryptosporidiosis in Lambs in three selected districts of Southern KPK.**

Factor	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P- Value
January	2/10	20	1/10	10	3/10	30	6/30	20^{c}	0.057
February	1/10	10	3/10	30	1/10	10	5/30	16.66^{c}	
March	2/10	20	3/10	30	2/10	20	7/30	23.33^{c}	
April	2/10	20	3/10	30	4/10	40	9/30	30^{abc}	
May	3/10	30	1/10	10	5/10	50	9/30	30^{abc}	
June	3/10	30	3/10	30	3/10	30	9/30	30^{abc}	
July	4/10	40	3/10	40	5/10	50	12/30	40^{ab}	
August	4/10	40	5/10	50	5/10	50	14/30	46.66^{a}	
September	3/10	30	1/10	10	4/10	40	8/30	26.6^{bc}	
October	1/10	10	3/10	30	4/10	40	8/30	26.6^{bc}	
November	2/10	20	3/10	30	1/10	10	6/30	20^{c}	
December	1/10	10	1/10	10	3/10	30	5/30	16.66^{c}	
Total	28/120	23.3%	30/120	25%	40/120	33.33%	98/360	27.22%	

$^{a,b,d,,ab,abc,abcd,\ bcd,cd}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

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Table 4.11: Season wise percent prevalence of cryptosporidiosis in Lambs reared in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall Prevalence		
	Infected / Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Winter	6/40	15	8/40	20	8/40	20	22/120	18.33^{b}	0.102
Spring	4/20	20	6/20	30	6/20	30	16/60	26.66^{ab}	
Summer	14/40	35	12/40	30	18/40	45	44/120	36.66^{a}	
Autumn	4/20	20	4/20	20	8/20	40	16/60	26.66^{ab}	
Total	28/120	23.33	30/120	25	40/120	33.33	98/360	27.22%	

$^{a,\ b,\ c,\ ab}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

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Table 4.12: Age wise Percent prevalence of cryptosporidiosis in Lambs reared in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
≤1-15 days	17/42	40.47	14/40	35	18/44	40.90	49/126	38.88^{a}	0.011
16-30 days	7/32	21.87	10/34	29.41	12/36	33.33	29/102	28.43^{a}	
≥31-60 days	4/46	8.69	6/46	13.04	10/40	25	20/132	15.15^{b}	
Total	28/120		30/120		40/120		98/360		

$^{a,\ b}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.13: Sex wise percent prevalence of cryptosporidiosis in Lambs reared in three selected districts of Southern KPK.

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Male	12/56	21.42%	12/58	24.13%	17/60	28.33%	40/174	22.98[a]	0.249
Female	16/64	25%	18/62	25.80%	23/60	38.33%	52/186	31.18[a]	
Total	28/120		30/120		40/120		98/360		

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

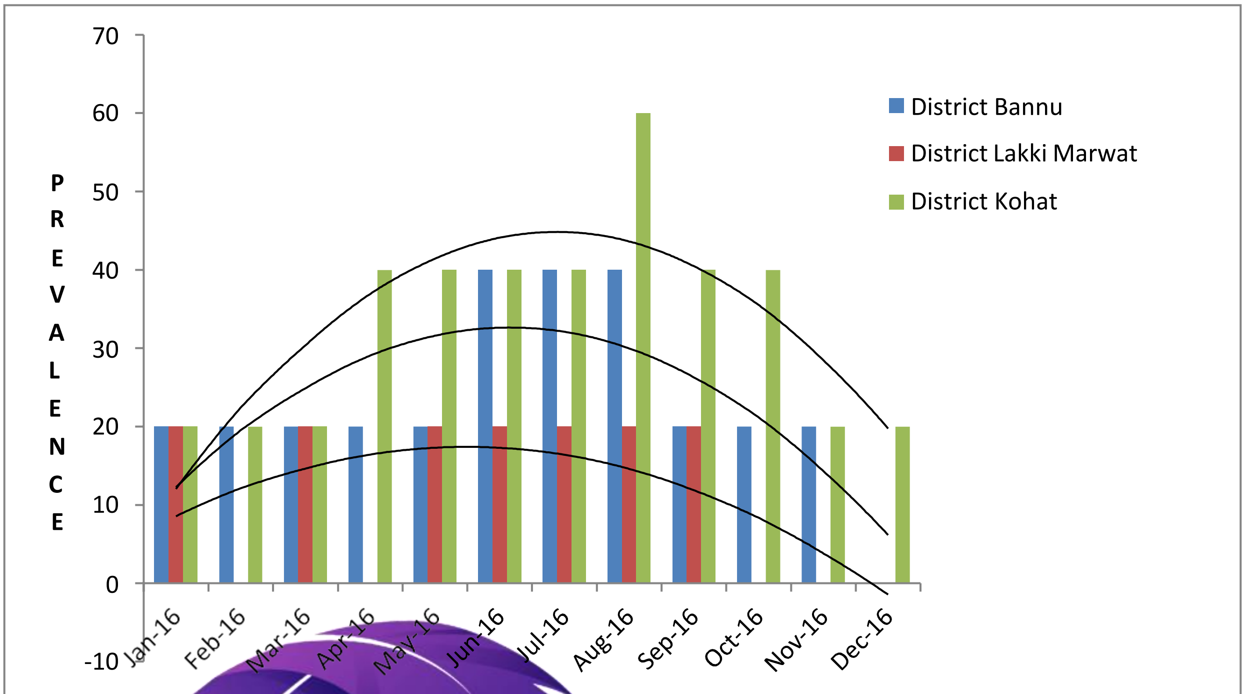

Figure 4.9: Month wise percent prevalence of cryptosporidiosis in Lambs reared (January 2016 to December2016) in three districts of Southern KPK (Kohat: $y = -0.001x^2 + 87.42x - 2E+06$ $R^2 = 0.684$; Bannu: $y = -0.000x^2 + 70.74x - 2E+06$ $R^2 = 0.571$; Lakki Marwat: $y = -0.000x^2 + 40.56x - 86218$ $R^2 = 0.326$).

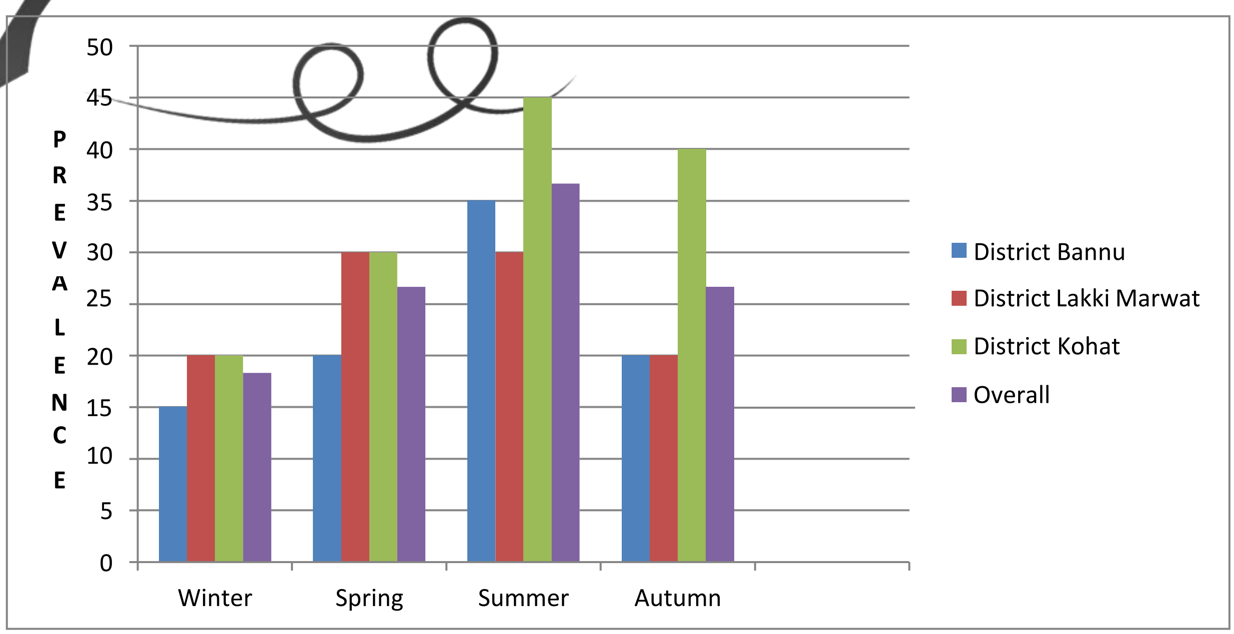

Figure 4.10: Season wise percent prevalence of cryptosporidiosis in Lambs in three selected districts of Southern KPK.

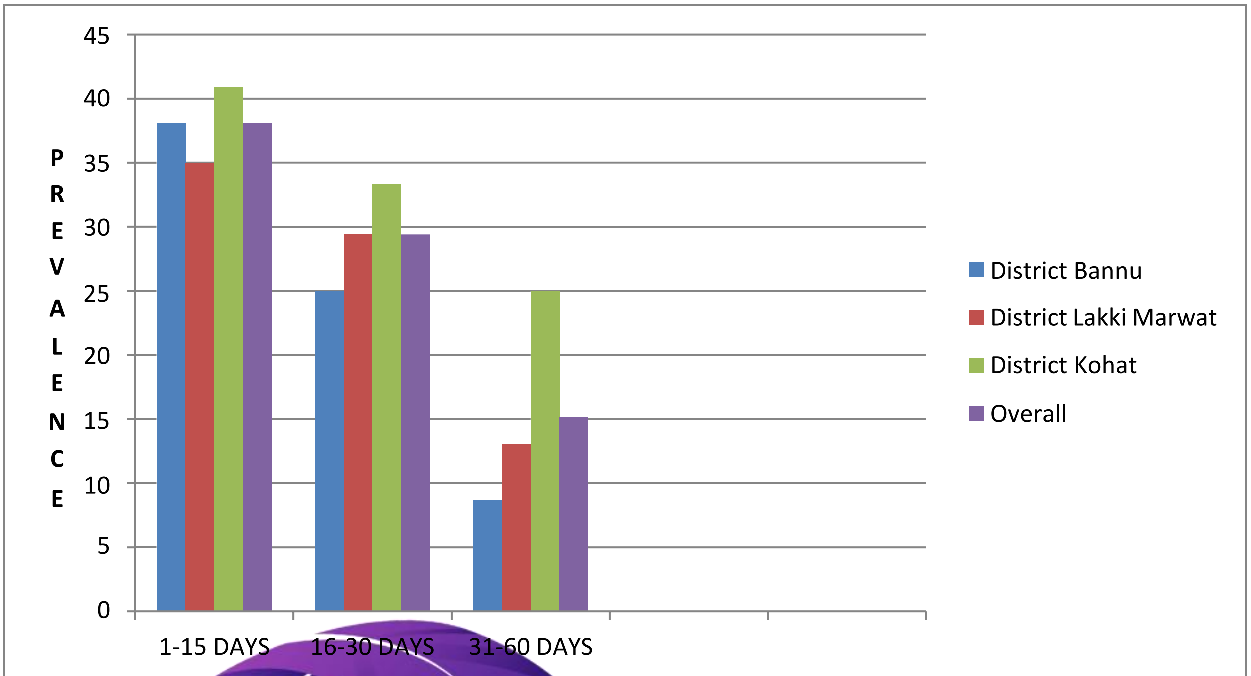

Figure 4.11: Age wise percent prevalence of cryptosporidiosis in Lambs reared in three different selected districts of Southern KPK.

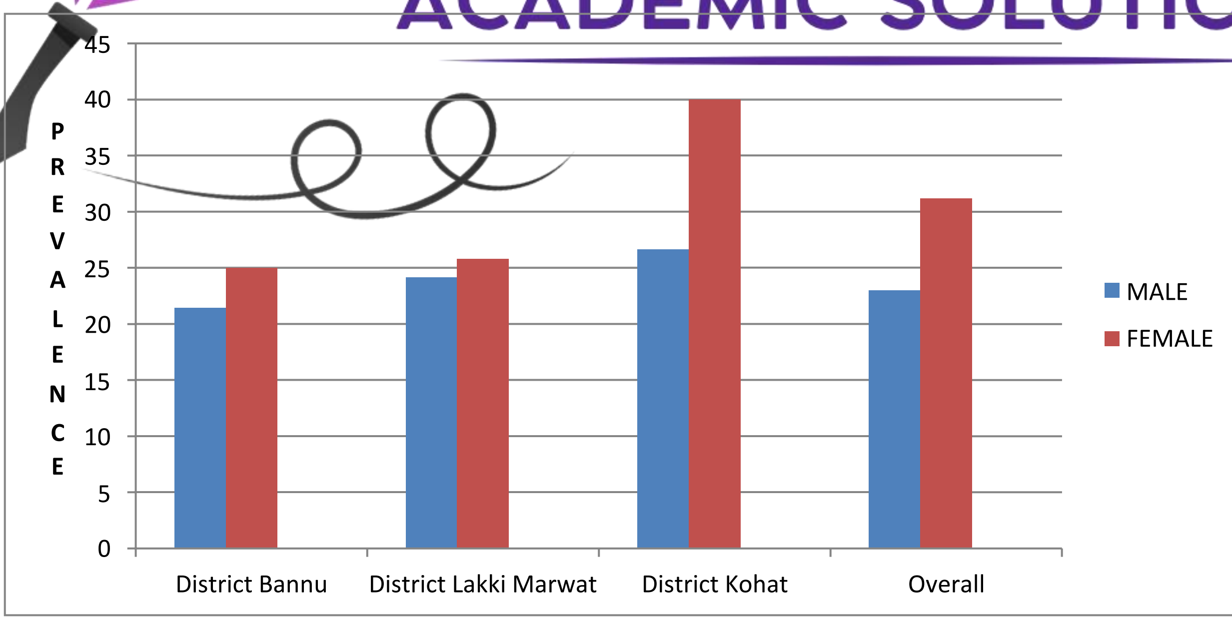

Figure 4.12: Sex wise percent prevalence of cryptosporidiosis in lambs reared in three different selected districts of Southern KPK.

4.5 : Percent Prevalence of cryptosporidiosis in Kids

In the present study, an overall percent prevalence of cryptosporidiosis was studied and as a result 20.55% prevalence was recorded in three selected zones of southern KPK. The highest percent prevalence of the *Cryptosporidium* infection was recorded in the District Kohat (23.33%) followed by District Bannu (20%) while the lowest percent prevalence was recorded in District Lakki Marwat (18.33%) and statistically significant difference (P< 0.028) was recorded in different months of the year.

4.5.1 : Month wise percent prevalence of cryptosporidiosis in kids

Data were analyzed on monthly basis to determine which month of the year had the highest percent prevalence of the *Cryptosporidium* infection in kids. The highest percent prevalence was recorded in the month of August (36.66%) followed by May (30%), June, July and October (26.66%), April (23%), September (20%), March (16.66%), November (13.3%), December (10%) while the lowest percent prevalence was recorded in the month of January and February (6.66%).

On the basis of statistical analysis, there was significance difference (P<0.05) was recorded in the month of August, January and December. Similarly statistically non-significant difference (P>0.05) was recorded in April, May, June, July, October, November and December All results of monthly prevalence have been shown in table (4.14) and curves showing high or low monthly prevalence as figure (4.13).

4.5.2 : Season wise percent prevalence of cryptosporidiosis in kids

The highest season wise percent prevalence of *Cryptosporidium* infection was recorded in the summer season (30.83%), followed by autumn and spring season (21.66%) while the lowest percent prevalence was observed in the winter season (9.16%). On the basis of statistical analysis, significant difference (P<0.0045) was recorded in percent prevalence of

Cryptosporidium infection in different seasons of the year. Different results for seasonal percent prevalence of *Cryptosporidium* infection in kids have been presented in table (4.15) and as a figure (4.14).

4.5.3 : Age wise percent prevalence of cryptosporidiosis in Kids

Different results about percent prevalence of cryptosporidiosis were recorded on the basis of age factor presented in table (4.16).In the present study, the highest percent prevalence was observed at the age of ≤1-15 days(33.92%), followed at the age of 16-30 days(17.69%) while the lowest percent prevalence was, recorded at the age of 31-60 days and above(15.25%). On the basis of statistical analysis, overall significant difference ($P<0.0045$) was recorded in three groups of different ages. Various results for age wise percent prevalence of cryptosporidiosis in kids have been presented in table (4.16) and the difference in three different age groups has been presented by a figure (4.15).

4.5.4 : Sex wise percent prevalence of cryptosporidiosis in Kids

The highest percent prevalence was recorded in females (20.98%) followed by male (19.19%). On statistical analysis, non-significant difference ($P >0.229$) was observed in female and male kids. Results for percent prevalence of cryptosporidiosis in relation to sex of the kids have been presented in table (4.17) and as a figure (4.16).

Table 4.14: Month wise (Jan-Dec. 2016) percent prevalence of cryptosporidiosis in Kids in three selected districts of Southern KPK

Factor	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
January	0/10	00	0/10	00	2/10	20	2/30	6.66^{d}	0.028
February	2/10	20	0/10	00	0/10	00	2/30	6.66^{d}	
March	1/10	10	3/10	30	1/10	10	5/30	16.66^{bcd}	
April	3/10	30	1/10	10	3/10	30	7/30	23^{abcd}	
May	4/10	40	2/10	20	3/10	30	9/30	30^{ab}	
June	2/10	20	3/10	30	3/10	30	8/30	26.66^{abc}	
July	3/10	30	4/10	40	1/10	10	8/30	26.66^{abc}	
August	3/10	30	3/10	30	5/10	50	11/30	36.66 a	
September	1/10	10	2/10	20	3/10	30	6/30	20^{abcd}	
October	3/10	30	2/10	20	3/10	30	8/30	26.66^{abc}	
November	1/10	10	0/10	00	2/10	20	3/30	13.33^{cd}	
December	1/10	10	2/10	20	2/10	20	4/30	10^{bcd}	
Total	24/120	20%	22/120	18.33%	28/120	23.33%	74/360	20.55%	

a, ab, abc, abcd, bcd, cd, d mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.15: Season wise percent prevalence of cryptosporidiosis in Kids in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall Prevalence		
	Infected / Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Winter	3/40	7.5	2/40	5	6/40	15	11/120	9.16^{a}	0.0045
Spring	5/20	25	4/20	20	4/20	20	13/60	21.66^{b}	
Summer	12/40	30	13/40	32.5	12/40	30	37/120	30.83^{b}	
Autumn	4/20	20	3/20	15	6/20	30	13/60	21.66^{b}	
Total	24/120		22/120		28/120		74/360		

$^{a,\ b}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.16: Age wise percent prevalence of cryptosporidiosis in Kids in three selected districts of Southern KPK.

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
≤1-15 days	12/36	33.33	10/32	31.25	16/44	36.36	38/112	33.92^{a}	0.0001
16-30 days	9/52	17.30	7/42	16.66	7/36	19.44	23/130	17.69^{b}	
≥31-60 days	3/32	9.37	5/46	10.86	5/40	15	18/118	15.25^{c}	
Total	24/120		22/120		28/120		74/360		

$^{a,\ b,\ c,\ ab}$ mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.17: Sex wise percent prevalence of cryptosporidiosis in Kids in three selected districts of Southern KPK

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-Value
Male	12/64	18.75	10/64	15.62	16/70	22.85	38/198	19.19^{a}	0.229
Female	12/56	21.42	12/56	21.42	12/50	24	34/162	20.98^{a}	

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

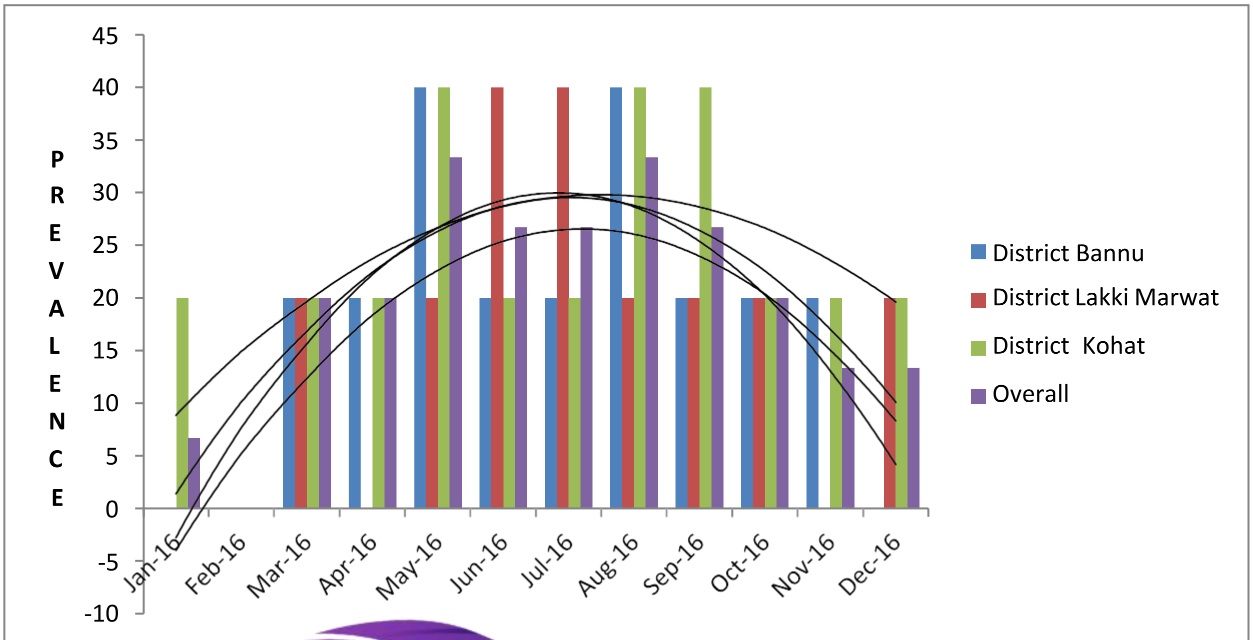

Figure 4.13: Month wise percent prevalence of cryptosporidiosis in kids from January, 2016 to December, 2016 in three selected districts of Southern KPK. (Bannu: $y = -0.001x^2 + 88.32x - 2E+06$, $R^2 = 0.655$; Lakki Marwat: $y = -0.000x^2 + 72.65x - 2E+06$, $R^2 = 0.445$; Kohat: $y = -0.000x^2 + 45.70x - 97280$, $R^2 = 0.317$; Overall: $y = -0.000x^2 + 71.46x - 2E+06$, $R^2 = 0.775$)

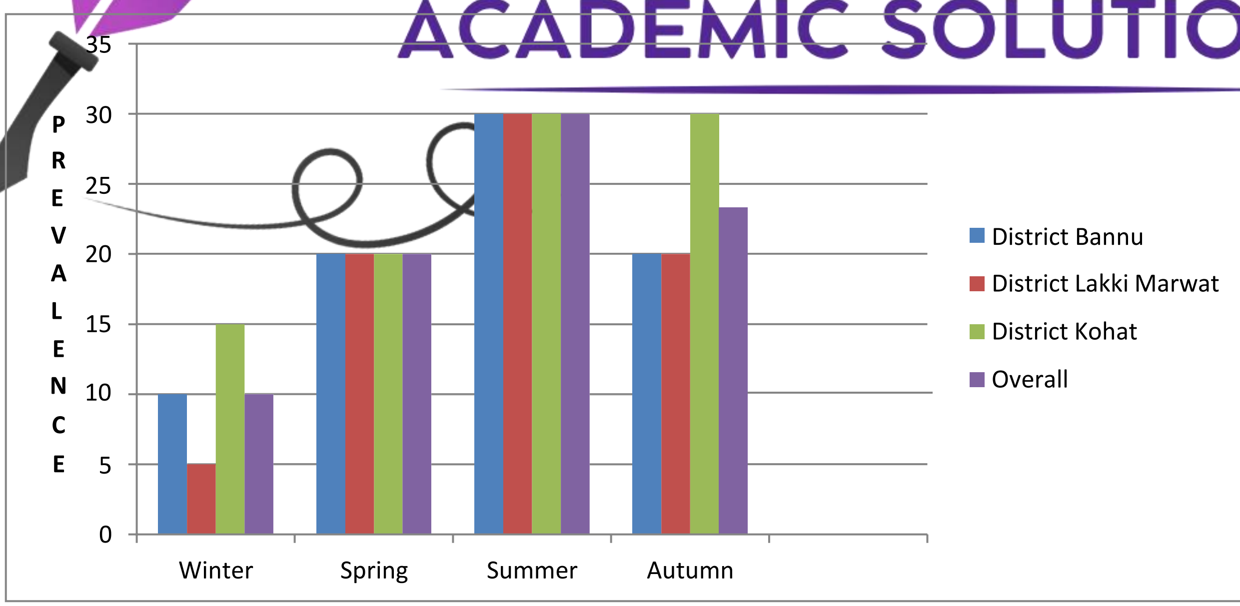

Figure 4.14: Season wise percent prevalence of cryptosporidiosis in kids in three selected districts of Southern KPK.

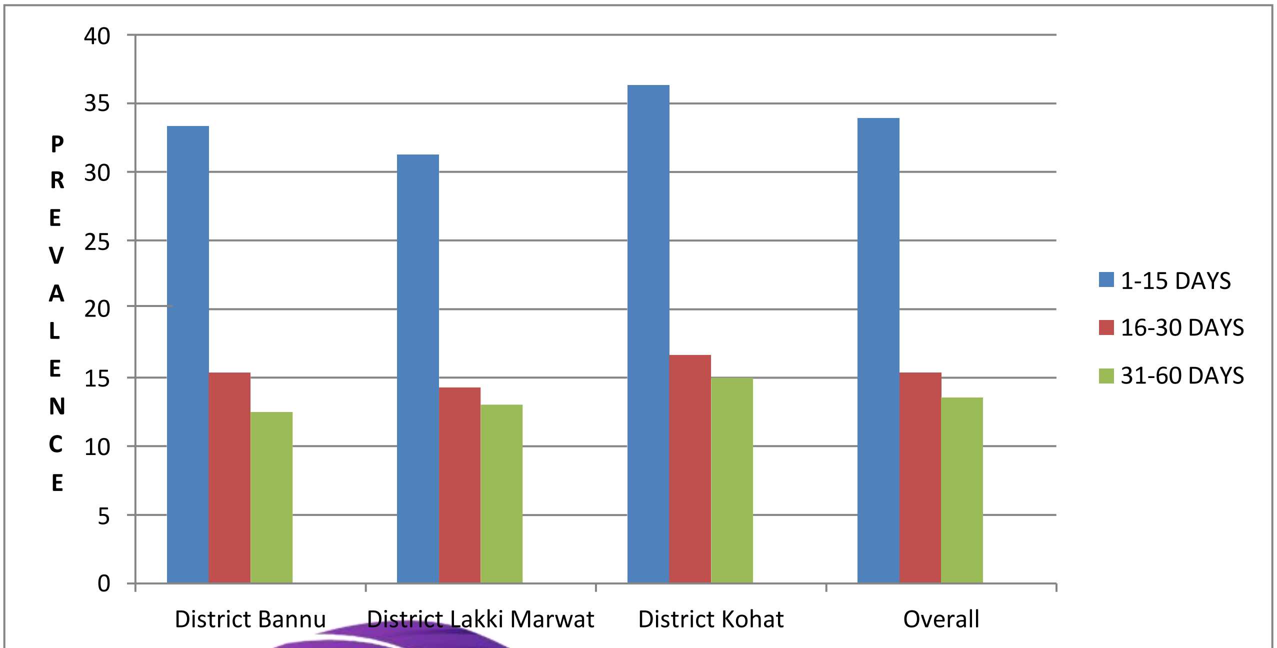

Figure 4.15: Age wise percent prevalence of cryptosporidiosis in Kids in three selected districts of Southern KPK.

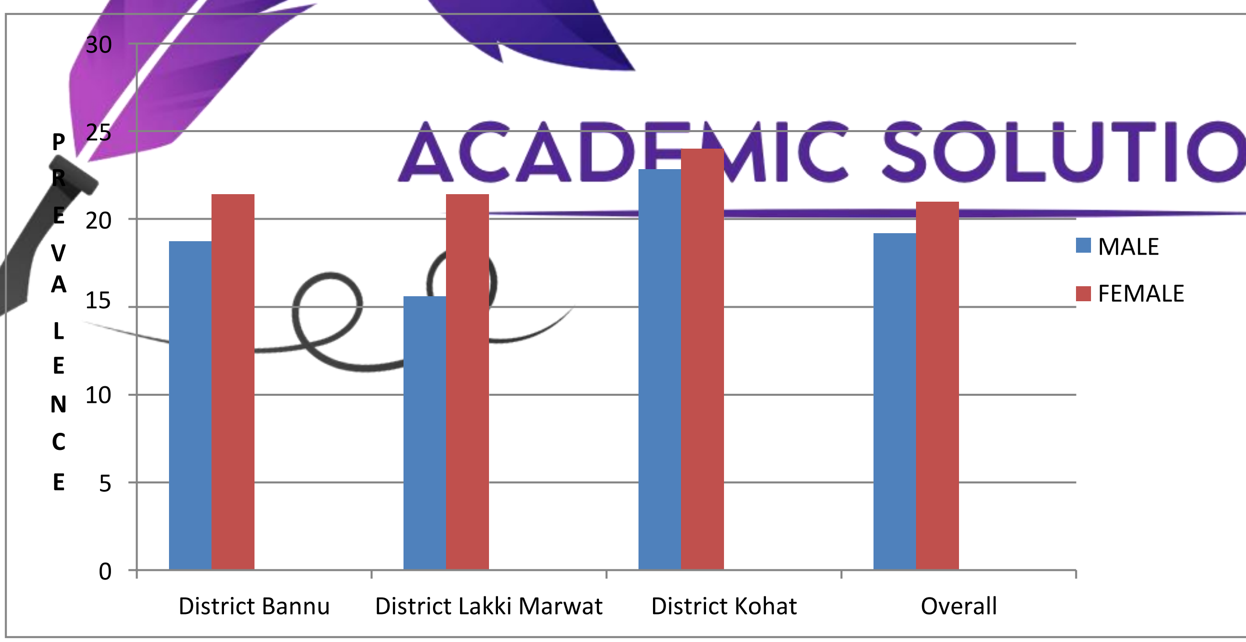

Figure 4.16: Sex wise percent prevalence of cryptosporidiosis in kids in three selected districts of Southern KPK

4.6: Molecular detection of *Cryptosporidium* infection

Fecal samples were collected from sheep, and were analyzed through simple conventional method for diagnosis of *Cryptosporidium* infection. After conventional method, the samples were reconfirmed through a highly sensitive molecular technique known as PCR for detection of *Cryptosporidium* infection. DNA extraction kit was used to isolate the DNA from *Cryptosporidium* oocyst. Polymerase chain reaction (PCR) was practically applied for detection of *Cryptosporidium* DNA then genus specific primers were used.

The amplified targeted nucleic acid was revealed at 435 base pairs (bp) of DNA band. A specific DNA band has been presented as a plate (4.1) The most common genes were18S rRNA where 99% similarity have been reported between different species of the *Cryptosporidium* and different primers have been designed for the detection of *Cryptosporidium* (Xiao et al.1999).

During the total study period, 360 samples of sheep were analyzed through simple PCR for presence of *Cryptosporidium* infection. Out of 180 samples, 90 were positive by PCR. The overall molecular percent prevalence was 24.99% recorded in sheep. The highest molecular percent prevalence was recorded in District Kohat(31.66%), followed by District Bannu (25%) while the lowest molecular percent prevalence was recorded in District Lakki Marwat(18.33%).

Different results in relation to different districts about season, sex and age of sheep's are presented in table (4.18). Bar presentation for molecular percent prevalence of cryptosporidiosis in sheep's, in relation to selected areas, season, sex and age are presented as figures (4.17, 4.18, 4.19 and 4.20 respectively).

Table 4.18: Molecular and Microscopic percent prevalence of cryptosporidiosis in relation to area, season, sex and age in sheep in three selected districts of Southern KPK Pakistan.

Factors		**NO. Of Positive Samples/ Total No of Samples Examined by PCR**	**Molecular (%) prevalence**	**Microscopic (%) prevalence**	**P-value**
Area wise prevalence	Bannu	30/120	25	**18.33**	**0.187**
	Lakki Marwat	22/120	18.33	15	
	Kohat	38/120	31.66	21.66	
Season wise prevalence	Winter	16/120	13.33	8.33	0.268
	Spring	16/60	26.66	18.33	
	Summer	40/120	33.33	27.5	
	Autumn	18/60	30	20	
Sex wise prevalence	Male	24/94	25.53	17.02	0.019
	Female	58/266	27.08	18.80	
Age wise prevalence	≤1 year of age	40/134	29.85	23.13	0.264
	1-2 years of age	32/122	26.22	18.85	
	≥2-3 years of age	18/104	17.30	11.53	

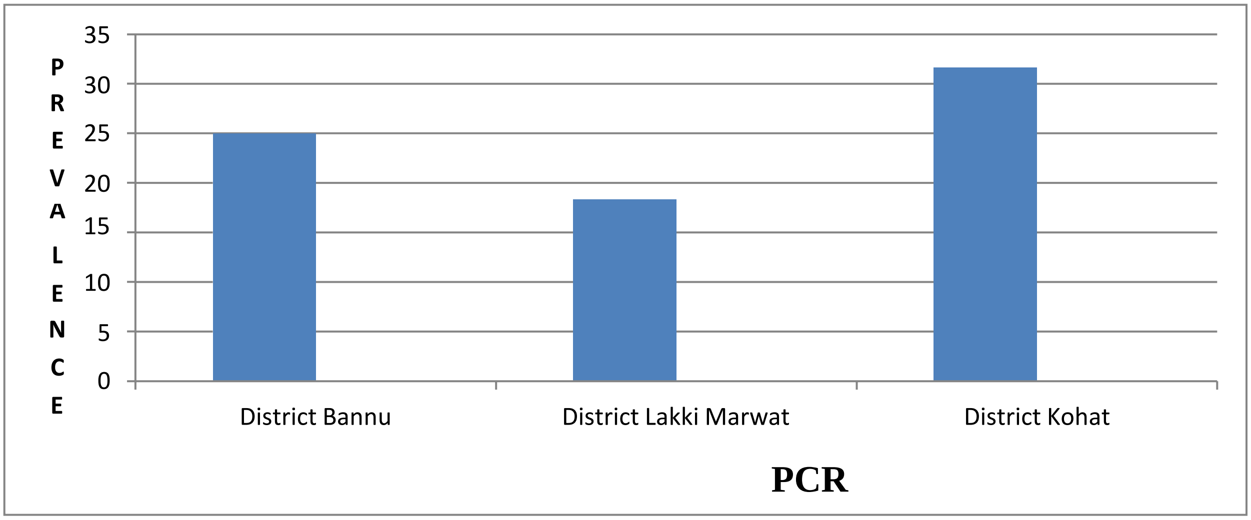

Figure 4.17: Molecular percent prevalence of cryptosporidiosis in sheep in three selected districts of Southern KPK.

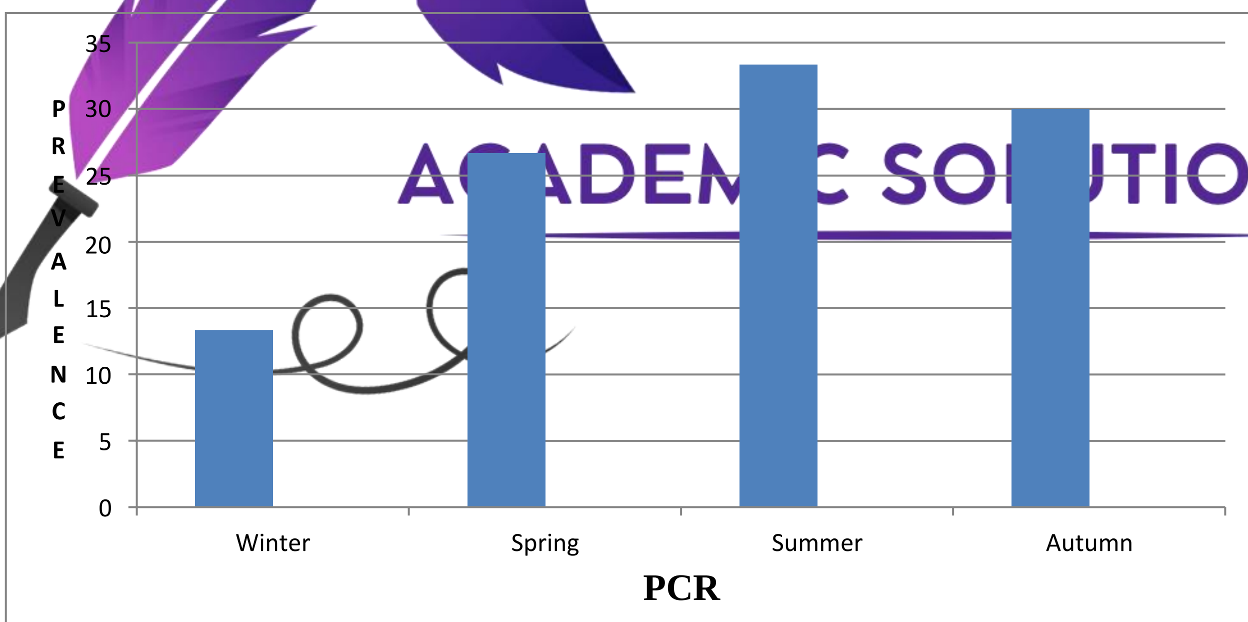

Figure 4.18: Season wise molecular percent prevalence of cryptosporidiosis in sheep (January- 2016 to December- 2016) in three selected districts of Southern KPK.

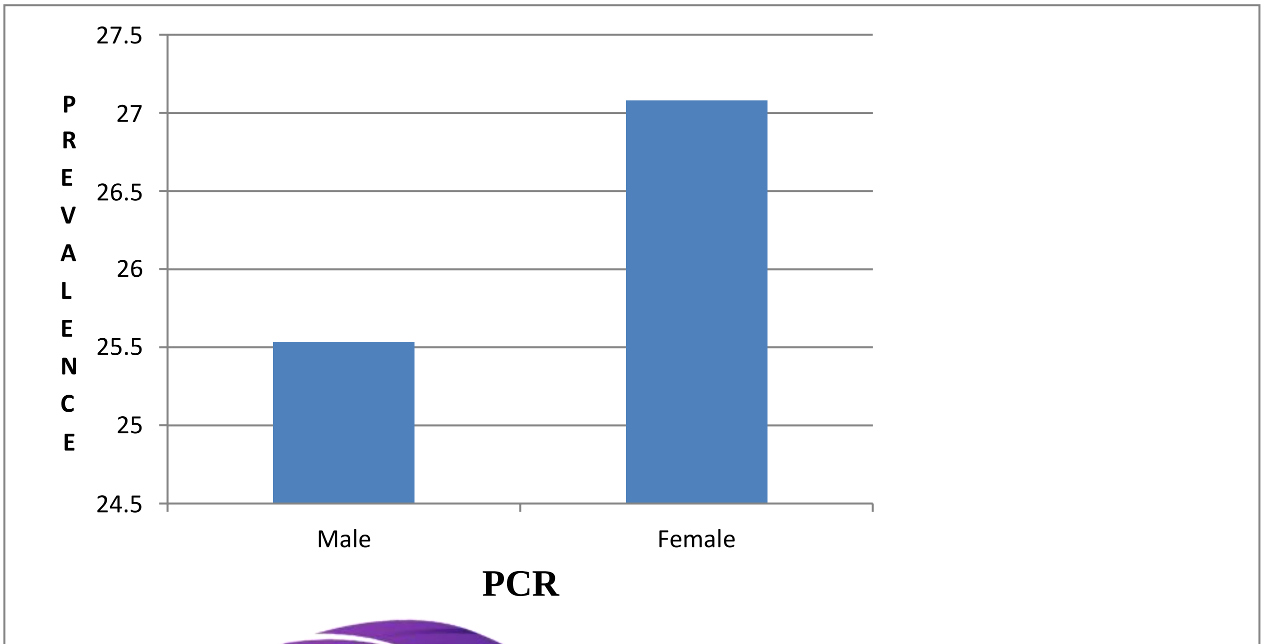

Figure 4.19: Sex wise molecular percent prevalence of cryptosporidiosis in sheep in three selected districts of southern KPK.

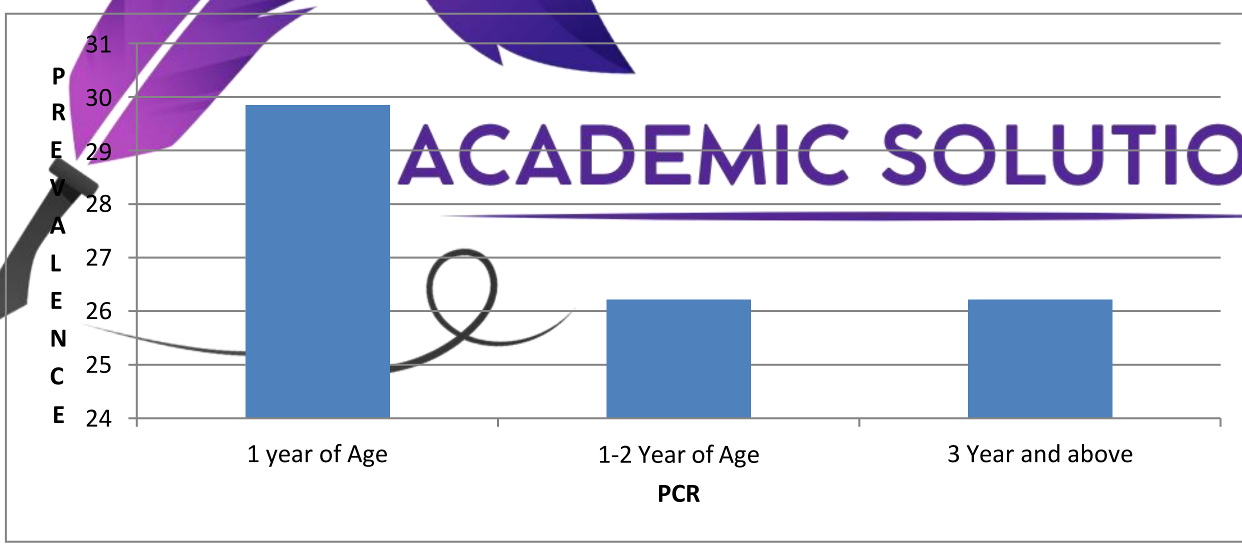

Figure 4.20: Age wise molecular percent prevalence of cryptosporidiosis in Sheep in three selected districts of Southern KPK.

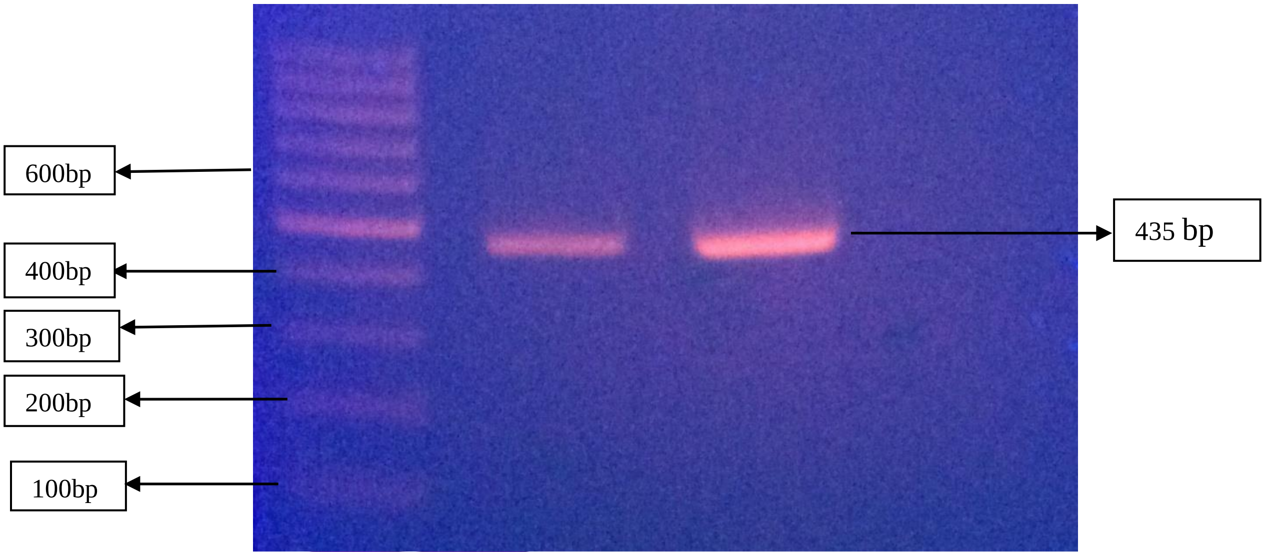

Plate 4.1: Agarose gel electrophoresis pattern of PCR amplicons of *Cryptosporidium* Oocyst.

4.7: Comparative sensitivity and specificity study of Molecular (PCR) and Simple microscopic Coprological techniques for detection of *Cryptosporidium* infection in sheep.

In the present study, overall molecular percent prevalence of cryptosporidiosis, determined by PCR (molecular technique) was higher (24.99%) than simple microscopic morphological identification (14.99%) in three districts of Southern KPK.

In the current study, the efficiency of PCR and simple microscopic techniques were compared to find the percent efficacy. As a result, the highest percent efficacy was reported by PCR (25%) followed by simple microscopic examination (18.33%) in detection of *Cryptosporidium* infection in District Bannu. In District Lakki Marwat, the highest percent prevalence was detected by PCR (18.33%) while the lowest percent prevalence (15%) was determined by simple microscopic examination. Similarly, in District Kohat, the highest percent prevalence was detected by PCR (31.66%) while the lowest percent prevalence (21.66%) was determined by simple microscopic examination. On the basis of statistical analysis, non-significant difference (P>0.187) was recorded in sensitivity of two diagnostic tools (simple microscopy and PCR) for detection of *Cryptosporidium* infection.

Similarly, season wise percent prevalence of *Cryptosporidium* infection was also determined by simple microscopic examination and PCR where the highest percent prevalence was determined by PCR than simple microscopic examination. In the **winter season**, the highest percent prevalence (13.13%) was detected by PCR while the lowest percent prevalence (8.33%) was determined by simple microscopic examination.

In **spring season**, the highest molecular percent prevalence was determined by PCR (26.66%) while the lowest percent prevalence (18.33%) was determined by simple microscopic examination. In **summer season**, the highest molecular percent prevalence was detected by PCR (33.33%) while the lowest percent prevalence (27.5%) was determined by simple microscopic examination. In **autumn season**, the highest molecular percent prevalence (30%) was detected by PCR while the lowest percent prevalence (20%) was determined by simple microscopic examination. On the basis of statistical analysis, non- significant (P>0.268) difference was observed.

In the present study **gender wise** molecular study was also conducted where the highest molecular percent prevalence was determined in male (25.53%) by using PCR technique than simple microscopic examination (17.02%) while in female, the highest molecular percent prevalence was determined by PCR (27.08%) than simple microscopic examination (18.80%). On the basis of statistical analysis, significant difference (P<0.019) was observed in sensitivity of both diagnostic techniques.

In the present study, age wise molecular percent prevalence was also determined by PCR and simple microscopic examination. The highest molecular percent prevalence was determined through PCR than simple microscopic examination. At the age of ≤1 year, the highest molecular percent (29.85%) prevalence was determined by PCR while the lowest percent prevalence (23.13%) was determined through simple microscopic examination.

At the age of 1-2 years and ≥ 2-3 years, the highest molecular percent prevalence (26.22% and 17.30%) prevalence was determined through PCR while the lowest percent prevalence (18.85% and 11.53%) was determined by simple microscopic examination. On the basis of statistical analysis, non-significant difference (P>0.264) was observed in sensitivity of two diagnostic techniques in detection of cryptosporidial infection in sheep.

Results for comparison of simple microscopy and PCR for identification of the *Cryptosporidium* oocysts in fecal samples of sheep has been presented in table (4.18 and 4.19).

Table 4.19: Comparative analysis of sensitivity and efficacy of two diagnostic techniques (Simple conventional method and PCR) for detection of cryptosporidiosis in small ruminants (N= 360).

Parameters	Microscopic (%) Prevalence	PCR (%) Prevalence	P –value
Area wise(overall)	18.33[a]	25.17[a]	0.187
Season wise(overall)	18.54 [a]	25.78 [a]	0.268
Sex wise(overall)	17.91[b]	26.30[a]	0.019
Age wise(overall)	17.89 [a]	24.45 [a]	0.264

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

4.8 : Relationship between prevalence of cryptosporidiosis and Meteorological factors

In District Bannu, maximum mean average temperature was 32.49°C, while the lowest mean average temperature was 9.78°C. Similarly in District Lakki Marwat, the maximum mean average temperature was 31.94°C while the lowest mean average temperature was 12.45°C.In District Kohat, the maximum mean average temperature was 32°C while the lowest recorded minimum temperature was 11°C. On daily basis, the maximum and minimum temperature was recorded and their averages were calculated on monthly basis. In District Bannu, the mean highest relative humidity was 69.01% during the month of July while the lowest humidity was 38.3%, recorded during the month of May, 2016.On daily basis, relative humidity was recorded at the time of morning and evening and calculates the monthly averages. The metrological information is presented in table (4.20). In District Lakki Marwat, the mean highest relative humidity was 72.72% during the month of August while the lowest relative humidity was 38.57% recorded during the month of July. The metrological information is presented in table (4.21). In District Kohat, the mean highest relative humidity was 65% recorded during the month of August while the lowest relative humidity was 43% recorded during the month of May, 2016. The metrological information are presented in table (4.22).

In District Bannu, overall highest (171mm) rainfall was recorded in the month of August while the lowest (0 mm) in the month of October. The meteorological information is available in table (4.20).In District Lakki Marwat, the overall highest (169mm) rainfall was recorded in the month of August while the lowest (0 mm) in the month of November and the metrological information is presented in table (4.21).In District Kohat, overall highest (182.3 mm) rainfall was reported in the month of August while the lowest (4.3mm) rainfall was recorded during the month of November. Overall the highest percent prevalence of cryptosporidiosis in sheep, Goats, lambs, and kids was 36.66%, 26.6%, 46.6%, and 33.33% respectively recorded during in the month of August in three districts of Southern KPK. Similarly, the highest rainfall and humidity was recorded in the month of August in three districts of Southern KPK where maximum prevalence of cryptosporidiosis was recorded in small ruminants. It was concluded that high temperature, relative humidity and heavy rainfall play an important role in heavy infestation of endoparasites in domestic animals.

Table 4.20: Mean month wise temperature, humidity and rain fall in District Bannu during the month of January, 2016 to December, 2016.

Months	Temperature(C^0)			Relative Humidity (%)			Rainfall (mm)
	Minimum	Maximum	Mean	Morning	Evening	Mean	
January	4.10	15.46	9.78	72.47	45.22	58.84	13
February	6.50	21.39	13.94	70.29	49.13	59.71	8.6
March	10.17	22.83	16.5	65.18	38.37	51.77	41.2
April	21.50	32.62	27.06	52.34	33.9	43.12	19.7
May	21.36	38.17	29.76	46.35	30.25	38.3	22.4
June	22.30	40.22	31.26	73.36	52.27	62.81	36.3
July	26.13	38.59	32.36	76.34	61.39	69.01	166.4
August	26.15	38.59	32.43	77.12	67.22	72.17	171
September	23.20	36.56	29.88	75.43	58.29	66.96	31.3
October	16.44	31.37	23.90	66.62	71.33	68.97	00
November	10..38	26.6	18.49	79.6	55.43	67.51	6
December	2.55	18.48	10.51	80.33	51.22	65.75	5
Mean	15.89	30.07	22.98	69.61	51.16	60.45	43.40

Table 4.21: Mean month wise temperature, humidity and rain fall in Lakki Marwat from January to December, 2016.

Months	Temperature(C^0)			Relative Humidity (%)			Rainfall (mm)
	Minimum	Maximum	Mean	Morning	Evening	Mean	
January	7.14	17.77	12.45	71.42	44.24	57.83	14.3
February	9.54	20.35	14.94	69.33	47.22	58.27	9.1
March	13.17	24.83	19	66.12	36.41	51.26	44
April	22.52	32.60	27.56	50.32	32.88	41.6	18.3
May	25.10	36.15	30.62	47.31	29.84	38.57	25.3
June	27.10	35.22	31.16	74.41	51.29	62.85	39.2
July	27.33	35.59	31.46	78.34	60.32	69.33	163.2
August	28.39	35.50	31.94	78.11	67.32	72.71	169.1
September	26.24	35.56	30.90	74.83	57.55	66.19	33.2
October	19.44	31.34	25.39	67.69	70.39	69.04	5.4
November	12.34	26.21	19.27	80.43	54.48	67.45	00
December	9.20	19.42	14.31	81.43	50.34	65.88	6.2
Mean	18.98	29.21	24.08	69.97	50.19	60.08	43.94

General Metrological information about District Kohat

The Kohat region is mainly composed of hilly areas and the average height above sea level ranges from 4900 ft (1500m). The climatic conditions of Kohat and its surroundings areas are hot from the month of May to September. The mean (max and min) temperature observed during the month of June is about 40° C and 28° C respectively. The winter season is cold. The mean (max and min) temperature recorded during the month of January is 17° C and 6° C respectively. The heavy rainfall is received all over the year with an average about 114mm.

During the month of August and December (summer and winter season) maximum humidity was recorded all over the year. The metrological data of District Kohat has been presented in a table (4.22).

Table 4.22: Mean month wise temperature, humidity and rain fall in Kohat during January, 2016 to December, 2016.

Months	Temperature(C0)			Relative Humidity (%)			Rainfall (mm)
	Minimum	Maximum	Mean	Morning	Evening	Mean	
January	6	16	11	58	51	54.5	26
February	8	18	13	60	50	55	22.7
March	13	22	17.5	65	47	56	58.4
April	19	28	23.5	58	42	50	28.9
May,	23	35	29	54	32	43	27
June	27	36	31.5	58	38	48	42.7
July	28	36	32	75	47	62	179
August	27	37	32	76	54	65	182.3
September	24	32	28	74	52	63	17.9
October	17	28	22.5	70	60	65	9.7
November	11	22	16.5	77	49	63	4.3
December	6	17	11.5	80	50	65	8.3
Mean	15.08	27.25	22.33	67.08	47.66	57.45	50.6(mm)

4.9 : Percent Prevalence of Cryptosporidiosis in humans

To determine zoonotic aspect of Cryptosporidiosis, the present study was designed to find out the prevalence of the *Cryptosporidium* infection in three selected districts of Southern KPK as reported by various researchers that Sheep and goats are the main source of reservoirs for *Cryptosporidium* infections in humans (Koinari et al. 2014).An outbreak with *Cryptosporidium* infection was documented in school children linked and contacted with lamb and goat kids (Lange et al. 2014).The *Cryptosporidium* species have been reported in different animals such as cattle, sheep, fish, birds and reptiles. There are 79 species of the *Cryptosporidium*. Some species can survive in a number of hosts whereas some species are adopted to host specific. Cryptosporidiosis was reported in 1976 as a first case of humans. Cryptosporidiosis is often fatal in those individuals suffering from AIDS (Tzipori S et al. 2008)

Study Design and Sampling Strategy

Human stool samples (n=360) suspected for cryptosporidiosis were collected from three selected districts headquarter hospitals of District Bannu, Lakki Marwat and Kohat during the month of January to June, 2016.In the present study, collected 20 samples per month per district. Out of 20 samples, 10 samples were collected from adult patients, suffering from abdominal disturbance such as acute or persistent diarrhea, vomiting while 10 samples were collected from children showing acute or persistent diarrhea, vomiting and abdominal cramps. All the samples were preserved in 10% formalin with a ratio of 2:3. Samples were collected into sterile wide mouthed plastic bottles properly labeled and transported to the refrigerator till analysis within one or two weeks. All the samples were analyzed in the Medicine laboratory, Department of Clinical Medicine & Surgery, University of Veterinary and Animal Sciences, Lahore. All the collected samples were properly stained with Modified Ziehl Neelsen acid fast satin after proper centrifugation and examined under microscope at 100X and screened out for the presence of *Cryptosporidium* oocysts. This study was just a simple descriptive type of study for the purpose to find out the frequency of the *Cryptosporidium* infection in young children and adults in relation to animals contact and other associated risk factors. All the samples were collected randomly with the help of simple convenience sampling method from those patients showing abdominal disturbance (abdominal pain, nausea, and vomiting, acute or persistent diarrhea, dysentery). The purpose of this study was to determine the percent prevalence of the

Cryptosporidium infection in children and adults in three selected areas of Southern KPK, Pakistan. Sampling strategy has been presented in Table (4.23).

Table: 4.23: Experimental sampling plan for collection of Human stools for detection of *Cryptosporidium* oocyst during January-June, 2016 from three selected districts of Southern KPK.

Months	District Bannu		District Lakki Marwat		District Kohat		Overall	
	Adults	Children's	Adults	Children's	Adults	Children's	Adults	Children's
January	10	10	10	10	10	10	30	30
February	10	10	10	10	10	10	30	30
March	10	10	10	10	10	10	30	30
April	10	10	10	10	10	10	30	30
May	10	10	10	10	10	10	30	30
June	10	10	10	10	10	10	30	30
Total	60	60	60	60	60	60	180	180

4.9. Overall percent prevalence of cryptosporidiosis in humans

In the present study, overall highest percent prevalence of the *Cryptosporidium* infection was reported in children (16.66%) followed by adults where the lowest (4.44%) percent prevalence was recorded. In District Bannu, the highest percent prevalence was recorded in children (18.33%) while the lowest percent prevalence was recorded in adults (5%). In District Lakki Marwat, the highest percent prevalence was recorded in children (15%) while the lowest percent prevalence (5%) was recorded in adults while in District Kohat, the highest percent prevalence was observed in children (16.66%) while the lowest percent prevalence was recorded in adults (3.33%) .On the basis of Statistical analysis, significant difference ($P<0.000$) was recorded in overall percent prevalence of cryptosporidiosis in adults and children and represented by table (4.24). In humans, C.oocyst has been shown on a slide and provided in form of plate (4.2).

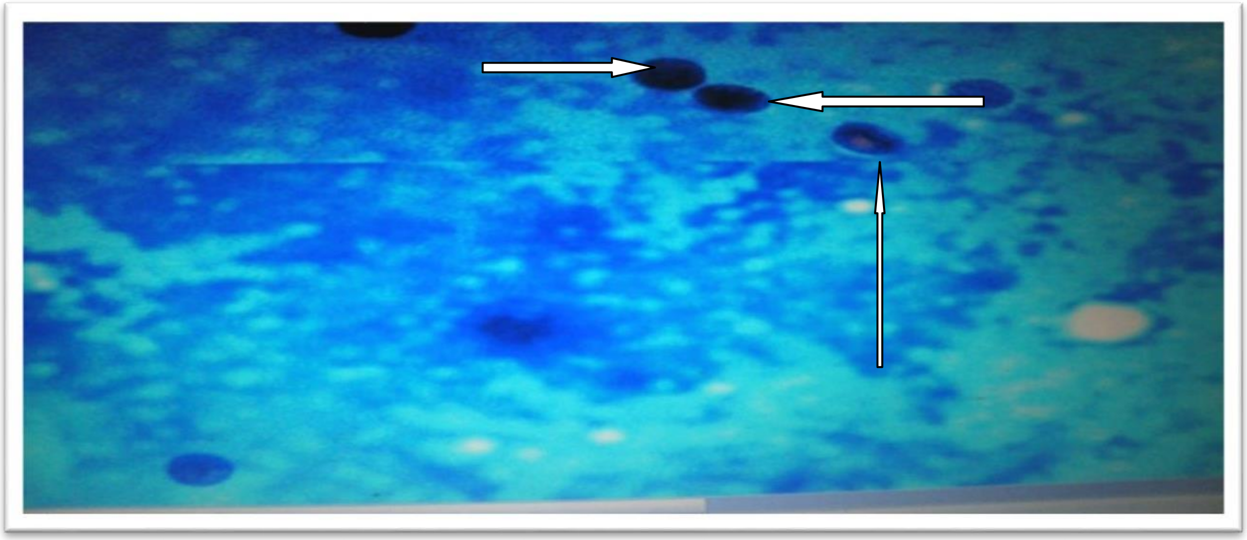

Plate 4.2: ***Cryptosporidium*** **Oocysts stained by MZN (Modified Ziehl Neelsen acid Fast stain) method at power of 400X magnification.**

4.9.2 : Age wise percent prevalence of cryptosporidiosis in children

Results of percent prevalence of cryptosporidiosis in relation to age of children are presented in table (4.25). In the present study, overall the highest percent (21.42%) prevalence was recorded at the age of ≤1-5 years of age followed at the age of 6-10 years (17.30%) years while the lowest percent prevalence was recorded at the age of11-15 years (10.34%).

On the basis of statistical analysis, significant difference (P<0.017) was recorded in three groups of different ages. Various results for age wise prevalence of cryptosporidiosis in young Children have been presented in table (4.25).

4.9.3 : Percent prevalence of cryptosporidiosis in relation to animals contact (yes) or not contact (No) in young children in three districts of Southern KPK.

In the present study, overall the highest percent prevalence(20%) was recorded in those Children having direct or indirect contact with animals followed by those Children having no direct or indirect contact with animal where recorded the lowest percent prevalence(12.94%) .

In District Bannu, the highest percent prevalence was recorded, in those children where there was direct contact with animals or contaminated area or fecal contact or contaminated drinking water by animal (20.58%) was recorded while the lowest percent prevalence (15.58%) was, recorded in all those children's where there was no direct or indirect contact with animal. In District Lakki Marwat, the highest percent prevalence (20.68%) was recorded in those children having direct animals contact while lowest percent prevalence (9.67%) was recorded where no direct contact was recorded. Similar results were observed in District Kohat where the highest percent prevalence (18.75%) was recorded in those children where there was direct contact with animals was recorded and vice versa. On statistical analysis, significant difference (P<0.020) was recorded between two groups" having animals contact or not having any animal contact. Various results for animal contact or not have been presented in table (4.26).

4.9:4 Percent prevalence of cryptosporidiosis in relation to acute or persistent diarrhea in young children in three selected districts of Southern KPK

In the present study, overall the highest percent prevalence (25.84%) was reported in those Children suffering from persistent diarrhea while the lowest percent prevalence (7.69%) was reported in those Children suffering from acute diarrhea. On the basis of statistical analysis, significant difference (P<0.000) was recorded on the basis of nature of diarrhea (acute or persistent).

Table 4.24: Percent prevalence of cryptosporidiosis in relation to children and adult humans in three selected districts of Southern KPK.

Factor	District Bannu		District Lakki Marwat		District Kohat		Overall Prevalence		
	Infected/Total Examined	Prevalence (%)	Infected/Total Examined	Prevalence (%)	Infected/Total Examined	Prevalence (%)	Infected/Total Examined	Prevalence (%)	P-value
Adults	3/60	5	3/60	5	2/60	3.33	8/180	4.44^{b}	0.000
Childrens	11/60	18.33	9/60	15	10/60	16.66	30/180	16.66^{a}	

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.25: Age wise percent prevalence of Cryptosporidial infection in children and adults in three selected districts of Southern KPK.

Factors (age)	District Bannu		District Lakki Marwat		District Kohat		Overall		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
≤0 -5 years	5/23	21.73	5/21	23.80	5/26	19.23	15/70	21.42^{a}	0.017
6-10 years	3/17	17.64	3/18	16.66	3/17	17.64	9/52	17.30^{a}	
≥11- 15 years	3/20	15	1/21	4.76	2/17	11.76	6/58	10.34^{b}	
Total	11/60		9/60		10/60		30/180		

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.26: Percent prevalence of cryptosporidiosis in relation to animals contact or not in childrens in three different districts of Southern KPK

Factors (Have animals contact Yes or No)	**District Bannu**		**District Lakki Marwat**		**District Kohat**		**Overall**		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Yes	7/34	20.58	6/29	20.68	6/32	18.75	19/95	20[a]	0.020
No	4/26	15.38	3/31	9.67	4/28	14.28	11/85	12.94[b]	
Total	11/60		9/60		10/60		30/180		

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.27: Percent prevalence of cryptosporidiosis in relation to nature of diarrhea in children in three selected districts of Southern KPK.

Factors (Diarrhea)	**District Bannu**		**District Lakki Marwat**		**District Kohat**		**Overall**		
	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	Infected/ Total Examined	Prevalence (%)	P-value
Acute	2/24	8.33	3/35	8.57	2/32	6.25	7/91	7.69[b]	0.000
Persistent	9/36	25	6/25	24	8/28	28.57	23/89	25.84[a]	
Total	11/60		9/60		10/60		30/180		

[a, b] mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

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4.10 : Chemotherapeutic trials

To carry out the therapeutic trials, a total of 50 goats of same weight and age naturally infected with cryptosporidiosis were selected randomly for the present study. All the goats were placed under same environmental conditions such as feeding, handling, management and behavior. All the goats were randomly divided in to five groups (A, B, C, D and E). Animals in groups A, B, C and D were treated with Azithromycin, Metronidazole, Herbal drug i.e. *Allium sativum* (garlic), and Paromomycin respectively while Group-E was placed as a positive control group. All the four groups such as s A, B, C, and D were treated with different drugs doses such as Azithromycin at dose rate of 10 mg per Kg body weight, Metronidazole at dose rate of 50 mg per kg body weight, extract of garlic at the dose rate of 50 mg / kg body weight and Paromomycin at the dose rate of 100mg/kg body weight. The OPG was counted for all groups at day 0, 7, 14, 21 and 28.The therapeutic protocol has been presented in a table (4.28).

Table. 4.28: Therapeutic strategy along with groups of animals, drugs, doses and time for reduction of OPG.

Groups (Goats)	**Therapeutic Trials with doses, administration and OPG counting**			
	Drug	**Dose**	**Administration(PO)**	**OPG counting Period**
Group A	Azithromycin	10mg/ kg Body Weight	5 consecutive days	At day 0,7, 14, 21, 28
Group B	Metronidazole	50 mg/ Kg body weight	5 consecutive days	At day 0,7, 14, 21, 28
Group C	Allium sativum(garlic)	50mg/ kg body weight	5 consecutive days	At day 0,7, 14, 21, 28
Group D	Paromomycin	100mg/ kg body weight	5 consecutive days	At day 0,7, 14, 21, 28
Group E (control Positive group)	NA	NA	NA	NA

The efficacy of different selected drugs was evaluated on the basis of reduction in OPG (oocyst per gram) after treatment in relation to time. The means of reduction in oocysts of treated groups and control groups were compared with each other.

4.10.1 : Azithromycin

Azithromycin was used at the dose rate of 10 mg/kg body weight. Results for percent efficacy of Azithromycin were evaluated on the basis of reduction in oocyst per gram of feces against *Cryptosporidium* infection in naturally infected goats. The different selected drugs with doses in relation to time have shown in table (4.28) while percent efficacy of different drugs has shown in table (4.32). In positive control group (Untreated group) showed an increase trend in OPG count. The percent efficacy of Azithromycin was 13.90%, 29.07%, 44.87% and 59.29% on 7^{th}, 14th, 21th and 28^{th} day post treatment. Statistically, significance difference (P<0.000)was recorded in reduction of OPG by Paromomycin, followed by Metronidazole, Allium sativum while the poor efficacy showed by Azithromycin as presented by table(4.29).Statistically , overall significant difference (P<0.000)was recorded in reduction of OPG at different days, presented by table(4.30).

4.10.2 : Metronidazole

Metronidazole was used at the dose rate of 50 mg/kg body weight under same experimental conditions, in goats, naturally infected with *Cryptosporidium* infection. The percent efficacy of Metronidazole was 25.74%, 46.70%, 68.14%, and 78.20% in reduction of oocysts per gram at days 7^{th}, 14th, 21th and 28th, respectively (table 4.30). Metronidazole showed better results in reduction of OPG than Azithromycin and Allium sativum but poor than Paromomycin. Statistically significant difference was observed with Azithromycin while nonsignificant difference (P>0.05) was recorded with Allium sativum as presented by table (4.29). Similarly, statistically significant difference (P<0.000) was observed in reduction of OPG at day 0, 7, 14, 21 and 28 (table.4.31).

4.10.3 : *Allium Sativum* (garlic)

During therapeutic trials, *Allium sativum* was used at dose rate of 50mg/ kg body weight. A single dose of Allium sativum caused a significant decrease in OPG at 7th day post treatment and onward. At the day 7th post treatment, the percent reduction in OPG was 27.06% recorded. Similarly percent efficacy of Allium sativum was 49.70%, 62.24%, and 77.00 %, at days 14, 21 and 28 respectively (Table: 4.32). On the basis of statistical analysis, significant (P<0.000) decrease was recorded at 7th, 14th, 21th and 28th day post treatment. Allium sativum showed better results than Azithromycin but poor results than Paromomycin and statistically significant difference (P<0.00) was observed. Allium sativum and Metronidazole showed similar results and statistically non-significant difference (P>0.05) was recorded as presented in (Table=4.29). On the basis of statistical analysis, significant difference (P<0.000) was recorded in reduction of oocysts at different days post treatment as shown in table (4.31). Effect of drugs on reduction of OPG and percent efficacy has been presented by table (4.29) and (4.32) respectively.

4.10.4 : Paromomycin

In the present study, Paromomycin was used as a single dose of 100mg/kg body weight (b.wt) was used against cryptosporidiosis in goats and showed significant (P<0.000) decrease in oocyst per gram (OPG) count at 7th day post treatment and onward treatment. Percent efficacy of Paromomycin counted at day 7, 14, 21 and 28th and observed 51.77%, 67.08%, 87.54% and 91.77% reduction in OPG respectively. As a result, the excellent outcome was observed with Paromomycin. On the basis of statistical analysis, significant difference (P<0.000) was observed in relation to reduction in OPG with other drugs. The results for percent efficacy have been shown in table (4.32).There was significance difference (0.000) at different days in reduction of oocysts as shown in table (4.31). On comparison, the percent efficacy of different allopathic and herbal drugs, the highest reduction in oocyst per gram (OPG) was recorded by the use of paromomycin at the dose rate of 100mg per kg body wt. of goats.

Table 4.29: Effect of different drugs on reduction of *Cryptosporidium* oocyst/gram in goats.

Independent variables		**OPG**
Drugs	Azithromycin	556.4[b]
	Metronidazole	467.6[c]
	Allium sativum	468.7[c]
	Paromomycin	391.6[d]
	Positive control	1207.3[a]
P- Value		0.000

[a, b, c, d] mean values carrying same superscript within the row are differ non-significantly ($P>0.05$) whereas with different superscripts are differ significantly at ($P< 0.05$).

Table 4.30: Mean values of oocysts/gram at different days as affected by drugs.

Independent variables		**OPG**
Days	Day-0	841.60[a]
	Day-7	729.40[b]
	Day-14	585.13[c]
	Day-21	481.53[d]
	Day-28	453.43[e]
P- Value		0.000

[a, b, c, d] mean values carrying same superscript within the row are differ non-significantly ($P>0.05$) whereas with different superscripts are differ significantly at ($P< 0.05$).

Table 4.31: Means of oocysts/gram as affected by different drugs at different days in goats.

Independent variables (Drug*Days)		**OPG**
Positive Control	Day-0	833.3^{f}
	Day-7	1095.3^{d}
	Day-14	1221.4^{c}
	Day-21	1379.3^{b}
	Day-28	1507.0^{a}
Azithromycin	Day-0	790.7^{g}
	Day-7	681^{h}
	Day-14	555.7^{j}
	Day-21	433.3^{k}
	Day-28	321.3^{l}
Metronidazole	Day-0	837.3^{f}
	Day-7	619.7^{i}
	Day-14	445^{k}
	Day-21	255.7^{mn}
	Day-28	180.3^{o}
Allium sativum	Day-0	862.7^{e}
	Day-7	626.3^{i}
	Day-14	428^{k}
	Day-21	234^{n}
	Day-28	192.30^{x}
Paromomycin	Day-0	884.3^{f}
	Day-7	624.7^{i}
	Day-14	276^{m}
	Day-21	105.3^{p}
	Day-28	68.0^{q}
P- Value		0.000

^{a,b,c,d,e,q,m,I,x,f,mn,g,k} mean values carrying same superscript within the column are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Table 4.32: Comparative percent efficacy of different allopathic and herbal drugs used at different doses against cryptosporidiosis in goats

Groups	Treatment	Dose rate: mg/ kg b. Wt.	Efficacy (%)				
			0 day	07 day	14 day	21 day	28 day
A	Azithromycin	10 mg/ kg b.wt	0	13.90	29.07	44.87	59.29
B	Metronidazole	50 mg/kg b.wt.	0	25.74	46.70	68.14	78.20
C	Allium sativum	50 mg/kg.b.wt.	0	27.06	49.70	72.24	77.00
D	Paromomycin	100 mg/kg.b.wt.	0	51.77	67.08	87.54	91.77

4.10.5 : Side effects of the selected drugs

During therapeutic trails, different side effects were also observed such as abdominal pain, anorexia, sweating and diarrhea by the use of selected drugs. Abdominal pain was observed when the *Allium sativum* was used in group C but the abdominal pain was mild type and rapid recovery was observed after short period of time. Similarly sweating was observed when paromomycin was used in group D. When Metronidazole and Azithromycin was used in group A & B, diarrhea was observed in some goats within 2-3 days. No other severe type of side effect was observed during treatment trails. Increase in body weight gain was recorded in groups A, B, C and D whereas significant difference was observed when the weight gain values were statistically compared with the group E (infected non treated group- positive control).Weighted all the animals under trials at day 0(Pre-medication) and at day 28(Post-medication).

4.11 : Haematobiochemical Profile

Blood samples were collected from 50 goats infected with *Cryptosporidium* infection and 50 noninfected goats (Healthy) from the same herd and area and were analyzed by automatic hematology analyzer. On statistical analysis, Significant difference ($P<0.002$) was observed in the values of lymphocytes in infected goats. The lymphocytes count was 47.18% in non-infected group while 54.45% count was recorded in infected group. Similarly, eosinophils count was 6.10% in infected group whereas 2.49% counts was recorded in non-infected group (Healthy). On the basis of statistical analysis, significant difference ($P<0.000$) was observed in count of eosinophil values in both healthy and non healthy groups. Hb level was lower (10.53gm %) in healthy group while it was higher (10.84gm %) in diseased group and statistically both values differed non-significantly ($P>0.611$). Monocytes count was 2.41%in healthy animals while it was 2.47% in infected group and statistically non-significant difference ($P>0.08$) was recorded. Basophils count was 1.59% in healthy group while it was 1.74% in infected group while statistically non-significant difference ($P>0.354$) was recorded. Results for hematology have been presented in table (4.33). PCV level was 42.94% in infected animals while it was 34.62% in normal healthy animals. On statistical analysis, significant difference ($P<0.000$) was observed in PCV (%) level in healthy and infected animals.

Table.4.33: Mean comparative hematological values of two groups (A & B) of goats, non-infected (A) and infected (B) with cryptosporidiosis

Goats (n=50)	Groups	Hb (gm/ 100ml)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Neutrophils (%)	PCV (%)	TEC (number×10^{12} /L)
n=50	A= Healthy	10.53^{a}	47.18^{b}	2.41^{a}	2.49^{b}	1.59^{a}	40.27^{a}	34.62^{b}	13.67^{a} million/cmm
n=50	B= Unhealthy	10.84^{a}	54.45^{a}	2.47^{a}	6.10^{a}	1.74^{a}	39.81^{a}	42.94^{a}	13.21^{a} millions/cmm
P- Value		0.611	0.002	0.81	0.000	0.354	0.800	0.000	0.200

[a, b,] mean values carrying same superscript within the column row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05).

Separation of Serum

Blood samples were collected from infected and noninfected goats in dry, clean and labeled test tubes and left the tubes at room temperature to clot and then allowed to centrifuged at 1500 rpm for 20 minutes. The sera were separated and kept in a sterile labeled test tube at -20C^{0} until further used.

Biochemical Examinations:

Total serum protein was determined with the help of Biuret Method while albumin was detected by using special reagent kits (Gornal et al.1949). The concentration of serum globulin was determined when subtracted the albumin value from the total proteins. Copper level was determined by using calorimetric methods with the help of reagent kits (Dreux, 1977). Serum sodium, Chloride and Potassium levels were determined with the help of atomic absorption spectrophotometer.

Table 4.34: Different biochemical parameters of healthy and infected goats compared for estimation of biochemical analysis.

Serum parameters	Healthy	Infected	P- Value
Total protein (gm %)	8.30[a]	6.22[b]	.003
Albumin (gm %)	8.2660[a]	6.1720[b]	0.0001
ALT(u/l)	34.798[a]	35.036[a]	0.814
AST(u/l)	42.366[a]	41.890[a]	0.689
ALP(u/l)	88.712[a]	73.220[b]	0.000
Ca (mg %)	8.356[a]	8.10[a]	0.24
Mg (mg %)	1.44[a]	1`.28[a]	0.192
Sodium(Meq/l)	133.35[a]	108.12[b]	0.000
Potassium(Meq/l)	3.41[b]	4.61[a]	0.0002
Chloride (mg %)	97.92[a]	93.79[a]	0.092
Zinc (mg %)	74.136[a]	52.400[b]	0.0001
Copper (mg %)	80.212[b]	91.640[a]	0.002
Urea (mg %)	23.5[b]	34.89[a]	0.000
Creatinine (mg %)	0.398[b]	1.2100[a]	0.000

CHAPTER 5
DISCUSSION

The *Cryptosporidium* is an important zoonotic enteric protozoan parasite belonging to the phylum *Apicomplexa* and family *Cryptosporidiidae* (Romero et al. 2001). The *Cryptosporidium* is a vital pathogen affecting both human as well as cattle (Bouzid et al. 2013). Infection with this protozoan may lead to great economic losses due to high morbidity and mortality (Casemore et al.1997; Lendner et al. 2011).The *Cryptosporidium* parvum is mainly responsible for causing a disease in sheep and goats, known as cryptosporidiosis and have the zoonotic importance due to its public health significance (Robertson et al. 2010; Wang et al. 2014). cryptosporidiosis is caused by a protozoan known as *Cryptosporidium* and clinically characterized by watery diarrhea, severe dehydration, emaciation, anorexia and weight loss (Panciera et al. 1971; Fayer et al. 2000; Santin et al. 2004). The *Cryptosporidium* infection mainly affects epithelial cells of small intestine and some other tissues of liver and lungs. The *Cryptosporidium* rarely affects stomach, liver, gall bladder, trachea and lungs of mammals including human beings (Pillar and Izqierdo et al. 1993, Hunter and Thompson, 2005). *Cryptosporidium* can cause infection in more than 170 species of vertebrates distributed globally (Romero et al. 2001). *Cryptosporidium* mainly transmitted through fecal-oral route (Fayer et al. 2000). In small ruminants, the disease is clinically characterized by severe diarrhea, anorexia and poor weight gain (Forety, 1990). It is more harsh and severe in young animals as compared to adult (Chalmeras et al. 2002). The infected animals can shed a huge number of the *Cryptosporidium* oocysts up to (10^8–10^9 /g) which is the main source of infection in humans and animals (Lee et al. 2001). In addition, cryptosporidiosis causes diarrhea in humans in acute form which is fatal in chronic form in immunocompromised patients(Wilhelm et al. 2014).Cryptosporidiosis has been also connected with a cancer in humans.(Lander et al. 2011; Benamrouz et al. 2012).Similarly it also causes diarrhea in lambs and kids which may lead to mortality in severe form (Tizpori et al. 1981;Kaminjolo et al. 1993;Thompson et al. 2005).However, little information are available about the *Cryptosporidium* infection in small ruminants and humans in southern areas of KPK, Pakistan. Therefore, it was needed to determine the *Cryptosporidium* infection in small ruminants and its zoonotic importance in three selected districts of southern KPK, Pakistan.

The present study was conducted under a series of four sub-experiments;

Study 1: In the first study the prevalence of cryptosporidiosis in small ruminants along with different risk factors such as different areas, season's wise prevalence, sex wise prevalence, month wise prevalence, age wise prevalence and effect of different environmental factors such as temperature, humidity and rainfall on prevalence of the cryptosporidiosis in small ruminants was studied.

Study 2: In the second study, the *Cryptosporidium* infection was detected from fecal samples of sheep by using two different diagnostic techniques i.e. Molecular (PCR) and Simple microscopic coprological techniques. Comparative sensitivity efficacy of both diagnostic techniques was also studied and compared their sensitivity in detection of the *Cryptosporidium* oocysts.

Study 3: In the third study, the zoonotic aspect of the *Cryptosporidium* infection was studied. Prevalence and risk factors were also studied in humans. Stool samples were collected from adults and children then examined under microscope after proper staining with MZN technique.

Study 4: Hematological analysis, was also conducted to study and compare different blood parameters (Hemoglobin estimation, total erythrocyte count, packed cell volume, total leukocyte count) of healthy and diseased goats. Similarly, goat serum analysis in healthy and diseased animals suffering from cryptosporidiosis was also conducted to find out the difference in ALT, AST and ALP etc.

Study 5: Efficacy (%) of different selected local herbal and allopathic drugs were evaluated on the basis of reduction in oocyst per gram (OPG) under field conditions through different therapeutic trials in goats.

5.1 : Prevalence of cryptosporidiosis in small ruminants

The prevalence of the *Cryptosporidium* infection was determined on the basis of simple microscopic examination of fecal samples, stained by using Modified Ziehl-Neelsen (MZN) acid fast staining technique. In Caprines, Ovines and bovines, diagnosis of the *Cryptosporidium* infection is mainly based on simple microscopic identification of the *Cryptosporidium* oocysts (Johnson et al. 1995; Potter and Esbroeck, 2010).Similarly, Park et al. 2006, detected *Cryptosporidium* oocysts with the help of simple microscopy. Modified Ziehl-Neelsen staining technique was also used by Maurya et al. (2013) for detection of the *Cryptosporidium* oocysts in fecal samples of calves and lambs in India. In the present study, the highest percent prevalence of the *Cryptosporidium* infection was recorded in lambs (27.22 %) followed by kids (20.56%), Sheep (18.33%) while the lowest prevalence was recorded in goats (12.22%) by using simple conventional identification method.

5.2 : Prevalence of cryptosporidiosis in Sheep

In the present study, overall 18.33% prevalence of the *Cryptosporidium* infection was recorded in sheep in three selected areas of the southern KPK, Pakistan. The highest percent prevalence of the cryptosporidiosis was recorded in District Kohat (21.66%), followed by District Bannu (18.33%) while the lowest percent prevalence was recorded in Lakki Marwat (15%).

Danladi et al. 2015, recorded 11.7% prevalence of the *Cryptosporidium* infection in sheep which is very similar to our results for District Lakki Marwat. However, in adult sheep, 4-55%

prevalence of the *Cryptosporidium* infection was documented by Himonas et al. 2013 and Panousis et al. 2008.

Similarly, Fasihi-Harandi and Fotohi-Ardakani; 2008 also documented, 4-85% prevalence of the *Cryptosporidium* infection in adult sheep. All the previous studies conducted about the percent prevalence of the *Cryptosporidium* infection in sheep, was based on diagnosis through simple microscopic examination after proper staining. According to the previous study, conducted in Ethiopia, 0% prevalence of the *Cryptosporidium* infection was reported in sheep by Ulutas and Voyvoda, 2004. Similarly, in Australia, 2.6% prevalence of the *Cryptosporidium* infection was recorded in sheep by Green et al. 2004. These two results are lower than present study observed in southern KPK, Pakistan. The percent prevalence of the *Cryptosporidium* infection was about 3.7%-47% in Brazil reported by Ozdal et al. 2009 and13.6%-46.5% in Turkey reported by Silva-Fiuza et al. 2011 which are higher than the results observed in the present study in three selected areas of southern KPK. Fresan et al. 2004, conducted a study in sheep about the prevalence of cryptosporidiosis and recorded 25.7% prevalence of the *Cryptosporidium* infection in Mexico while 29% prevalence was documented in Greece by Zorana et al. 2006. Theses both results are very close to the findings observed in the present study for District Kohat. In Serbia, 42.1% prevalence was reported by Seri et al. 2009 where higher percent prevalence was recorded than the present study conducted in our southern area.

The prevalence of the *Cryptosporidium* infection was 67.5% in sheep reported in the Veracruz (Perote) in Mexico while 34.3 % prevalence was recorded in northern Mexico (Alonso-Fresán et al. 2005). Sheep and goats serve as a major source of reservoirs for the *Cryptosporidium* infection in humans (Koinari et al. 2014).In Spain, 31-59% prevalence was recorded in sheep by Causape et al. 2002 and Castro-Hermida et al. 2007 which are higher than the present findings. In Poland, 10.1% prevalence of the *Cryptosporidium* infection was determined in sheep by Majewsla et al. 2008. Mahfouz et al. 2014, documented the lowest percent prevalence (2.5%) of the *Cryptosporidium* infection in sheep in Egypt that were naturally infected. In Iran, Gharekhani et al. 2014; conducted a study and found 11.3% prevalence whereas similar close percent prevalence was also recorded in District Lakki Marwat. In Iraq, 13.3% prevalence of the *Cryptosporidium* infection was recorded in sheep by Mahdi and Ali, 2002 and similar results we observed and reported in the present study in District Lakki Marwat. In Tunisia, 11.2% prevalence of the cryptosporidiosis was recorded in sheep by Soitan et al. 2007 while similar results were recorded in the present study where, 15% prevalence of the *Cryptosporidium* infection was recorded in sheep in District Lakki Marwat. The lowest percent prevalence of the *Cryptosporidium* infection was1.8%, recorded in India as reported by Mauya et al. 2013 and 2.2% prevalence was recorded in Guinea by Koinari et al. 2014. These both observations are not similar to the findings reported in the present study. The variation in prevalence of the *Cryptosporidium* infection in sheep in different areas may be due to different managements, climatic conditions, feeding, study design, heavy rainfall, humidity and poor hygienic conditions. The variation in the frequency of the *Cryptosporidium* prevalence in sheep raised in various geographical locations can be the effect of differences in infectivity of the environment. It is also possible that the poor quality of hygienic conditions during grazing practices, might influence the animal's susceptibility to the *Cryptosporidium* infection (Ramirez et al. 2004)

5.2.1 : Month wise percent prevalence of cryptosporidiosis in Sheep

. The data was analyzed on monthly basis to find out which month of the year has the highest percent prevalence of the *Cryptosporidium* infection in sheep. The highest overall percent prevalence was recorded in the month of August (36.66%) followed by April (26.66%), May, July and September (23.33%), June (20%), October (16.66%), February, March and November (10%) while the lowest percent prevalence was recorded in the month of December and January (6.66%). It appears from the results that the highest prevalence was recorded in warm and wet months of the year while the lowest prevalence was recorded in cooler months of the year while similar findings were documented by other authors reporting significant relationship between incidence of the *Cryptosporidium* infection and seasonal variations. The changing pattern of temperature, humidity and heavy rain in different months of the year affects the percent prevalence of the *Cryptosporidium* infection in sheep (Green et al. 2004, Jafari et al. 2013). Cryptosporidiosis mainly occurs in rainy and warm season of the year (Perch et al. 2001) and reaches its highest point in spring and summer season (Lake et al. 2008). The highest percent prevalence was recorded in the summer season due to high intake of water, increased outdoor activities (swimming activities increased during the summer season in recreational water in form of community swimming and enhances chances for fecal-oral transmission (Sunderland et al. 2007).There are few risk factors such as poor quality of pool design, water quality, don't use of disinfectant and lack of filtration facility are responsible for cryptosporidiosis (Fayer et al. 2000). In some countries the extensive outbreak of cryptosporidiosis may be due to shortage of water in some dry season of the year.

As a result of water shortages, utilize other sources of water where hygienic conditions are poor such as wells that may have maximum concentrations of the C. oocysts (Gatei et al. 2006). Additionally, daily use of recreational waters by infected children, adults, wild bird and animals may enhance the risk for water-born transmission in the summer season (Fayer et al. 2000).

5.2.2 : Season wise percent prevalence of cryptosporidiosis in Sheep

The *Cryptosporidium* infection is worldwide in distribution and commonly found in all wet and warm seasons of the year as reported by (Green et al. 2004; Jafari et al. 2013). The highest percent prevalence of cryptosporidiosis in sheep was recorded in the summer (27.50%) and autumn season (20%), followed by spring season (18.33%) while the lowest percent prevalence was recorded in the winter season (8.33%). Our results are consistent and agree with the results of other researchers and investigators reported a strong correlation between the warm and wet seasons with the infection rate. Gharekhani et al. 2014, documented (17.3%) the highest percent prevalence in the summer season in Mazandaran province of Iran where rainfall was maximum (Green et al. 2004). Some other researchers also reported similar results of the Cryptosporidial prevalence in rainy and warm season of the year (Perch et al. 2001) that reach to the highest point in spring and summer season (Lake et al. 2008). The highest percent prevalence was recorded in the summer season due to high intake of water and increased outdoor activities such as swimming which occurs mainly in summer activities increased during the summer season in recreational water in the form of community swimming and enhancing the chances for fecal-oral transmission (Sunderland et al. 2007).

Different management conditions and hygienic conditions, climates, study design and different geographical areas might be the possible reason of variations in results (Ulutas and Voyvoda et al. 2004; Ramirez et al. 2004).

5.2.3 : Age wise percent prevalence of cryptosporidiosis in sheep

. In the current study, overall the highest percent prevalence was recorded at the age of ≤ 1 year (23.3 %) followed by at the age of 1-2 years (18.85%) while the lowest percent prevalence was recorded at the age of ≥2-3 years and above (11.53%). Similar results were found by Gharikhani et al. 2014, where the highest percent prevalence was recorded at the age of 1 year (16.7%) while the lowest percent prevalence was recorded at the age of more than 1 year (8.8%). The results are in consistent with the results of other researchers indicating a strong correlation between age and infection rate (Dezfouli et al. 2002; Ulutas et al. 2004; Mokhber- Heidari and Gharakhani, 2006; Ozdal et al. 2009).The variation in different age factors may be due to immature immune system in neonatal animals and highly sensitivity to the *Cryptosporidium* infection (Fasihi-Harand et al. 2008). Age is the key risk factor responsible for spreading and distribution of the Cryptosporidiosis in different species (Sari et al. 2009). The highest morbidity has been recorded in neonatal animals at an early age of life (Panousis et al. 2008).

5.2.4 : Sex wise percent prevalence of cryptosporidiosis in Sheep

Overall percent prevalence of the *Cryptosporidium* infection was high in female (18.80%) than male (17.02%) reared under similar management conditions in three selected areas. In District Bannu, the sex wise percent prevalence ratio of male and female was 18.75% and 19.04% respectively. In District Lakki Marwat, the sex wise percent prevalence ratio of male and female was 14.28% and 15.21% respectively whereas in District Kohat, the sex wise percent prevalence ratio of male and female was 20% and 22.22% respectively. Our findings are opposite to other investigator (Gharikhani et al. 2014) where 14.3% prevalence was recorded in male while10.3% prevalence was found in female. Our findings are similar to other investigators where highest percent prevalence was reported in female as reported by (Dezfouli et al. 2002;

Heidari and Gharakhani, 2006; Ozdal et al. 2009; Vahedi et al. 2009; Jafri et al. 2013). Danladi et al. 2015, also reported 9.2% and 14.5% prevalence ratio in male and female respectively. A study was designed by Pam et al. 2013, to find out percent prevalence in sheep on sex basis and recorded 13.3% prevalence in male and 18.7% in female which supports the present study. Our findings are also in agreement with observations of Ayinmole and Fagbemi, (2010) who reported the highest percent prevalence in female (38.10%) than male (17.75%).

The higher percent prevalence in females in sheep might be due to hormonal disturbance during pregnancy, weakness, poor immune system and lactation stress (Danladi et al. 2015).

5.3: Percent prevalence of cryptosporidiosis in Goats

The Cryptosporidium oocysts were detected and identified on the basis of morphological characters of oocysts through simple microscopic examination of stained thin smear. Overall percent prevalence rate was 12.22% in goat, in three different selected areas of study. In the present study, the highest percent prevalence was recorded in District Kohat (18.33%) followed by District Lakki Marwat (11.66%) while the lowest percent prevalence was recorded in District Bannu (6.66%). Our results are close to the findings reported by Danladi et al. 2015; who documented 17.7% prevalence of the *Cryptosporidium* infection in goat while in present study recorded 18.33% prevalence in District Kohat. A study was conducted in Sokoto and Plateau where 3.3% and 24% prevalence of the *Cryptosporidium* infection was reported by Faleke et al. 2006 and Pam et al. 2013, respectively while in the current study reported 6.66%, prevalence in Bannu area. In Spain, a study was conducted by Castro-Hermida et al. 2007; where 30% prevalence of the *Cryptosporidium* infection was recorded in goats while observed lower percent prevalence in the present study. In Poland, 0% prevalence of the *Cryptosporidium* infection was documented in goats by Majewska et. al. 2000 while these results are lower than the findings observed in the present study. In Iraq, a study was conducted by Mahdi and Ali et al. 2002; where 17.7% prevalence of the *Cryptosporidium* infection was reported in goats while similar results were also observed in the present study in District Kohat where percent prevalence was 18.33% while overall prevalence was 12.22% in three selected districts. In Papua New Guinea, a study was designed to find out the percent prevalence of the *Cryptosporidium* infection in goats where 4.4% prevalence was reported by Koinari et al. 2014 whereas in current study observed 6.6% prevalence in District Bannu.

However, care should be taken to compare the prevalence of the cryptosporidiosis in goat in different countries of the world because different risk factors affect the percent prevalence of the cryptosporidial infection in small ruminants such as raising conditions, characteristics of animals, immune status of the animals, study design, temperature, humidity, rainfall, hygienic conditions of the area and managemental conditions are different from each other (Fayer et al. 2000).

5.3.1 : Month wise percent prevalence of cryptosporidiosis in goats

The highest percent prevalence was observed in the month of August (30%), followed by July (23.33%), June (20%), May (16.66%), March and September (13.33%), April and November (10%), January, February and October (3.33%) while the lowest percent prevalence was recorded in the month of December (0%).Lower percent prevalence of the *Cryptosporidium* infection in goats may be attributed to certain essential factors such as the environmental and ecological factors of the study area, where relative low humidity and short period of rainfall was reported. This is supported by recommendations and suggestions documented by Yu and Seo (2004) and Fayer et al. 2000, that high rainfall and relative high humidity are responsible for high prevalence (%) of cryptosporidiosis because oocyst can survive for long period in such environmental conditions. The highest percent prevalence of the *Cryptosporidium* infection was observed in those months of the year where the highest ambient temperature and relative humidity was recorded.

5.3.2 : Season wise percent prevalence of cryptosporidiosis in goats

Considerable seasonal variations are reported for prevalence of cryptosporidiosis in goats (Green et al. 2004, Jafari et al. 2013). Our results are in consistent with the results of other researchers who reported positive strong correlation between the warm and wet seasons of the year with high prevalence of infection. Our results agreed with the results of Jafari et al. 2013 where overall the highest percent prevalence was recorded in warm and hot seasons. In the present study, the overall highest percent prevalence of cryptosporidiosis was recorded in the summer season (20.83%), followed by spring (13.33%), autumn (11.66 %) while the lowest percent prevalence was recorded in the month of winter season (3.33%). Similar findings were reported by Masood M et al. 2013 in cow calves where the highest percent prevalence of the *Cryptosporidium* infection was recorded in the summer season followed by autumn, spring while the lowest in winter season. Some other researchers also documented similar results for the highest percent prevalence in hot and humid season (Perch et al. 2001) and similar findings were reported in the spring and summer season by certain other researchers (Lake et al. 2008).

Environmental conditions such as high relative humidity, high ambient temperature, heavy rainfall and moisture are necessary for the survival of the oocysts of the parasite. These oocysts are the constant source of infection for both animals and humans. As it is water born disease and use of water for drinking and water bath is at maximum in hot and summer season. Therefore it might be possible that the high prevalence percentage was due to excessive contact with contaminated water. In summer season, there might be a shortage of water in some areas of the world resulting in the use of utilize the stored water (Fayer et al. 2000; Sunderland et al. 2007).

5.3.3 : Age wise percent prevalence of cryptosporidiosis in Goats

In the current study, recorded the highest percent prevalence at the age up to ≤1 year (18.58%) followed by 1-2 years of age (10.20%) while the lowest at the age of ≥2-3 years (5.95%) in adults. Similar study was also conducted on the basis of age factor to find out the percent prevalence of cryptosporidiosis in goats by Akinkuotu et al. 2015, who concluded the highest percent prevalence in pre-weaned kids (62.7%), followed by Post weaned kids (30.4%) while the lowest in adults (22.5%).Our results are agreed with the fact that highest percent prevalence of the *Cryptosporidium* infection occur at an early age of life while the lowest percent prevalence occur at higher ages. Pam et al. 2013, reported prevalence of 9% was reported in goats at the age of 0-10 months while in the present study, higher prevalence (18.58%) was recorded.

The difference may be due to different hygienic conditions, environmental factors such as heavy rain fall, high relative humidity, high temperature, study design, immune status of animal, feeding and watering management conditions of the area (Ozdal et al. 2009).

5.3.4 : Sex wise percent prevalence of cryptosporidiosis in goats

Overall percent prevalence of the *Cryptosporidium* infection was recorded in both sexes of goats and little higher prevalence was recorded in male (12.30 %) than female (12.17%) in three zones of the Southern KPK. A similar study was also conducted by Akinkuotu et al. 2015, where significant difference ($P<0.05$) was recorded in prevalence (%) of the *Cryptosporidium* infection in both sexes who recorded the highest percent prevalence of the *Cryptosporidium* infection in female (47.5%) while lowest in male (29%). Our findings are not agreed with the findings reported by Akinkuotu et al. 2015. Similar study was also conducted by Pan et al. 2013, who recorded the highest percent prevalence in female (25.3%) and lowest in male (22.7%). Our findings are not agreed with these findings because we found little higher prevalence in male than female. The variations in results may be due to different environmental conditions and study design. The constancy of the higher percent prevalence rates in females than male in goats may be due to some hormonal disturbance during pregnancy, immune conditions of the body and lactation stress (Danladi et al. 2015).

5.4 : Prevalence of cryptosporidiosis in Lambs

The cryptosporidiosis mostly occurs in lambs at an early age of life than adult sheep and the severity of the infection in lambs is more often than adult sheep and the intensity of infection is high in young lambs than adult sheep (Majewska et al. 2000). There is significant difference ($P<0.05$) in prevalence of the *Cryptosporidium* infection in young and old (Noordeen et al. 2000).

Infected lambs, goat kids and other young animals are the main source of contamination. Clinically infected lambs and goat kids, along with other young animals, are a major source of environmental contamination.

In the present study, an overall percent prevalence of cryptosporidiosis was 27.22% reported in lambs, in three selected zones of study area. The highest percent prevalence of the *Cryptosporidium* infection was recorded in District Kohat (33.33%) followed by District Lakki (25%) while the lowest percent prevalence was recorded in District Bannu (25%). Similar study was also conducted in Spain to find out the prevalence of different enteric pathogens in lambs and goat kids. As a result, the highest percent prevalence was recorded in lambs (45%) than diarrhoeic kids (42%) (Munoz et al.1996). There are certain variations in percent prevalence in our study and other researchers that might be due to some factors such as study design, breed variation, immune status of the animal, area, hygienic conditions and other environmental conditions such as humidity, temperature and rain fall (Majewska et al. 2000).

5.4.1 : Month wise percent prevalence of Cryptosporidiosis in Lambs

The *Cryptosporidium* infection is worldwide in distribution and commonly found in all wet and warm months of the year where the highest ambient temperature, relative humidity and high rainfall was recorded (Green et al. 2004; Jafari et al. 2013). The data was analyzed on monthly basis to find out which month of the year has the highest percent prevalence of the *Cryptosporidium* infection in lambs. The overall highest percent prevalence was recorded in those months of the year where high temperature, relative humidity and heavy rainfall was recorded such as in August (46.6%), followed by other months of the year such as July (40%), April, May and June (30%), September and October (26.66%), November and January (20%) while the lowest prevalence was recorded in the month of February and December (16.66%) where lower temperature, humidity and minimum rainfall was recorded.

5.4.2 : Season wise percent prevalence of cryptosporidiosis in Lambs

In the present study, the overall highest percent prevalence of cryptosporidiosis was recorded in the summer season (36.66%), followed by spring and autumn (26.66%) while the lowest in the winter season (18.33%). Our results are similar to the findings reported by Causape et al. 2002; where the highest prevalence was observed in the summer season as observed in the current study. Our results are not agreed to the prevalence reported in the winter season by Causape et al. 2002 where the highest prevalence (92.8%) was recorded. In the current study, 26.66% prevalence was recorded in the spring and autumn season while Causape et al. 2002 reported the highest percent prevalence in spring and autumn season (79.3% and 75%). The variation in results might be due to different hygienic conditions, environmental factors, study design and immune status of the animals (Ozdal et al. 2009).

5.4.3 : Age wise percent prevalence of cryptosporidiosis in Lambs

It is the fact that the age related resistance to Cryptosporidial infection is exists naturally in lambs and kids. During the first two weeks of age, infection is more severe and obvious decrease has been reported in the severity of the clinical signs as the age increases during infection (Ortega-Mora et al.1999, Causape et al. 2002; Giadinis et al. 2007). A study was conducted in Spain by Ortege-Mora et al. 1999; and it was estimate that 20,000-444000 oocysts/ day can be excreted by an asymptomatic ewe. It was observed that 1 oocyst/ lamb can cause the infection which is the minimum infective dose whereas the average numbers of oocysts were 5/lamb to cause infection. Therefore, it was recorded that 4,000-110,000 infective doses per day were shed by ewe without any clinical symptoms (Ortega-Mora et al. 1999).

Age is the main risk factor responsible for spreading of cryptosporidiosis in lambs and goat kids (Sari B et al. 2009); and age factor is also a risk of infection and high morbidity mainly occurs in neonatal animals such as lambs and kids (Panousis et al. 2008).

Our results are in agreement with the above statements mentioned by various researchers that age is the key factor in spreading of the *Cryptosporidium* infection so similarly the highest percent prevalence was recorded in an early age of lambs as in our study at the age of ≤1-15 days (38.09%) followed by lambs at the age of 16-30 days (29.41%) while the lowest percent prevalence was recorded at the age of ≥31-60 days and above (15.15%).

Our results are similar to the findings reported by Baris et al. 2008, where the highest prevalence was recorded at the age of first week(44.4%), followed by two weeks of the age(37.5%), 3 weeks (32.3%) of the age while the lowest prevalence was recorded at the age of 4 weeks(22%) in lambs.

Our results are not agree to the findings reported by Misic Zorana et al. 2006, who recorded the highest percent prevalence at the age of 30 days (45.3% in lambs) while in the present study recorded, lower percent prevalence at the age up to 4 weeks (29.41%). Misic Zorana et al. 2006, also recorded 42.1% prevalence of the *Cryptosporidium* infection in lambs while in the current study, recorded "27.22%" overall prevalence in three selected zones of southern KPK. The variations in results might be due to certain factors as reported by Fasihi-Harandi M and Fotohi-Ardakani et al. 2008; that at early age, high percent prevalence of the *Cryptosporidium* infection might be due to poorly developed immune system and their maximum sensitivity to the *Cryptosporidium* infection.

5.5: Percent prevalence of cryptosporidiosis in goat Kids

The *Cryptosporidium* infection in goat kids has been recorded in many countries by a different number of researchers. The prevalence and incidence rates vary commonly form area to area, and country to country on the basis of geographic distribution and sample size. As first time in Australia, a report about prevalence of cryptosporidiosis was recorded in goat kids while 5-35% prevalence of cryptosporidiosis has been also reported by several different investigators (Robertson, 2009). The zoonotic specie such *Cryptosporidium parvum* has been reported in different countries of the world including Zambia, Belgium, Australia and Cyprus in small ruminants (Geurden et al. 2008).

In the present study, "20.55%"overall percent prevalence of the *Cryptosporidium* infection was recorded in goat kids in three selected areas of study. The highest percent prevalence of the *Cryptosporidium* infection was recorded in District Kohat (23.33%), followed by District Bannu(20%) while the lowest percent prevalence was observed in District Lakki

Marwat(18.33%). Nektarios et al. 2015; reported 76.6%, prevalence of the *Cryptosporidium* infection in kids and theses findings were not agreeing with our results because our results are lower. A study was conducted by Amam Zonaed Siddiki et al. 2015 to find out prevalence (%) in goat kids where15% prevalence of the *Cryptosporidium infection was* observed. This diagnosis was just based on simple microscopic examination by using Modified Ziehl-Neelsen staining technique which is not actually 100% sure confirmatory test because several acid fast bacteria such as *Mycobacterium* species also interfere with the results of the test. In UK, a study was conducted by Sturdee et al. 2003; to determine percent prevalence of the *Cryptosporidium infection* in kids and as a result observed 23% prevalence of the *Cryptosporidium* infection in kids. In the present study, similar results were also recorded in the area of District Kohat (23.33%) and overall percent prevalence was 20.55% recorded in all three selected areas. In Romania; 24%, China 24.2% while in Trinidad and Tobago 20% prevalence of Cryptosporidiosis was recorded by Castro-Hermida (2002) and Causpe et al. 2002; ; Mir et al. 2013; Stzanidakis et al. 2014).We also observed similar results in Bannu (20%), Lakki Marwat(18.33%) and Kohat(23.33%) while in some parts of the world, the lowest percent prevalence was reported such as in Greece(7.1%), Belgium(9.5 %) and Iran(17%). These results are not agreed with our all findings (20.55%) because our results are comparatively higher than Greece and Belgium whereas close to Iran. A study was conducted in Spain who recorded 40-70% prevalence of the *Cryptosporidium* infection in kids while 20.55% prevalence was recorded in the current study. The variation in prevalence percentage may be due to different in hygienic conditions, study design, poor health status of the animal, poor immunity and different feeding; watering and managemental conditions are the factors responsible for variation (Ozdal et al. 2009).

5.5.1 : Month wise percent prevalence of cryptosporidiosis in kids

The data was analyzed on monthly basis to determine which month of the year has the highest percent prevalence of the *Cryptosporidium* infection in kids. The highest overall percent prevalence was recorded in the month of August (36.66%), followed by May (30.66%), June, July and October (26.66%), April (23%), September (20%), March (16.66%), November (13.3%), December (10%) while the lowest percent prevalence was recorded in the month of January and February (6.66%). The highest percent prevalence was recorded in the District Kohat (23.33%) followed by District Bannu (20%) while the lowest percent prevalence was recorded in the District Lakki Marwat (18.33%). The variation in different months might be due to heavy rains and warm environment favorable for oocysts while cooler months are less favorable for the survival of the oocyst (Perch et al . 2001).High intake of water in warm months from streams, ponds and rivers might be the factor responsible for the highest percent prevalence of *Cryptosporidium* infection in warm months of the year (Sunderland et al. 2007).

5.5.2 : Season wise percent prevalence of cryptosporidiosis in kids

The highest percent prevalence of the *Cryptosporidium* infection was recorded in the summer season (30.83%), followed by spring and autumn (2.66%), while the lowest percent prevalence was observed in the winter season (9.16%). The prevalence of cryptosporidiosis seems to show strong seasonality correlation and it was reported that the highest prevalence was recorded during the hot, warm and rainy season of the year as reported by Perch et al. 2001.Our results are agreed with this statement and we recorded the highest percent prevalence of the *Cryptosporidium* infection in those seasons of the year where heavy rainfall and ambient temperature was recorded. The Cryptosporidiosis is worldwide in distribution and commonly found in all wet and warm seasons of the year as described by Jafari et al. 2013 and Green et al. 2004. Our results are in consistent and agree with the results of other researchers and investigators who reported a strong correlation between the warm and wet seasons of the year and the rate of prevalence of the infection.

5.5.3 : Age wise percent prevalence of Cryptosporidiosis in goat kids

In current present study, the highest percent prevalence was recorded at an early age of kids. As a result, the highest percent prevalence was recorded at the age of ≤1-15 days (33.92%), followed by 16-30 days (17.69%) while the lowest percent prevalence was recorded at the age of ≥31-60 days (15%). Our results are lower than a study conducted by Nektarios et al. 2015, where percent prevalence of the *Cryptosporidium* infection was 76.4% in diarrheic kids. In French, Delafosse et al. 2006 also documented high prevalence of the *Cryptosporidium* infection in a goat kids suffering from diarrhea.

Similar study was designed by Amam et al. 2015 in Bangladesh who reported the percent prevalence of the *Cryptosporidium* infection in kids on the basis of age factor where the highest percent prevalence was recorded up to the age of 3 months of old kids that were highly susceptible than adults.

A study was conducted by Misic Zorana et al. 2006, in goat kids at Serbia who documented 31.81% prevalence of the *Cryptosporidium* infection and it was concluded that small ruminants act as a source of reservoirs for the transmission of the *Cryptosporidium* infection to humans and other susceptible animals. The difference in percent prevalence of the *Cryptosporidium* infection in young and adult might be due to poorly developed immune system and their highly sensitivity as described by Fasihi-Harandi and Fotohi-Ardakani et al. 2008.

5.5.4 : Sex wise percent prevalence of cryptosporidiosis in goat Kids

In the current study, the highest percent prevalence of the *Cryptosporidium* infection was reported in female kids (20.98%) than male's kids (19.19%). Similar study was conducted by Amam et al. 2015 where the highest percent prevalence of the *Cryptosporidium* infection was recorded in female goat kids than male. As a result, it was concluded that females are highly susceptible than male. This could be on the basis of variations in different hormones that can donate and enhance the susceptibility of the *Cryptosporidium* infection to different goat kids of either sex (Danladi et al. 2015).

5.6 : Prevalence of cryptosporidiosis in small ruminants and environmental risk factors

Month wise percent prevalence of the cryptosporidiosis in small ruminants was correlated to the relative high or low humidity, high or low ambient temperature and heavy rainfall. In the present study, it was concluded that high temperature, humidity and heavy rainfall play an important role in higher loads of endoparasites in small ruminants. In the month of August, $32C^0$, $31.94C^0$ and $32C^0$ temperature was recorded in District Bannu, Lakki Marwat and Kohat respectively while in the month of December, lowest temperature, humidity, and rainfall was recorded therefore lowest percent prevalence was recorded in three selected areas of the southern KPK. Our results are in agreement with the findings observed by Donovan et al. 1989 where the highest percent prevalence of the *Cryptosporidium* infection was recorded in summer season at different farm and animal level while the lowest prevalence was recorded in the winter season. The highest percent prevalence of the *Cryptosporidium* infection was reported in the rainy season in those patients showing diarrhea in Bangladesh while the highest prevalence was recorded in the autumn season in Saudi Arabia (Donovan et al. 1998).

Maurya et al. 2012; reported that seasons have a big effect on the prevalence of the *Cryptosporidium* infection in animals and humans. These observations and findings are very similar with the present study. Katsumata et al. 1998; documented different risk factors that are responsible for prevalence of the cryptosporidiosis such as overcrowding, heavy rainfall, flood and contact with cats or other small ruminants.

5.7 : Molecular study

In addition to coprological examination, the same samples were also processed by PCR to detect the *Cryptosporidium* DNA .

On application of PCR (molecular detection technique) little higher prevalence was detected than simple microscopic examination. PCR based detection of the *Cryptosporidium* infection was more effective and confirmatory technique than simple traditional technique concerning with certain specific stains such as modified Ziehl- Neelsen stain (MZN). Although conventional approach was comparatively less costly and no need of highly scientific instruments as well as other facilities. The sensitivity of the conventional approach was lower than molecular techniques that may result in false positive result. During simple microscopic examination, some acid fast bacteria also stained with MZN stain which gave false positive results. Therefore modern PCR techniques might replace the conventional approach with all technical facilities.

During the current study, simple PCR approach was additionally applied to find out the percent prevalence of *Cryptosporidium* infection as the technique was more reliable and sensitive than conventional approach and similar findings were also observed by Mathis et al. 1996; and McGlade et al. 2003. DNA band was revealed at 435 base pair (bp) when amplified targeted nucleic acid.

Molecular diagnostic technique (PCR) as sensitive diagnostic tool was first times used in 1991, for detection of *Cryptosporidium* oocysts in fecal and water samples. Different molecular diagnostic sensitive and specific methods such as Simple PCR, RT-PCR, PCR-RFLP and nested PCR methods were used for detection and identification *Cryptosporidium* species (Aslan et al.

2012, Dreelin et al. 2014). The *Cryptosporidium* oocysts could be identified in feces, bile and sputum. Several diagnostic techniques were used for the detection of the *Cryptosporidium* oocysts though the molecular diagnostic techniques were most sensitive and specific for the detection of the parasites. Experienced people could do the microscopic diagnosis successfully but inexperienced people can made mistakes to diagnose properly. PCR methods and ELISA were highly sensitivity and specificity for the rapid detection of the *Cryptosporidium* antigen. The *Cryptosporidium* has two target antigens and the molecular weight of two antigens is 15-17 kDa. There were two main target antigens for the *Cryptosporidium* with a molecular weight of 15-17 kDa. These two antigens were also known as Cp17 and 27 kDa antigens. These two antigens were also known as Cp 23 antigens.

By using molecular techniques, different species of the *Cryptosporidium* were reported in the fecal samples of lambs in various countries such as Tunisia (Soltane et al. 2007), United Kingdom (Mueller-Doblies et al. 2008), Spain (Quílez et al. 2008), USA (Fayer and Santín, 2009), Australia (Yang et al. 2009), Italy (Paoletti et al. 2009), Brazil (Feres et al. 2009) and China (Wang et al. 2010). The main *Cryptosporidium* species responsible for causing infection in small ruminants were *Cryptosporidium parvum*, *Cryptosporidium ubiquitum* and *Cryptosporidium xiaoi* (Fayer and Santin, 2009; Fayer et al. 2010).

DNA was extracted from 90 samples out of total 360 samples of sheep and was declared positive after amplification through PCR. At molecular level, 24.99%, an overall percent prevalence of the *Cryptosporidium* infection was determined in sheep in three selected areas of southern KPK. The highest molecular percent prevalence was recorded in District Kohat (31.66%), followed by District Bannu (25%) while the lowest molecular percent prevalence was recorded in Lakki Marwat (18.33%). The highest season wise percent molecular prevalence was recorded in the month of summer (33.33%), followed by autumn (30%), spring (26.66%) while the lowest prevalence was in winter season (13.33%).

Sex wise molecular percent prevalence of cryptosporidiosis was higher in female (27.08%) while lower in male (25.53%). On the basis of Statistical analysis, non-significant difference was recorded at sex level in prevalence of the *Cryptosporidium* infection in sheep.

The highest molecular percent prevalence was recorded at early age of 1 year(29.85%) followed by at the age of 1-2 years (26.22%) while the lowest percent prevalence was recorded at the age of 3 years or above(17.30%). The current study confirmed that young's were more frequently infected than adults with the *Cryptosporidium* infection and similar findings were also observed by Xiao et al;1993 while Olson et al;1997,reported that prevalence of the *Cryptosporidium* infection was higher in those animals older than six months. However already reported information about percent prevalence of the *Cryptosporidium* infections are ranged from 23-100% in young sheep in different parts of the world which were higher than that we observed in our findings (Tzipori et al., 1981; Xiao et al., 1993; Munoz et al., 1996; Olson et al., 1997).

The difference in percent prevalence of the *Cryptosporidium* infection in sheep raised in different geographical regions may be due to zoohygienic conditions, grazing practices, drinking water, level of contamination of the environment with *Cryptosporidium* oocysts and immune status of the animals.

5.7.1 : Comparative analysis of sensitivity and specificity of two diagnostic tools for rapid detection of the *Cryptosporidium* infection in small ruminants

The highest percent prevalence of the *Cryptosporidium* infection was determined by PCR (24.99%) while the lowest percent prevalence was determined by microscopic morphological identification (14.99%) in sheep in three selected areas of Southern KPK. However, on statistical analysis, non-significant difference (P>0.05) was recorded in molecular and coprological technique.

According to area wise percent prevalence, the highest percent prevalence was determined by PCR (25%) followed by simple microscopic technique (18.33%) in District Bannu. In District Lakki, the highest percent prevalence was determined by PCR (18.33%) followed by simple microscopic technique (15%). Similarly, in District Kohat, the highest percent prevalence was determined by PCR (31.66%) followed by simple microscopic examination (21.66%).

Our results are very close to the findings as reported by Zucatto et al. 2015, where 15% molecular prevalence was observed while in the current study, 18.33% molecular prevalence of cryptosporidiosis was reported in sheep in the area of Lakki Marwat. Similarly, a study was also conducted in Australia, where 24.5% molecular prevalence of the *Cryptosporidium* infection was detected in sheep while 25% molecular prevalence was recorded in Brazil by yang et al. 2009 and Silva (2007) respectively. On the other hand, similar results were also observed in the present study in District Bannu (25%). Our results are lower than the results reported by Santin et al. 2007 where the highest molecular percent prevalence (77.4%) was recorded. Our results are also not agreed to the findings reported by Fiuza et al. 2011 where the lowest molecular percent prevalence was recorded in sheep. In Norway, a study was designed by Robertson et al. 2010; and reported similar findings that were observed in the present study.

On the basis of statistical analysis, non-significant difference (P<0.187) was recorded in comparative sensitivity of two diagnostic techniques (microscopic examination and PCR) for detection of cryptosporidial infection in sheep. However the highest percent prevalence was recorded by molecular techniques (PCR) than simple microscopic examination.

Similarly, season wise percent prevalence of cryptosporidial infection in sheep was also determined by using simple microscopic examination and PCR and found higher percent prevalence at molecular level than simple microscopic examination.

In the present study, the highest percent prevalence was determined by PCR (13.33%) followed by simple microscopic (8.33%) in winter season while in spring season, 20% prevalence was determined by simple microscopic examination while 26.66% prevalence was determined by PCR technique. In the summer season, 23.33 percent prevalence was determined by using simple microscopic examination while 33.33 percent prevalence was detected by PCR. In autumn season, 23.33 percent prevalence was determined by simple microscopic examination while 30 percent was detected by molecular technique. Statistically non-significant difference ($P>0.268$) was observed in two diagnostic techniques.

On sex based analysis, in male, 17.02 percent prevalence was determined by coprological examination while 25.53% prevalence was determined by molecular technique. In female, the highest percent prevalence (27.08%) was determined PCR while the lowest percent prevalence was determined by simple microscopic (18.80%). On the basis of statistical analysis, significant difference ($P<0.019$) was recorded in comparative efficacy of both diagnostic techniques at gender wise percent prevalence.

Similar findings were also recorded in relation to age of the sheep, where higher percent prevalence was recorded by molecular technique (PCR) followed by simple microscopic examination and statistically non-significant difference ($P>0.264$) was detected in sensitivity of two diagnostic techniques. Our results are similar with the findings observed by Masood S et al. 2013 where higher percent prevalence of the *Cryptosporidium* infection was observed by PCR technique followed by simple microscopic coprological examination.

Our results are agreed to the findings reported by Santin et al. 2007 where 20.6% prevalence was reported in sheep with IFA technique while 50.8% prevalence was diagnosed through PCR.

In the present study, PCR technique was used in addition to simple microscopic examination for the detection of the *Cryptosporidium* infection in fecal samples of sheep. PCR skill provides an alternative offers to conventional diagnostic techniques for detection of the *Cryptosporidium o*ocysts in clinical and environmental samples. We have to compared simple microscopic examination by using Ziehl Neelsen Modified acid fast staining procedure with a currently developed PCR technique that can neither only detect only a single oocyst of the *Cryptosporidium* but can also distinguish between different species of the protozoan on the basis of genetic characterization.

Our results are similar to the findings reported by Morgan et al. 1998, where 36 samples were declared positive by PCR and 29 samples out of 511 were declared positive after confirmation by microscopy. A total of five samples were declared positive by microscopy while PCR declared negative and declared the result as false positives. Direct immunofluorescent-monoclonal antibody staining is more sensitive than acid fast staining and increases the chances of detection rate over acid-fast staining (Alles et al. 1995) while Modified Ziehl Neelsen acid -fast staining has been proved 83.7% sensitivity and 98.9% specificity when compared to the molecular technique known as PCR (Morgan et al. 1998). PCR technique is doubtless, highly sensitive, specific and gives more advantages than other diagnostic methods. Furthermore PCR enables genotyping of the different *Cryptosporidium* strains. However, recently PCR is limited to research work mostly than diagnostic labs.

The findings based on simple microscopic examination by MZN staining technique is not 100% positive because this is not only the oocysts of the *Cryptosporidium* but other organisms such as several types of acid fast bacteria (Mycobacterium species) may also get the same stain. The most important advantage of the PCR technique is to differentiate between different strains of the *Cryptosporidium* but through simple microscopy can't differentiate different species. PCR is more useful than microscopy due to highly sensitivity, specificity, easiness to use, genotyping ability and batch testing adaptability have made PCR a useful tool for future analysis and survey on molecular epidemiology of cryptosporidiosis in humans and animals. Due to lack of proper information at molecular level, detection of the percent prevalence of the *Cryptosporidium* infection in sheep could not be compared with other countries of Southeast Asia or the subcontinent.

5.8: Zoonotic potential of the *Cryptosporidium* Infection

In developing countries, endoparasites are very common problem in children and adults and one of the most common prevalent enteric protozoan is the *Cryptosporidium* that mainly infests the epithelial layer of small intestine that result in loss of villous enterocytes, atrophy, malabsorption and diarrhea. Other clinical symptoms include diarrhea, dehydration, loss of weight, abdominal cramps, nausea, fever and vomiting (Huang et al. 2004).

The *Cryptosporidium* oocyst is highly resistant to different disinfectants in tape water systems so, it was reported that chlorinated water having oocysts are responsible for an outbreak of cryptosporidiosis in humans. In 1989, an outbreak was reported in UK where 5000 peoples were infected with the *Cryptosporidium* infection, while in the USA more than 400,000 people in 1993 were affected by an outbreak of the *Cryptosporidium* (Mac Kenzie et al. 1994).

Diarrheal diseases are one of the most leading cause of morbidity and mortality in children under 5 years of age throughout of the world (Walker et al. 2013). Exposure to animals, Poor sanitation and hygienic conditions are the factors responsible for transmission of the parasitic diseases.(Fletche et al. 2012; Zambrano et al. 2014) and these factors are highly prevalent in subcontinent where the feces of the domestic animals are dispersed and open defecation of stools provides a favorable environment for exposure to *Cryptosporidium* infection (UNICEF, WHO, 2014).

In the present study, stool samples were collected from human beings and screened out for presence of the *Cryptosporidium* oocyst through simple microscopy and recorded 16.66% overall prevalence in children followed by adults (5.55%). In District Bannu, the highest percent prevalence was 18.33% in children while the lowest percent prevalence was 15% in District Lakki Marwat. Our results are similar to those reported by different researchers where 21.4% prevalence was found in southern city of Iran (Hamedi et al. 2005; Khalili et al. 2006; Mohammadi Ghaleh Bin B et al. 2006; Berenji F et al. 2007; Khalil et al. 2007.; Mirzaei, 2007; Nahrevanian et al. 2008) and our results are higher than the results reported in northern city of Iran.

According to findings observed in the current study, it was reported that the *Cryptosporidium* infection is common in children of southern areas of KPK. Our results are also similar to the findings reported by Al-Hashimi and Al-Najar et al. 2002, Al-Warid et al. 2012; in Baghdad, where 13.80% and 14.79% prevalence was reported respectively. Our results are also in agreement with the findings reported by Alsake et al. 2004 and Ali at al. 2013, in Pediatric Teaching Hospital in Sulaimai where the reported rate of infection was 13.3% and 15.2% respectively.

Our results are not agree with the findings reported by Mahdi et al. 1996 where lower rate of infection (8.8%) was recorded in children and similarly 6.8% prevalence was observed by Abdullah et al. 2010.

The highest infection rate was reported in veterinary students, small children and veterinary practitioners having close contacts with animals (Kiang et al. 2006). We also observed similar findings in the present study where the highest prevalence (20%) was recorded in those humans having close contacts with the animals while lower prevalence was recorded in those humans where no direct or indirect contact was observed with the animals.

The variations in percent prevalence reported by different researchers, may be due to differences in diagnostic tools, study population, considering age range, environmental risk factors, time of study (winter vs. summer), immune and nutritional status of the children & other risk factors such as type of feeding, life style, overcrowding, contact with animals, drinking water source (Khoshzaban et al. 1998; Huang et al. 2004; Roy et al. 2004).

5.9: Hematological and biochemical parameters in healthy and unhealthy goats suffering from the *Cryptosporidium* infection

5.9.1: Study of hematological parameters in healthy and unhealthy (Goats)

In the present study, recorded 47.18% total lymphocytes count in healthy goats while 54.45% was recorded in those goats, which were naturally infected with *Cryptosporidium* infection. Our results are similar to the findings reported by Kadria et al. 2015 where WBCs count (number×109/L) was 8.41 ± 0.07 in apparently healthy calves while WBCs count was 9.52 ± 0.06 in calves naturally infected with *Cryptosporidium* infection. Eosinophils count was also high (6.10%) in those goats that were naturally infected with *Cryptosporidium infection* while lower count (2.49%) was recorded in healthy goats and statistically significant difference (P<0.000) was observed. During every parasitic infestation, there is rise in eosinophil numbers which is one of the most well known diagnostic characteristic of parasitic infection and the same findings were recorded by Jain (1986) where higher numbers of eosinophils were recorded during *Cryptosporidium* infection.

In the present study, increased level of Hb was recorded in infected group as compared to healthy animals and similar results were also reported by Kadria et al. 2015; in ruminants suffering from *Cryptosporidium* infection. Our results are also agreed to the findings reported by Osman and Sadiek, 2008; while in some study reduction was also recorded in the Hb concentration as reported by El-Dessouk and El-Masry, 2005 which are not similar to our findings as we recorded in the current study. There are certain factors such as anorexia, anemia, low iron level, severe dehydration, hemoconcentration and long standing diarrhea are also responsible for reduction in Hb concentration (Bailey et al. 2004).

Our results are not agreeing to the findings reported by Shobhamani (2005) where low level of hemoglobin and TLC count was recorded in cow calves suffering from cryptosporidiosis. The difference may be due to diarrhoeic conditions, anemic factors and health status of the diseased animals (Bailey et al. 2004). In the present study, high level of Packed cell volume (39.22%) was documented in diseased goats suffering from *Cryptosporidium* infection while lower level of PCV (34.41%) was observed in healthy animals. Our results are similar with the findings reported by Osama and Sadiek (2008) where mean values of PCV showed highly significant increase in infected group. On statistical analysis, significant difference ($P<0.05$) was observed in PCV (%) level in non -infected (healthy) and infected animals.

Similar observations were also reported by Kadria et al. 2015, where highly significant ($p<0.001$) increase of PCV (%) was recorded in ruminants suffering from *Cryptosporidium* infection.

5.9.2 : Biochemical parameters of goat serum infected with *Cryptosporidium* infection

There was highly significant(P<0.000) decrease in total protein, albumin and ALP (u/l)(Alkaline phosphatase) in goats serum suffering from *Cryptosporidium* infection and similar findings were also reported by Kadria et al. 2015 in ruminants suffering from *Cryptosporidium* infection. With reference to the mean values of total protein and albumin levels, significant decrease was recorded in ruminants suffering from *Cryptosporidium* infection in comparison to the healthy ones. These observations were also supported by some other researchers having similar findings (Mohge, 1994; Awadala, 1996; El-Dessouky and El- Masry, 2005).

The decrease in total protein (gm%) and albumin (gm%) in ruminants suffering from *Cryptosporidium* infection may be due to poor absorption of nutrients and breakdown of excessive protein and excessive loss of albumin (Maline et al. 1994).

In the present study, it was documented that there was non-significant difference (P>0.814), (P>0.689) recorded in the quantity of ALT (u/I) (Alanine Aminotransferase) and AST (u/l) (Aspartate Aminotransferase) in serum of healthy and diseased goat.

Statistically highly significant (P<0.000) decrease in sodium ions were observed in infected goats than apparently noninfected (healthy) goats while low percent quantity of chloride ions were recorded while statistically, non-significant difference (P>0.092) was observed. Our results are similar to the findings reported by El-Dessouky and El-Masry (2005), Osman and Sadiek (2008) where low level of sodium and chloride ions were recorded in ruminants suffering from *Cryptosporidium* infection. These results may be due to excessive loss of sodium and chloride ions in watery diarrhea (Fadl-Alla; 1989).

There was highly significant increase in potassium, copper, Creatinine and urea level was recorded in serum of goats suffering from cryptosporidiosis while low level was recorded in healthy animals. Statistically significant difference (<0.000) was observed. These observations are agreed with Tawfik et al. 2004, where highly significant increase was reported in potassium level in infected animals (cryptosporidiosis).

There was highly significant increase was recorded in copper level in those goats suffering from *Cryptosporidium* infection and statistically significant difference (P<0.002) was observed. Our results are not similar with the findings reported by El-Dessouky and El-Masry (2005) where low level of copper was observed in diseased animals. This might be due to decrease in beta globulin levels which has the capacity to bind with the mineral as reported by Nasr, 1993. Lower quantity of Ca (8.10mg %) and Mg (1.28mg %) ions were reported in diseased animals while higher quantity was observed in healthy animals. There was non-significance difference (P>0.24) was recorded in calcium (Ca) level. Similarly, statistically non-significant difference (P>0.192) was observed in magnesium (Mg) level in blood serum of infected and healthy goats.

5.10 : Comparative efficacy of different allopathic and herbal agents against *Cryptosporidium* infection in small ruminants

In the present study, different drugs were evaluated on the basis of their percent efficacy on the basis of reduction in OPG, for the purpose to determine drug of choice in our local community for better treatment of the Cryptosporidial infection in small and large ruminants.

5.10.1* : *Azithromycin

A single dose of *Azithromycin* (10 mg/ kg b.wt) was used against cryptosporidiosis in naturally infected goats and the efficacy was determined on the basis of reduction in OPG count. The percent efficacy of *Azithromycin* was 13.90 %, 29.07 %, 44.87 % and 59.29% at 7th, 14th, 21th and 28^{th} day post treatment. On statistical analysis, significant difference in OPG reduction was recorded in relation to time (P>0.000) at different days. But the Azithromycin showed poor reduction in OPG when compared to other allopathic and herbal drugs and statistically significant difference (P<0.000) was observed in efficiency of the drug. Similar findings in relation to the efficacy of different macrolids against cryptosporidiosis were reported by other researchers such as Tams, 1996; who reported the efficacy of Tylosine against cryptosporidiosis in dogs. Spiramycin (Galvagno et al. 1993), Clarithromycin (Cama et al. 1994) and Azithromycin (Elitok et al. 2005) were found to be effective against cryptosporidiosis. On the basis of statistical analysis, mean OPG count was same after treatment with the control group because the poor efficacy of the drug and self-limiting characteristic of the cryptosporidiosis.

5.10.2 : Metronidazole

In the current study, a single dose of *Metronidazole* at the dose rate of 50 mg/kg body weight PO was used against group B, where goats were naturally infected with cryptosporidiosis and caused a significant decrease (P<0.000) in oocyst per gram (OPG) at 7^{th} day post treatment and onward. At day 7 post treatment, 25.74% decreases was observed in oocyst count. Similarly the percent efficacy of *Metronidazole* was 46.70 %, 68.14%, and 78.20 % at days 14, 21 and 28, respectively. On statistical analysis by two way ANOVA, significant (P<0.000) decrease in oocyst per gram (OPG) was recorded at day 7^{th}, 14^{th}, 21th and 28^{th} day post treatment while statistically non-significant difference (P>0.05) was observed with herbal drug i.e. *Allium sativum.* Metronidazole showed poor efficiency than Paromomycin and statistically significant difference (P<.000) was observed in reduction of OPG. Our findings are very similar to the observations found by Masood et al. 2013; who reported that *Metronidazole* is highly efficient drug in reducing of *Cryptosporidium* oocysts.

5.10.3 : *Allium sativum* (Garlic)

Allium sativum was used at the dose rate of 50mg/ kg body weight as described by Castro-Hermida et al. 2004. A single dose of *Allium sativum* caused a significant decrease in OPG count at 7th day post treatment and onward. At day 7th post treatment, recorded 27.06% reduction in OPG count. Similarly percent efficacy of *Allium sativum* was 49.70%, 62.24%, and 77.00% at days 14, 21 and 28, respectively. Statistically significant difference (P<0.000) was observed at different days while statistically non-significant difference (P>0.05) was observed in reduction of OPG with Metronidazole. *Allium sativum* showed better results than Azithromycin while poor results than Paromomycin and statistically significant difference (P<0.000) was observed. Our results are similar to the findings as reported by Kadria et al. 2015 that garlic is the most effective herbal agent against cryptosporidiosis in ruminants and similar findings were also observed in the current study. The mode of action of *Allium sativum* against Cryptosporidiosis as a prophylaxis and treatment was explained by different researchers. Masamha et al. 2010; reported that *Allium sativum* disrupts the normal physiology of the parasite such as movement, absorption of food and reproductive activities. Sutton and Haik, 1999; reported the phagocytic activities and natural killer cell activity which improve the immune system activities and make strong the body defense system, increased during the treatment period by *Allium sativum* . Adetumbi et al. 1983, reported that *Allium sativum* blockage the lipid synthesis by microbes so act as an antimicrobial agent. When *Allium sativum* was used in HIV patients for the treatment of cryptosporidiosis by Fareed et al. 1996, complete recovery was reported in some patients while partial recovery was recorded in others.

In the present study, it was concluded that *Allium sativum* is one of the excellent prophylactic and gifted therapeutic agent against the *Cryptosporidium* infection and similar suggestions were carried out by Maha Reda Gaafar, 2012 that further investigations should carried out to find out applications of garlic as a part of complementary medicine in proper treatment and management of cryptosporidiosis in animals and humans.

***5.10.4* : Paromomycin**

During the current therapeutic trials, *Paromomycin* was used at the dose rate of 100mg/kg body weight in goats suffering from *Cryptosporidium* infection and statistically significant (P<0.000) decrease was observed in Oocyst per gram (OPG) count at 7^{th} day post treatment and onward treatment. Post treatment percent efficacy of *Paromomycin* was calculated at day 7th, 14th, 21th and 28th and recorded 51.77%, 67.08%, 87.54% and 91.77% efficacy respectively in reduction of OPG count. On the basis of statistical analysis, significant difference (P<0.000) was observed in relation to reduction in OPG count at different selected days. Paromomycin showed better results in reduction of OPG than all other drugs and statistically significant difference (P<0.000) was observed. Similar results were also observed by Tzipori et al. 1994; Verdon et al. 1994; Sharling et al. 2010; Leitch and He, 2011.

Masood et al. 2013, suggested that *Paromomycin* is the most effective and expensive drug for the treatment of Cryptosporidiosis based on the clinical trials and similar findings were also observed by (Griffiths, 1998).

Under experimental conditions, *Paromomycin* was used by Masood et al. 2013; against *Cryptosporidium* infection and showed superior results than *Metronidazole* and *Albendazole.* Hahn and Capuano, 2010, recorded that anti-cryptosporidial activities are present in *Paromomycin* only.

Paromomycin showed more useful results, when used at the dose rate of 25-35 mg/kg body weight per day but partial effect upon shedding of oocyst and stool frequency in patients suffering from AIDS (Chawla et al. 2011). It was concluded that paromomycin is the most effective drug for treatment of cryptosporidiosis in human beings. On comparison, the efficacy of different allopathic and herbal drugs, the maximum reduction in oocyst per gram (OPG) was observed when paromomycin was used at the dose rate of 100mg per kg body weight of goats.

CONCLUSIONS

- Cryptosporidiosis is highly prevalent in small ruminants in southern Khyber Pakhtunkhwa, Pakistan.
- All the studied risk factors were highly significantly associated with *Cryptosporidium* infection.
- Molecular technique for detection of *Cryptosporidium* infection was more precise and sensitive than simple conventional methods.
- Children were more prone to infection having close contact with small ruminants.
- *Cryptosporidium* infection was also responsible to alter some hematobiochemical parameters.
- *Paromomycine* was highly effective against cryptosporidiosis.
- *Cryptosporidium* infection was highly prevalent in small children than adults.
- *Allium sativum* was highly effective as a herbal drug against cryptosporidiosis.
- Small ruminants were the potential source of zoonosis under field conditions.

RECOMMENDATIONS:

- There is need of study to find out prevalence of cryptosporidiosis in all vertebrates in different parts of the country at regular intervals.
- Adaptation of proper effective control measures are necessary to handle with the high load of *Cryptosporidium* infection.
- Avoid direct close contact with small ruminants because the potential source of cryptosporidiosis.
- Always used the drug of choice against *Cryptosporidium* infection such as *Paromomycine* (allopathic) and *Allium sativum* (herbal) when needed.
- Proper adaptation to hygienic environment can reduce the prevalence of the disease.
- There is need of further study for proper sequencing and characterization of the *Cryptosporidium at species level*

CHAPTER 6
SUMMARY

Cryptosporidiosis is one of the most important parasitic enteric protozoan infection affecting all vertebrates. The current study was designed to determine the percent prevalence of cryptosporidiosis in small ruminants and humans along with associated risk factors.

An **overall highest** percent prevalence of cryptosporidiosis recorded in all four categories of small ruminants were 27.22%, 20.56%, 18.33% and 12.22% in lambs, kids, Sheep and goats respectively.

In the current study, 21.55%, 18.33% and15% prevalence of the *Cryptosporidium* infection was recorded in **sheep** in District Kohat, Bannu and Lakki Marwat respectively. In the present study, the highest **month** wise percent prevalence in sheep, was observed in the month of August (36.66%) followed by April (26.66%), June (26.66%), May, July and September (23.33%), February (10.66%), March (10%), November (10%) while the lowest percent prevalence was observed in the month of December and January (6.66%). In sheep, **season** wise percent prevalence was also studied where highest prevalence was recorded in summer and autumn season (23.33%), followed by spring (20%) while the lowest percent prevalence was found in the winter season (10%). In sheep, **age wise** percent prevalence was also studied where highest percent prevalence was found at the age of 1 year (22.38%) followed by 1-2 years (18.03%) while the lowest at the age of 2-3 years (13.46%). In sheep, **sex wise** percent prevalence was also documented where highest percent prevalence was recorded in female (18.80%) followed by male (17.02%) where lowest percent prevalence was recorded.

In goats, the percent prevalence of the *Cryptosporidium* infection was also studied in three selected areas where recorded 6.66%, 11.66% and 18.3% prevalence in District Bannu, Lakki Marwat and Kohat respectively. Similarly, **in goats,** overall highest **month** wise percent prevalence was recorded in the month of August (30%), followed by July (23.33%), June (20%), May (16.66%), March and September (13.33%), April and November (10%), January, February and October (3.33%) while the lowest percent prevalence was recorded in December (0%). In the current study, the **season wise** prevalence was also studied in goats where highest percent prevalence was recorded in the summer season (20.83%), followed by spring (13.33%), autumn (11.66%) while the lowest prevalence was observed in winter season (3.33%). The highest a**ge** wise percent prevalence was recorded at the age of 1 year (18.58%) followed by 1-2 years (10.20%) while the lowest at the age of 2-3 years or above (5.95%). According to the **sex wise** percent prevalence, the highest percent prevalence was recorded in male (12.30%) while the lowest in females (12.17%).

The **overall highest percent prevalence** of the *Cryptosporidium* infection was also recorded in lambs in three areas where 33.33%, 25% and 23.33% prevalence was recorded in Kohat, Lakki Marwat and Bannu respectively. The highest **month** wise percent prevalence was recorded in the month of August (46.6%), followed by other months of the year such as July (40%), April, May and June (30%), September and October (26.66%), November and January (20%) while the lowest in the month of February and December (16.66%) in lambs. The S**eason wise percent prevalence** was recorded in lambs where highest percent prevalence was recorded in summer season (36.66 %), followed by spring and autumn (26.66%) while the lowest in winter season (18.33%). According to the **age wise** percent prevalence in lambs, the highest prevalence was recorded at the age of 1-15 days (38.09%) followed by 16-30 days (29.41%) while the lowest at the age of 31-60 days or above (15.15%). In lambs, the highest **sex wise** percent prevalence was recorded in females (31.18%) while the lowest percent prevalence was observed in males (22.98%).

In **kids**, overall highest percent prevalence was 20.55% recorded in three selected districts where the highest prevalence was recorded in District Kohat (23.33%), followed by District Bannu (20%) while the lowest in District Lakki Marwat (18.33%). In kids the month wise percent prevalence was also studied where the highest percent prevalence was recorded in May and August (33.33%), followed by June, July and September (26.66%), March, April and October (20%), November and December (13.33%) while the lowest percent prevalence was recorded in the month of the January (6.66%). The S**eason wise** percent prevalence was also recorded in **kids**, where the highest percent prevalence was observed in the summer season (30%), followed by autumn (23.33%), spring (20%) while the lowest prevalence was recorded in winter season (10%). The highest **age wise** percent prevalence in kids was also recorded at the age of ≤1-15 days (33.92%), followed by 16-30 days (15.38%) while the lowest at the age of ≥31-60 days or above (13.55%). S**ex wise** percent prevalence was also determined in goat kids where, the highest percent prevalence was recorded in female (20.98%) followed by male kids (19.19%).

To conduct **molecular study**, 360 fecal samples of sheep were analyzed for presence of the *Cryptosporidium* oocysts through simple microscopic method first then confirmed by PCR. **DNA was extracted** with the help of DNA extraction kit (Made in USA, GFC vivantis). The targeted gene of parasite was 18s rRNA which result in amplification of a segment of genomic DNA at 435 bp. The following **primers** sequence was used for Forward primer: (5-AAGCTCGTAGTTGGATTTCTG- and reverse primers (5-TAAGGTGCTGAAGGAGTAAGG-3. An overall **molecular percent prevalence** of the *Cryptosporidium* infection was 24.99% in sheep in three selected zones of southern KPK. The highest molecular percent prevalence was 31.66%, 25% and 18.33% in District Kohat, Bannu and Lakki Marwat respectively.

The highest **season wise molecular percent prevalence** was also recorded where the highest percent prevalence was recorded in the summer (33.33%), followed by autumn (30%), spring (26.66%) while the lowest in the winter season (13.33%). Molecular percent prevalence was higher in females (27.08%) than male (25.53%). On the basis of **environmental factors,** overall the highest percent prevalence was recorded in the month of August where highest ambient temperature, relative humidity and heavy rain fall was recorded.

To find out **Zoonotic aspect** of the *Cryptosporidium* infection, the overall highest percent prevalence was recorded in children (16.66%), followed by adults (5.55%).

The highest percent prevalence was recorded in diarrhoeic children where direct contact with small ruminants was observed while the lowest prevalence was recorded in those children where no direct or indirect contact was observed.

To conduct the **therapeutic trials**, a total of 50 goats were selected of the same weight and age that were naturally infected by *Cryptosporidium* under field conditions. All the goats were placed under same feeding and management conditions and randomly divided into five groups such as A, B, C, D and E. All animals in groups A, B, C and D were treated with Azithromycin (10mg/kg b.wt), Metronidazole (50mg/Kg b.wt), *Allium sativum* (50mg/Kg b.wt) and Paromomycin (100mg/kg b.wt) respectively while Group-E was placed as a positive control group. The highest percent efficacy in reduction of OPG was shown by different drugs such as Paromomycine (91.77%) followed by Metronidazole (78.20%), *Allium sativum* (77.00%) while the lowest percent efficacy was shown by Azithromycin (59.29%). On the basis of **hematological** study, lower lymphocytes count was (48.39%) recorded in non-infected animals while higher (54.33%) count was recorded in infected animals. Similarly higher eosinophil count was (6.73%) recorded in infected group while lower (50 %) counts were recorded in non-infected group. Hb level was higher in infected group than healthy animals. PCV level was higher (42.94%) in infected animals while low (34.62%) in healthy animals. **Biochemical analysis** of the serum showed, higher quantity of total protein, albumin, ALP, Sodium, Potassium, Chloride, Zinc, Copper, Urea and Creatinine was recorded in infected goats while lower quantity was observed in healthy goats.

CHAPTER 7
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CHAPTER 8
ANNEXURE

Annexure 1

Composition of PCR Reaction mixture

S.No	Reagents	Quantity
1	PCR buffer	5µl
2	Deoxy nucleotide triphosphat (dNTPs)	5µl
3	MgCl	5µl
4	DNA	5µl
5	Forward Primer	2µl
6	Reverse Primer	2µl
7	De-ionized water	25.5µl
8	Taq DNA Polymerase	0.5µl
Total Volume of the mixture		50µl

Annexure 2

Composition of Tank buffer (1.5% Agarose gel electrophoresis)

S. No	Chemicals	Composition
1	AGAROSE	DNA grade
2	10X TAE	0.4 M Tris acetate.20 M EDTA,PH 8
3	Ethidium bromide	10 mg/ml
4	6X DNA loading dye	50% glycerol, 6X TAE, 1% bromophenol blue)
5	DNA ladder mix	10 kb

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DATA CAPTURE FORM

Title of Research: Epidemiology, Molecular detection, Zoonotic potential, Hematology and chemotherapy of cryptosporidiosis small ruminants in southern KPK.

Basic information:

Farmer's name: ____

Location: ____

Contact No: ____

Observations:

Animal species. Ovines ☐ Caprines ☐

Breed____________ **Age.** _______________ **Sex.** ___________

Season

Spring ☐ Summer ☐ Autumn ☐ Winter ☐

Management

1. **Feeding:** Grazing/browsing ☐ Stall fed ☐
2. **Water source:** Deep well ☐ Stream ☐ Pond ☐
3. **Colostrum intake:** Fed ☐ Deprived ☐
4. **Deworming:** Yes ☐ No ☐

Environment:

Temperature.________°C **Humidity.______%, Avg rainfall __________**

Animal History:

Previous infection.____

Treatment: Yes ☐ No ☐

Present history:

Body temperature_____ _°F. **Diarrhea.** Present ☐ Absent ☐

Vet. Services. Availed ☐ Not availed ☐

Any home remedy: Yes ☐ No ☐ Name.____________________________

Lab investigations: Yes ☐ No ☐ Diagnosis.________________________

Sample ____

Gross findings: Color_____________ Consistency___________ _

Blood_____________ Mucous ______________

Lab findings:

Microscopic examination of stained films:

PCR results
