EPIDEMIOLOGY, MOLECULAR DIAGNOSIS AND CHEMOTHERAPY OF GIARDIASIS IN BOVINE.

By

SULTAN AYAZ
2005-VA-212
DVM, M.Sc (Hons)

A THESIS SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PARASITOLOGY

Department of Parasitology,
UNIVERSITY OF VETERINARY & ANIMAL SCIENCES, LAHORE
2009
To

The Controller of Examinations,
University of Veterinary and Animal Sciences,
Lahore.

We, the Supervisory Committee, certify that the contents and form of the thesis, submitted by SULTAN AYAZ, have been found satisfactory and recommend that it be processed for the evaluation by the External Examiner(s) for the award of the degree.

CHAIRMAN _____________________________________________
Prof. Dr. Azhar Maqbool

MEMBER _______________________________________________
Prof. Dr. Zafar Iqbal Chaudhry

MEMBER _______________________________________________
Dr. Aftab Ahmad Anjum
IN THE NAME OF ALLAH THE COMPASSIONATE, THE MERCIFUL
DEDICATED

To

My

Parents

And

Three Little angels

Samia

Qandeel

And

Hamad
ACKNOWLEDGEMENTS

All praises to ALMIGHTY ALLAH, who guides us in darkness and thankful to my ALLAH, who has conferred me with potential and ability to complete this research study.

I feel enormous intensity of obligation to my reverend Supervisor, Professor Dr. Azhar Maqbool, Department of Parasitology, University of Veterinary and Animal Sciences (UVAS), Lahore for his valuable guidance, stimulating ideas and extreme patience with my work, which proved to be a panacea in the completion of this thesis.

I have deep sense of appreciation to the members of my Supervisory Committee, Prof. Dr. Zafer Iqbal Chaudhry ex-Dean faculty of veterinary science, university of veterinary and animal sciences Lahore and Dr. Aftab Ahmad Anjum Assistant Professor Department of Microbiology, for their personal interest and cooperation.

I extend my thanks to Professor Dr. Mansoor u Din, chairman Department of Microbiology for provision of laboratory during my research work and thanks to Mr. Imran Najeeb, Lecturer Department of Microbiology for his assistance during my work . I am also thankful to Dr. Mohammad Lateef Associate Professor, Dr. Kamran Ashrif Assistant Professor and Mr. Awais Anees lecturer, department of Parasitology and other staff members (long list) of the Department of Parasitology for their support and cooperation which enabled me to bring this work to a logical conclusion.

Finally I am very much thankful for the cooperation and help of Miss Sabiqaa Masood ex lecturer department of Parasitology and friends in the pursuit of my studies.

SULTAN AYAZ
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>DEDICATION</th>
<th>i</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter No.</th>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>REVIEW OF LITERATURE</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>MATERIALS AND METHODS</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>RESULTS</td>
<td>42</td>
</tr>
<tr>
<td>5.</td>
<td>DISCUSSION</td>
<td>98</td>
</tr>
<tr>
<td>6.</td>
<td>SUMMARY</td>
<td>106</td>
</tr>
<tr>
<td>7.</td>
<td>LITERATURE CITED</td>
<td>110</td>
</tr>
<tr>
<td>8.</td>
<td>ANNEXURE</td>
<td>131</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Overall prevalence (%) of Giardiasis in calves of Lahore from August 2007 – July 2008.</td>
<td>44</td>
</tr>
<tr>
<td>2.</td>
<td>By season, sex and age prevalence % Giardiasis of calves in different form/areas of Lahore from August 2007 – July 2008.</td>
<td>49</td>
</tr>
<tr>
<td>3.</td>
<td>Month Wise Prevalence of Giardiasis in Cattle at Lahore From August 2007 to July 2008</td>
<td>54</td>
</tr>
<tr>
<td>4.</td>
<td>Prevalence of Giardiasis by season, sex and age in cattle at Lahore from August 2007 – July 2008</td>
<td>57</td>
</tr>
<tr>
<td>5.</td>
<td>Overall prevalence (%) Of Giardiasis in Buffaloes at Lahore From August 07 to July 08</td>
<td>61</td>
</tr>
<tr>
<td>6.</td>
<td>Relation of Giardia infection with stool consistency</td>
<td>67</td>
</tr>
<tr>
<td>7.</td>
<td>Molecular Diagnosis Of Giardiasis In Cattle At Lahore From August 07-July 08</td>
<td>71</td>
</tr>
<tr>
<td>8.</td>
<td>Mean Month wise Temperature, Humidity and Rain Fall at Lahore During 2007-2008</td>
<td>76</td>
</tr>
<tr>
<td>9.</td>
<td>Comparison Of Microscopic And PCR Based Diagnosis Of Giardiasis In Cattle At Lahore.</td>
<td>81</td>
</tr>
<tr>
<td>10.</td>
<td>Microscopic and PCR based by area prevalence of Giardiasis in cattle.</td>
<td>82</td>
</tr>
<tr>
<td>11.</td>
<td>Cyst per gram of faces in Cattle treated with different drugs on different days</td>
<td>89</td>
</tr>
<tr>
<td>12.</td>
<td>Comparative efficacy (%) of different drugs against Giardiasis in cattle</td>
<td>90</td>
</tr>
<tr>
<td>13.</td>
<td>Comparison of Body Weight (Kg) and Feed Intake (Kg/day) Before and After Treatment of Cattle</td>
<td>93</td>
</tr>
<tr>
<td>14.</td>
<td>Mean Comparative Hematology of infected and non-infected cattle (n=30)</td>
<td>94</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Fig No</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Prevalence of Giardiasis in calves of Lahore from August 2007 –July 2008.</td>
<td>45</td>
</tr>
<tr>
<td>1.2</td>
<td>Prevalence of Giardiasis in calves at Gawala Colonies Lahore</td>
<td>47</td>
</tr>
<tr>
<td>1.3</td>
<td>Prevalence of Giardiasis in calves at Govt. Dairy Farm Lahore</td>
<td>47</td>
</tr>
<tr>
<td>1.4</td>
<td>Prevalence of Giardiasis in calves at Household dairies Lahore</td>
<td>48</td>
</tr>
<tr>
<td>1.5</td>
<td>Prevalence of <em>Giardiasis</em> by age group in Calves at Lahore</td>
<td>50</td>
</tr>
<tr>
<td>1.6</td>
<td>Season wise Prevalence of Giardiasis in Calves</td>
<td>50</td>
</tr>
<tr>
<td>1.7</td>
<td>Prevalence of Giardiasis by sex group in Calves at Lahore.</td>
<td>51</td>
</tr>
<tr>
<td>2.1</td>
<td>Prevalence of <em>Giardiasis</em> in Cattle at Lahore from August 2007 to July 2008</td>
<td>55</td>
</tr>
<tr>
<td>2.2</td>
<td>Prevalence of Giardiasis in Cattle at Lahore</td>
<td>55</td>
</tr>
<tr>
<td>2.3</td>
<td>Month Wise Prevalence of Giardiasis in Cattle at Lahore</td>
<td>58</td>
</tr>
<tr>
<td>2.4</td>
<td>Overall Prevalence of Giardiasis in Cattle at Lahore</td>
<td>58</td>
</tr>
<tr>
<td>2.5</td>
<td>Prevalence of Giardiasis by season in Cattle at Lahore</td>
<td>59</td>
</tr>
<tr>
<td>2.6</td>
<td>Prevalence of Giardiasis by sex in Cattle at Lahore</td>
<td>59</td>
</tr>
<tr>
<td>3.1</td>
<td>Month Wise Prevalence of Giardiasis in Buffaloes at Lahore (From August 07- July 08)</td>
<td>62</td>
</tr>
<tr>
<td>3.2</td>
<td>Overall Prevalence of Giardiasis in Buffaloes at Lahore (August 07- July 08)</td>
<td>63</td>
</tr>
<tr>
<td>3.3</td>
<td>Prevalence of Giardiasis by sex in Buffaloes at Lahore (August 07- July 08)</td>
<td>65</td>
</tr>
<tr>
<td>3.4</td>
<td>Prevalence of Giardiasis by season in Buffaloes at Lahore</td>
<td>65</td>
</tr>
<tr>
<td>4.1</td>
<td>Relation of Giardia infection with stool consistency</td>
<td>68</td>
</tr>
<tr>
<td>5.1</td>
<td>Molecular Diagnosis by season in Cattle at Lahore from August 07-July 08</td>
<td>72</td>
</tr>
<tr>
<td>5.2</td>
<td>Comparison of PCR Based detection of Giardiasis in Cattle in Different Farms at Lahore</td>
<td>72</td>
</tr>
<tr>
<td>5.3</td>
<td>Molecular Prevalence of Giardiasis by age in Cattle at Lahore</td>
<td>73</td>
</tr>
<tr>
<td>5.4</td>
<td>Overall Molecular Prevalence of Giardiasis in Cattle at Lahore from August 07 – July 08</td>
<td>73</td>
</tr>
</tbody>
</table>
6.1 Mean Month wise Temperature (°C), Humidity (%) and Rain Fall (mm) at Lahore During 2007-2008 78

7.1 Prevalence of Giardiasis by PCR & Coprological Based Techniques 83

7.2 Prevalence of Giardiasis by PCR & Microscopic Based Techniques 84

8.1 Comparative Efficacy of Different Drugs (Medium Dose) Against Giardiasis in cattle 91

9.1 SDS PAGE electrophoresis of *Giardia* protein extracts of different cysts /ml concentrated sonicated samples. Lane M is the molecular size (K Da) marker while lane 1, 2, 3, 4, and 5 indicates the different sample run.

9.2 Standard Curve of known protein samples. This curve was made by plotting, the absorbance at 280 nm wavelength versus concentration of the bovine serum albumen standard. A stock solution containing 300mg / 3ml of BSA was made in normal saline which was further diluted to prepare concentrations of 10 %, 20%, 40%, 60%, 80% and 100% mg/ml.
Chapter 1

INTRODUCTION

*Giardia lamblia*, also called *Giardia duodenalis* or *Giardia intestinalis*, is a protozoan parasite which inhabits small intestine and causes extensive morbidity worldwide. *Giardia* was seen under microscope first time by Antony Van Leeuwenhock during 1681 (Dobel, 1920). Later on it was described by Vilim Lambli in 1859. However, most of the research workers in this field use the name *Giardia* (Meyer, 1990) and extensive work has been undertaken on its epidemiology, pathophysiology and treatment (Meyer, 1994). Outbreaks of waterborne giardiasis had been reported in Europe and United States during years 1960s and 1970s (Craun, 1990; Farthing, 1992). The clinical symptoms of the giardiasis in human are diarrhoea, dehydration, abdominal pain, nausea, vomiting, and weight loss (Thompson and Monis, 2004). *G. duodenalis* infection is depending on host, parasite, and environment (Thompson et al., 1990).

*G. duodenalis infection* is reported from a wide variety of mammalian hosts as well as birds, reptiles and amphibians (Thompson et al., 1993). Water is an important sources of spreading of the *Giardia* infection to people and is a major health concern of water utilities of the developed and developing countries of the world (Levine et al.,1990; Hogue et al.,2002)). *G. duodenalis* has been observed in different species of farm animals (Xiao, 1994; Olson et al., 1997). Livestock can excrete high numbers of Giardia cysts which become a source of water contamination and environment(Weniger et al., 1983; Craun, 1990). Livestock can play a potential
role to act as a reservoir for human outbreaks of giardiasis (Buret, et al., 1990; O, Handley, et al., 2000b).

*Giardia* can be transferred from domestic animals to human beings and is a potential Zoonotic agent (Davis and Hibler, 1979; Majeweska, 1994). *G. duodenalis* isolated from humans and animals is genetically comparable which confirm its zoonotic nature (Meeloni et al., 1995). *Giardia* had caused non-viral diarrhoea in humans and was responsible for epidemics in developed as well as developing countries (Lane and Lloyd, 2000; Thomson et al., 2000).

*Giardia* multiplies by asexual reproduction on luminal surface of small intestine in a wide range of vertebrate species (Thomson et al., 2000; Thomson et al., 2004). Life cycle of *G. lamblia* has two forms: trophozoite and cyst. Cysts are relatively resistant in environment and can survive in cold humid conditions whereas trophozoites cannot. After ingestion, excystation is initiated by contact with acidic gastric contents and is followed by a highly coordinated sequence of events leading to the release of one or two trophozoites (Bingham and Meyer, 1979; Hetsko et al., 1998). A parasite-derived protease may be activated during the excystation process (Ward et al., 1997). Trophozoites infect duodenum and upper intestine, which has a favorable alkaline pH, and give rise to clinical sequelae. While passing from small intestine to colon trophozoites are encysted. Encystations can be achieved by *in vitro* culture of parasites in low concentration of bile salts and cholesterol followed by higher concentrations at an alkaline pH (Lujan et al., 1997). Cyst proteins are then transcribed, secreted into encystment-specific vesicles and transported to newly forming cell wall in 14 to 16 hours (Erlandsen et al., 1996).
G. duodenalis is the only species observed both in human beings and animals including dogs, cats, bovines, pigs, sheep and equines (Thomson et al., 2000; Thomson, 2004). Infection spreads through ingestion of Giardia cysts excreted in faeces of infected animals (Monis and Thomson, 2003). Giardia is cosmopolitan in distribution and its prevalence has been reported in young cattle and less known in the adult cattle (Xiao and Herd 1994; Olson et al., 1997). Giardia infection occurs in group housed calves as well as housed within individual pens (Olson et al., 1997a; Handley et al., 1999). There are some reports on low Giardia infection rates (Oviedo et al., 1987) and others as major cause of diarrhoea in calves (Deshpande and Shastri, 1981). Other enteropathogens have not been tested with Giardia infection in calf (Xiao et al., 1994; Ruest et al., 1995). Giardiasis is associated with infection by particular genotypes in animal and humans as well (Nash et al., 1987). Infected livestock can excrete over $10^6$ Giardia cysts per gram of faeces which may contaminate the environment and can act as potential reservoir for human outbreaks of Giardiasis (Buret et al., 1990; Handley, 2000b).

Animal and human diseases have distinct seasonal patterns. Seasonal peaks of diarrhoeal and respiratory diseases in humans as well as animals have been documented (Hancock, 1983). Information on possible seasonal variation of Giardia infection in domestic animals is scarce. Some reports have indicated the seasonal trends in Giardia infection in humans (Kasim and Elheulu, 1983). Giardia infection has been detected frequently during winter in calves at Canada (Ruest et al., 1995). It has been reported in one study that hutch-reared calves had a higher rate of Giardia
infections in spring than in summer whereas no seasonal difference in infection rates was observed in calves grouped in pens (Xiao et al., 1993).

Chemotherapy is the most effective way to eliminate *Giardia* infection of livestock and human beings from a particular area (Reynolds et al., 1998; Thompson, 1998; Handley et al., 2001).

Diagnosis of *Giardia* by conventional microscopic methods following faecal concentration techniques, particularly zinc sulphate and zinc chloride floatation and centrifugation (Zajac et al., 2002) remains a reliable indicator of infection status. However, there is a need for a sensitive and specific diagnostic method to detect etiological agents of disease. For *Giardia*, molecular technique such as polymerase chain reaction (PCR) is an alternate method for specific detection of pathogens in stool. Sensitivity of detection by PCR is greater than that compared with microscopic evidence (McGlade et al., 2003).

Giardiasis is one of the major zoonotic threats in Pakistan. So far, no study has been undertaken to pin point zoonotic potential and molecular diagnosis of Giardiasis in humans and animals. In view high prevalence of Giardiasis in bovine a study was designed to accomplish following objectives.

1. To study the epidemiology of *Giardiasis* in bovines to compare its prevalence among buffaloes, cows and calves.

2. To develop a PCR based technique for diagnosis/detection of *Giardia*.

3. To study the zoonotic importance of *Giardia*, being most hazardous to human health.
4. To characterize *Giardia* isolates through SDS-PAGE (protein profile).

5. To evaluate efficacy of different chemotherapeutic agents against *Giardia* to point out the most effective, appropriate and economical drug of choice to eliminate the infection.
Chapter 2

REVIEW OF LITERATURE

*Giardia duodenalis* has a world wide distribution causing an estimated $2.8 \times 10^8$ cases per annum (Lane and Lloyd, 2002) and is a commonly intestinal parasite of mammals including human and morphologically indistinguishable from one another and grouped into assemblages (genotypes) based on molecular characteristics (Thompson, *et al.*, 2000). It has been reported that more than 200 million people have symptomatic giardiasis in Latin America, Asia and Africa (WHO, 1996). Outbreaks of waterborne giardiasis had been reported in Europe and United States during years 1960s and 1970s (Craun, 1990; Farthing, 1992).

*Giardia duodenalis* is recovered from a wide variety of mammalian hosts including humans (Kulda and Nohynkova, 1995). The water borne giardiasis outbreaks in humans have reported due to unfiltered surface or ground water systems contaminated by surface run off or sewage discharges (Jakubowski and Graun, 2002).

*Giardia* is transmitted through trophozoite and cysts between different species (Hewlett *et al.*, 1982). Giardiasis is the most frequently diagnosed water borne disease and the major public health concern of water utilities in the developed and developing nations (Levine *et al.*, 1990; Hogue *et al.*, 2002).

*Giardia* infecting humans and ruminants are morphologically and antigenically similar. It is postulated that domestic ruminants may be a reservoir for
human infection and vice versa, thus classifying giardiasis as a zooanthroponotic disease (Buret et al., 1990).

ETIOLOGY

Human giardiasis is caused by the intestinal flagellate *Giardia duodenalis* and is considered a zoonotic infection because of these subgenotypes of *Giardia*, namely A1, A2, A3, A4 and B3, were found to be associated with infections of humans, dogs and calves, which supported the role of these animals as a source of infection for humans (Lalle and Pozio, 2005). *Giardia* is the recognized etiological agent for traveler diarrhoea (Craun, 1990). Giardiasis should be considered as an etiologic agent of diarrhoea in calves (St. Jean et al., 1987).

Giardiasis is the most frequently diagnosed water borne disease and the major public health concern of water utilities in the developed and developing nations, water is an important vehicle for the transmission of *Giardia* to humans and livestock (Levine et al., 1990; Thurman et al., 1998; Hogue et al., 2002).

Giardiasis is a notifiable disease of high prevalence in New Zealand and 40 *Giardia* isolates from calves and 30 from humans living in the same region. *G. duodenalis* genetic assemblages A and B were identified in calves and humans, increasing the number of domestic cattle harboring genotypes potentially capable of causing infections in humans (Winkworth et al., 2008). *Giardia* has been implicated as an etiological agent alone and in combination with other enteric pathogens in calf diarrhoea (Xiao and Hard, 1994; Olson et al., 1995; Handley et al., 1999).
TAXONOMY AND MORPHOLOGY

*Giardia* was seen under the microscope and first time discovered by Antony Van Leeuwenhoek during 1681 (Dobel, 1920). More than 50 species of *Giardia* have been described between 1920 and 1930 (Kulda and Nohynkova, 1996; Thompson *et al.*, 1990; Thompson, 2002). The taxonomic rationalization which paved the way for recognition of Giardia and based on the morphological similarity of the species proposed by Filice (1952) and zoonotic potential of *Giardia* was recognized by World Health Organization as a result of epidemiological data and cross infection experiments (WHO, 1979). The proposed species were *G. agilis* (amphibians), *G. muris* (mice), *G. duodenalis* (vertebrates) and G.psittaci (Birds).

*G. duodenalis* is a flagellated protozoan parasite that can infect many species in the animal kingdom including mammalian, avian and reptilian wildlife, domesticated animals and humans (Thompson, 2004; Applebee, *et al.*, 2005). The genus *Giardia*, family Hexamitidae and the order Diplomonadida. Majority of wild and domestic animals carry *Giardia* species which are morphologically indistinguishable from human isolates (Meloni *et al.*, 1995).

The recently PCR based procedures have enabled the characterization of previously inaccessible genotypes (Hopkin *et al.*, 1997; Monis *et al.*, 1998; Van keulen *et al.*, 1998; Hopkin *et al.*, 1999). It has been possible to elucidate the fundamental genetic division within G.duodenalis by using PCR based procedures and a number of laboratories in different countries have contributed to this work and a
consensus has emerged (Hopkin et al., 1997; Monis et al., 1998; Hopkin et al., 1999; Monis et al., 1999).

*Giardia* is a ubiquitous and well-known enteric parasite affecting humans and a wide range of domestic and wild mammals. It is one of the most common parasites of domestic dogs and dairy cattle and a frequently recognized waterborne pathogen. A single species has been recognized as causing disease in humans and most other mammals, molecular characterization of morphologically identical isolates from humans and numerous other species of mammals has confirmed the heterogeneity of this parasite and provided a basis for a clearer understanding of the taxonomy and zoonotic potential of *Giardia* (Thompson et al., 2000). *Giardia* cysts from calves (average measurements: 13.7 x 9.1 microns) and lambs (13.8 x 9.2 microns) were indistinguishable both morphologically and morphometrically (Taminelli and Eckert, 1989). *G. lamblia* from humans is morphologically indistinguishable from *Giardia* isolates originating from several species of domestic and wild mammals (Eckert, 1993).

*Giardia* is the intestinal tract parasite of the vertebrate host. The trophozoite of *Giardia* is bilaterally symmetrical, piriform to ellipsoidal in shape 12-15µm x 6-8 µm, with a convex dorsal surface and large adhesive or sucking desic on the ventral surface and contain two nuclei. They multiply asexually by longitudinal binary fission in upper intestine and encyst in posterior part of intestine. The cysts are smaller in size than trophozoites. Cysts are more resistant than trophozoites and can survive for weeks in cool, humid condition where trophozoites can not (Bingham et al., 1979; Sampson, et al., 2002).
PATHOPHYSIOLOGY

The most important clinical symptoms of giardiasis are diarrhea and malabsorption (Adam, 2000). The mechanisms of host responses are poorly understood, but a number of abnormalities have been observed after *Giardia* infection, including changes in sodium uptake and digestive enzyme activities the pathogenesis of *Giardia* is not clearly understood and the symptoms including acute and chronic diarrhoea, dehydration, and abdominal pain and weight loss are highly variable. (Thompson, et al., 1993).

Recent research has provided information on the diversity of processes that triggered by the infection with *Giardia*, which contribute to a complex pathophysiological process (Buret et al., 2002a). The epithelial permeability of host intestine is altered by the direct cytopathic effect induced by the product of the parasite (Buret et al., 2002b).

The increased epithelial permeability leads to an inflammatory response, digestive and absorptive changes, that correlate with the brush border injury and disaccharidase deficiencies (Scott et al., 2002). The increased intestinal permeability may result in uptake of luminal antigen which creates the allergic disorders; a complication also reported in humans infected with *Giardia* (Scott et al., 2002).

Pathology induced by *Giardia* is attributed to multifactor pathophysiological process that both parasite and host dependent is variable and the changes occur in host infected with Giardia depending on the nutritional and immune status of the host.(Thompson, 2000). The loss of absorptive surface area of the epithelial cell is
correlated with deficiency of the brush border enzyme, disaccharidase and enzyme activity (Buret et al., 1990). In young animals and birds that are nutritionally compromised or exposed to stress through overcrowding or low temperature, *Giardia* may be an additional factor that culminates in the expression of severe disease (McRoberts et al., 1996). The risk factors for clinical giardiasis may involve host and environmental factors as well as strain of the parasite.

**TRANSMISSION**

*Giardia* infections are transmitted by accidental ingestion of cyst in contaminated food or drinking water or directly in environments where hygiene level is low. The concept of interspecies transmission of *Giardia* has demonstrated that infection can be transmitted by the trophozoite form as well as by cysts (Hewlett et al., 1982). The recent transmission studies of *Giardia* suggest that in Switzerland domestic animals may serve as a reservoir of human *Giardia* infections and the cross-transmission between humans and animals is likely to occur (Stranden et al., 1990). *G. lamblia* from humans is morphologically indistinguishable from *Giardia* isolates from several species of domestic and wild mammals supports the hypothesis that zoonotic transmission of *Giardia* may occur (Eckert, 1993). *G. lamblia* genotypes A and B are widespread and possibly zoonotic (Van Keulen et al., 2002). There was evidence that calves with *Giardia* were more likely to develop scouring. Restricting cattle from surface water during periods of high shedding may reduce watershed contamination (McAllister et al., 2005; Tim et al., 2005). Studies on the post-weaned calves, amongst 9/14 farms identified Assemblage A Giardia. Therefore, calves should be considered as a potential source of human infectious cysts in the
environment, with some farms representing a much higher risk than others (Trout et al., 2005). A single species has been recognized as causing disease in humans and most other mammals, molecular characterization of morphologically identical isolates from humans and other species of mammals has confirmed the heterogeneity of Giardia and provided a basis for a clearer understanding of the taxonomy and zoonotic potential of Giardia (Thompson et al., 2000). Isolation of Giardia spp. in deer suggests that deer could be a potential source of infectious cysts for humans and cattle (Trout and Santin, 2003). A study was reported that giardiasis in domestic cattle has the potential to contaminate surface and ground waters through manure distribution on fields and pasture run-off (Applebee et al., 2003).

**LIFE CYCLE.**

*Giardia* undergoes two important types of differentiation: encystation is required for survival outside the host, and excystation is required for infection. *Giardia* takes advantage of host conditions throughout its descent through the human gastrointestinal tract (Gillin, et al., 1996). The cyst persists for months in cold fresh water, and ingestion of as few as 10 cysts can initiate infection. After entry into the small intestine and stimulation by intestinal pH, bicarbonate, and protease, the parasite emerges into two equivalent binucleate flagellated trophozoites. If they are carried downstream, they must encyst to survive outside the host (Adam, 2000).

**INCUBATION PERIOD**

The incubation period may depend the age of the host and infective dose. The Giardia infection can be caused by ingestion of 10-100 cysts or less numbers in
humans within 8-10 days of incubation period (Rendtoff et al., 1954). Following infections with 1.5-5.1 x 10^6 Giardia cysts After a prepatent period of 7-8 days all calves excreted high numbers of Giardia cysts for 60-112 days (Taminelli et al., 1989). A Swiss bovine Giardia cyst-isolate was transmitted to 4 Giardia-free maintained lambs which excreted Giardia cysts after prepatent periods of 10-21 days for 31-61 days (Taminelli et al., 1989).

The incubation period 8-10 days was observed in cattles and humans (Jokipi, et al., 1977; Kulda and Nohynkova, 1977). Two to four days old calves were experimentally infected with 2x10^6 Giardia cysts developed diarrhoea 3-5 days post infection which lasted for 18-26 days (Pavlasek, 1989). Giardia infections in calves were developed with intermittent diarrhea after a period of 5 days (Ruest, et al., 1995). The infection pattern of Giardia was similar in all breed of cattle (Handley, et al., 1999; Ralston, et al., 2003).

**EPIDEMIOLOGY**

Giardiasis in humans and farm animals, particularly in bovines has shown a widespread prevalence throughout the world (Deshpande and Shastri, 1981; Xiao and Hard, 1994). The bovine giardiasis was reported in first report from South Africa and attributed to Giardia bovis (Fanthum, 1921).

The prevalence of G. duodenalis in relation to age and season was investigated on a dairy farm in Netherlands. Shedding of G. duodenalis was found in all age categories but peaked in animals 4-5 months old (54.5%). Herd prevalence of shedding for G. duodenalis varied from 0.8% in June and 15.5% in February (Huetink
et al., 2001). The information based on the prevalence and intensity of shedding of *G. duodenalis* cysts in cattle grazing in the vicinity of the Bwindi Impenetrable National Park, Uganda. The prevalence of giardiasis was 12% and cysts shedding intensity 110 to 270/g (Nizeyi et al., 2002).

A longitudinal study was undertaken to determine the spread of naturally acquired *Giardia* in dairy cattle at Western Australia. *Giardia* was the most prevalent in calves 4–7 weeks of age. *Giardia* was detected in 89% of sampled calves and calf-to-calf contact appears to be the most likely source of transmission (Becher et al., 2004).

*Giardia* infections are exceptionally high in young livestock particularly in calves at the first week of life (Thompson, 2000; Olson et al., 2004). Few studies have been reflected different infection rates among various age groups whereas others were not reported (1994; Mockbee, 1995; Olson et al., 1997). A high prevalence of *Giardia* infection has been reported between 4 and 12 weeks of age in calves as compared with adult cattle (Mockbee, 1995; O, Handley, et al., 1999).

The prevalence and risk factors were studied for *Giardia* infection in cows and calves during the calving season in western Canadian cow-calf herds. Prevalence for *Giardia* was much higher and detected in 17.0% (95/560) of the cow and 22.6% (137/605) of calf faecal samples. Data describing herd management practices, treatment and disease history, age, gender, breed and faecal consistency were gathered to assess potential risk factors associated with shedding (Gow and Waldner, 2006).
A molecular epidemiological study was conducted on 100 dairy (499 calves) and 50 beef (333 calves) farms in Belgium to estimate the prevalence of different *Giardia duodenalis* assemblages in calves younger than 10 weeks of age. The prevalence was 22% (95% Probability Interval (PI): 12-34%) in dairy calves and 45% (95% PI: 30-64%) in beef calves. Overall *G. duodenalis* assemblage E was 64% and assemblage A was 59% in the dairy calves (Geurden and Geldhof, 2008).

A quantitative and semi quantitative method was used to estimate the level of infection. The shedding patterns in 7-8 weeks old calves were observed for a period of 62 days (Gasser *et al.*, 1987). The sucrose concentration method revealed mean *Giardia* cysts shedding of $1.2 \times 10^3$ to $18 \times 10^3$ cysts/gram faeces (Deshpande and Shastri, 1981). The cysts shedding intensity was high in young calves and found from $4.1 \times 10^3$ to $3 \times 10^3$ cysts/gram of faeces. The samples were examined by zinc chloride ($\text{Zn Cl}_2$) floatation technique (Taminilli and Eckert, 1989).

Seasonal patterns of giardiasis in human and animal have been found different. (Hancoek, 1983). *Giardia* infection in humans was reported high in winter than summer. Human giardiasis in Saudi Arabia was more common (16%) in September. While infection was more in children as compared with adults (Kasim and Elhelu, 1983). There is little seasonal variation of *Giardia* infection in calves (Ruest, *et al.*, 1995). A low rate of *Giardia* infection was observed in calves during summer than spring (Xiao, *et al.*, 1993).

*Giardia* is a protozoan, flagellated parasites associated with diarrhoea in humans and its role as a possible cause of diarrhoea is unclear (Thompson *et al.*,}
1993). Some cross sectional studies attributed diarrhoea to *Giardia* infection in calves (Deshpande and Shastri, 1981). *Giardia* cysts were found in diarrhoeic stools but no detection was made to identify the enteropathogens (Ruest et al., 1995). Experimental infection of six week old lambs resulted in prolonged shedding of *Giardia* cysts and a significant increase in weight gain (1.96 Kg/ lambs) between infected and normal groups (Olson et al., 1995).

**DIAGNOSIS:**

Diagnosis of *Giardia* by conventional microscopic methods following the application of faecal concentration techniques, especially Zinc sulphate flotation and centrifugation remains a relatively reliable indicator of infection (Zajac et al., 2002). The detection of *Giardia* by microscopy or faecal ELISA is of limited epidemiological value. The development of the direct immunoflourence microscopy has improved the sensitivity of detecting and quantitating the feacal *Giardia* cysts and more accurate prevalence rate and cysts excretion intensities as compared to the conventional microscopy (Handley et al., 2002).

There is need for a sensitive and specific diagnostic procedure for detecting the etiological agent of infectious disease, with *Giardia*, molecular techniques particularly PCR based procedures have greater sensitivity and specificity than the conventional diagnosis that are reliant on microscopy or immunodiagnosis (McGlade et al., 2003). One of major advantage of PCR based techniques is the ease of interpretation which usually involves the visualization of small number of bands on a gel (McGlade et al., 2003). The prevalence of *Giardia duodenalis* genotypes was
determined in adult dairy cows. Specimens, cleaned of faecal debris and concentrated using CsCl density gradient centrifugation, were subjected to PCR and DNA sequence analysis. *G. duodenalis* infection, the prevalence was ranged from 3% to 64%, with an average prevalence of 27%. DNA sequence analysis of the 16S rRNA gene revealed the presence of Assemblage A (2%) and Assemblage E (25%) in *G. duodenalis* infection (Trout and Santin, 2007). Similarly, a survey of dogs in India found a 3% prevalence using microscopy compared to 20% with PCR (Traub *et al.*, 2004).

A greater awareness of parasite contamination of the environment and its impact on health has precipitated the development of better detection methods for water borne pathogens such as *Giardia* (Slifko *et al.*, 2000). In addition, molecular techniques can provide genotypic characterization of the parasites isolated from the faecal sample or water, thus providing valuable data for determining the source of contamination.
CHEMOTHERAPY

Nitroimidazoles and Benzimidazoles are effective antigiardial drugs for treating infection in human and companion animals (Barr et al., 1998; Zajac et al., 1998), have been used for the treatment of *Giardia* infection in calves (Deshpande and Shastri, 1981; Wilson et al., 1982; Xiao et al., 1993). Symptomatic improvement was achieved in all treated animals. No control animals were used and the parasitological cure was not evaluated. The adverse effect of the drugs was not described in the calves (Zimmer and Burrington, 1986; Zajac et al., 1992). Metronidazole and Furazolidone are mutagenic and easily cross the placenta, these drugs have not been approved for use in food animals in most developed countries. The alternative drugs are highly desirable for the treatment of *Giardia* infection in farm animals.

In livestock, benzimidazole (fenbendazole and albendazole) has shown to be effective in the elimination of *Giardia* from both housed and free range calves (Xiao et al., 1996; Handley et al., 2001). Treating calves with fenbendazole was also able to improve the mucosal microvillus structure and function within seven days of initiating treatment (Handley et al., 2001).

Recent studies suggested that albendazole and mebendazole have clinical efficacy against human giardiasis (Zhong et al., 1986; Wang et al., 1988; Al-Waili and Hasan et al., 1992). The claims for mebendazole were disputed (Al-Waili, 1990; Gascon and Corachan, 1990). Although chemotherapy may be highly effective in eliminating the infection, re-infection frequently occurs if the sources of
environmental contamination are not eliminated and the frequency of transmission is high. This applies to both human and animal infections (Thompson et al., 1998; Handley et al., 2001). The efficacies of albendazole and fenbendazole appeared to be effective in suppressing cyst excretion by *Giardia* infected calves (Xiao et al., 1996). Benzimidazoles are well known inhibitors of the polymerization of tubules to microtubules because the microtubules are the major components of the four pairs of flagella, the median body and the ventral desk of *Giardia* trophozoites. It is likely that these drugs exert their activities against *Giardia* through the inhibition of their attachment to the intestinal mucosa (Reynoldson et al., 1992; Morgan et al., 1993).
Materials and Methods

Chapter 3

MATERIALS AND METHODS

Duration of the study was one year i.e. from August 2007 to July 2008.

(1) A- Observational study:

Questionnaire was developed to gather information during each visit to the study farm/household dairies for the collection of faecal samples. The questionnaire is attached in annex. (1).

B- Animals examined:

A total of 2160 buffaloes, cattle and calves of various ages and of both sexes at Government dairy farm, Military dairy farm, Gawalla colonies and Household dairies at Lahore were randomly selected and examined for the prevalence of giardiasis.

These animals were randomly divided into three subgroups i.e. A, B and C. In group, A there were buffaloes, group B contained cows, while in group C there were calves. A total 2160 samples were studied i.e. 60 samples per month per group were screened for the presence of giardiasis.
Materials and Methods

RESEARCH WORK PLAN

<table>
<thead>
<tr>
<th>Place</th>
<th>Types of Animal</th>
<th>Months of the year</th>
<th>Total No of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>1      2      3    4    5    6    7    8    9    10   11   12</td>
<td></td>
</tr>
<tr>
<td>Military Farm</td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td>G. Dairy Farm</td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td>Gwalla Colonies</td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td>Household dairies</td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15     15     15   15    15    15    15    15    15    15   15   180</td>
<td></td>
</tr>
</tbody>
</table>

Grand Total: 2160.

Sources and collection of specimen

A total of 2160 faecal samples were collected randomly from the buffaloes, cows and calves of Government dairy farm, Military dairy farm, Gawala colonies and House hold dairy animals at Lahore during the period August 2007 to July 2008. The faeces were collected directly from rectum of each animal using disposable gloves in sterilized plastic bags were used for each animals and these were labaled information with regarding animal tag No, date of collection and name of the farm were furnished.
In addition attempts were made to collect fresh faeces or freshly passed faeces from the soil.

Human positive stools samples were collected from Mayo Hospital Lahore for studying of the zoonotic potential. After collection, these samples were stored in a cooler on ice and immediately transported to the postgraduate laboratories at the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore.

**Isolation of cyst**

*Giardia* cysts were isolated from the fecal samples using the method described by Handley et al (1999). The procedure was modified for the buffaloes, cows and calves samples to accommodate a faecal size of 20 grams. The fecal samples were mixed in 35 ml of phosphate buffered saline in a Petri dish after thorough mixing the slurry was filtered three times through cheesecloth and poured into 50ml centrifuge tubes and layered over 15ml of sucrose (specific gravity 1.13). The samples were centrifuged at 800 x g for 5 minutes. The upper layer and the interface layer were transferred through the pipette into another clean centrifuge tube and an equal amount of phosphate buffered saline added and recentrifuged at 800 x g for 5 minutes. The supernatant was discarded and the pellet was resuspended with PBS to a final volume of 1 ml in a clean ependorff tube.

**Preparation of Haematoxylin stain**

The stain was prepared on the following way,

Solution 1: - Haematoxylin = 10 gm

Absolute ethyl alcohol = 1 liter
Materials and Methods

The stain was placed in sunlight for 1 month.

Solution 2: -  
Ferrous ammonium sulphate = 10gm  
Ferric ammonium sulphate = 10gm  
HCl (conc.) = 10ml  
Distilled water = 1 liter

Solutions 1 and 2 were mixed in equal volumes as required.

Staining procedure

1. Fresh smear was prepared from the feacal pellet and air dried.
2. The slides were placed in 70% ethyl alcohol
3. The slides were placed in 70% ethyl alcohol containing iodine (5%) for 5 minutes.
4. Washed with distilled water
5. Immersed in iron Haematoxylin for 5 minutes.
6. Washed with distilled water.
7. Placed in 70% and 95% ethyl alcohol for 5 minutes each.
8. Placed in xylene for 5 minutes. The slides were mounted and observed under the microscope at 10x, 40x and 100x magnification.

Cyst examination and counting

The resuspended pellet (20 µl) was taken and placed on a slide, made smear and dried the slide warmer at 37°C for 10 minutes. The slides were stained with iron Haematoxylin stain using the standard procedure. One slide was examined for each sample. The slides were examined at 10x, 40x, and 100x. The slides were compared
with positive slide of *G. duodenalis* and parasites were identified on the basis of size and morphology (Lindsay, *et al.*, 2000).

The number of cysts per gram of faeces was calculated using the formula adopted by Handley *et al.*, (1999).

\[
N = \frac{C \times 50}{20}
\]

Where,

\(N\) = Cysts per gram faeces

\(C\) = Count in 20 µl x 50 (20µl is 1/50 of 1 ml) ÷ 20 (Cyst in 1 gm and 20gm)

If 20µl of supernatant is examined, then the count obtained needs to be multiplied by 50 to give number of cysts in 1ml. If this 1ml contains all cysts from 20 gm of faeces then this count needs to be divided by 20 to give the number of cyst per gram.

**DNA Extraction:**

The faecal samples were resuspended and subjected to DNA extraction by the method of Saliva, *et al.*, (1999) with slight modification. The tissue DNA extraction kit GFC Vivantis (company name) was used throughout the experiment. Ependorff were weighed by electric balance and averaged 0.97gm and to each was added the resuspended fecal material to half fill the ependorff and again weighed at an average 1.32gms for each sample. Samples were numbered before going into an ependorff and each was added 200ul TL buffer, 5 µl proteinase K and 200µl cell lysis enhancer from the tissue DNA extraction kit samples were incubated at 37° c for 24 hours. After incubation, 200 µl TB buffer was added and mixed thoroughly and placed in a
Materials and Methods

water bath at 65°C for 20 minutes. After this process, the samples were centrifuged at 12000 x g for 30 minutes and the supernatant was collected in a clean molecular grade ependorf and a further 200μl absolute ethanol mixed immediately and centrifuged at 12000 x g for 35 minutes. The pellet was resuspended in 750μl wash buffer and centrifuged at 12000 x g for 30 minutes. The supernatant was discarded and pellet washed 2-3 times until a clear pellet of DNA was obtained. The pellet was suspended in TBE buffer and stored at -80 °C.

The confirmation of the pellet DNA was made through electrophoresis. The samples were loaded in the wells of 1% agarose gel, 5μl bromophenol blue dye, mixed with 10μl DNA sample and similarly, 15μl sample loaded in each well of the gel. Current was applied at 100 volt and run for 40 minutes. The gel transferred to gel DOC and confirmed DNA samples were preserved at -80ºC for amplification.

Amplification of DNA (PCR) and detection

The following reagents were prepared before the PCR reaction.

1. dNTP- the stock dNTP available was 100mM, for the experiments, 2.5 mM strength dNTP was required. So, four dNTP vials were needed for this purpose. Thus 90μl deionized water was added from each vials of dNTP The net volume was 100μl, for each PCR reaction.

2. Primers- A 753 bp products of Giardia primers were amplified. The forward sequence of primers was G-7 (5AAGCCGACGACCTCACCCGCAGTGC-3) and
Materials and Methods

synth ID No. 265257 and reverse primers sequence was –G759 (5-GAGGCGGCCCTGGATCTTCGAGACGA-3) and synth ID No. 265259.

The forward primer in one vial stock was 55.1 nmol and pmol was needed for the reaction. So

\[
\frac{55.1 \text{ nmol} \times 10}{100} = 5.51 \mu l
\]

Added the 551µl TE buffer was added to each vial of the forward primers. Similarly, the reverse primer was 39.1 nmol and 391 ml of TE buffer was added. Both primers were kept frozen at -80°C and were then defrosted for the reaction.

3. 1X TBE buffer-

The 1XTBE buffer was prepared for gel tank. In the stock 50XTBE buffer was available and the required buffer in the experiment was 1xTBE buffer. The following formula was applied for the said purpose;

\[
C_1V_1 = C_2V_2
\]

\[
50XV_1 = 1X100
\]

\[
V_1 = 1X100/50 = 2
\]

So, 2 ml of 50x TBE buffer was dissolved in 98ml of distilled water and the net solution was 100ml 1XTBE buffer was obtained, which was used in PCR sample loading in gel electrophoresis.
4. 1% Agarose Gel

I gm of molecular grade agarose was mixed with 100ml distilled water in a sterilized flask. The bottle was incubated in the oven at 100ºC for 2 minutes. The flask was cooled in open air to 40-45ºC, then 5µl ethidium bromide solution was added to the flask and shaken gently. The solution was poured in the gel apparatus and the comb was fixed. The gel was stored in the refrigerator at 4ºC for setting. After I hour the gel was removed from the refrigerator and cut into two equal pieces. One piece was placed in the gel electrophoresis assembly and the other was wrapped in the aluminum foil to store in refrigerator at 4ºC for later use.

5. PCR Reaction Mixture-

The reaction mixture was prepared on the following ratio throughout the experiment.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR buffer</td>
<td>5µl</td>
</tr>
<tr>
<td>dNTPs</td>
<td>5µl</td>
</tr>
<tr>
<td>MgCl</td>
<td>5µl</td>
</tr>
<tr>
<td>Primer-F</td>
<td>2µl</td>
</tr>
<tr>
<td>Primer-R</td>
<td>2µl</td>
</tr>
<tr>
<td>DNA</td>
<td>5µl</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>H2O (Deionized water)</td>
<td>25.5 µl</td>
</tr>
<tr>
<td>Total mixture</td>
<td>50µl</td>
</tr>
</tbody>
</table>
Mixture was prepared for five reactions during the PCR and mixing was carried out in an ice jar in a safety cabinet. PCR was performed with the initial temperature 94°C and then set the programme file,

T= 65°C
G= 10

and run for 35 cycles. It was completed in two hours. The thermocycler was regulated to 4°C then PCR tubes were removed. Parafilm were taken out, and fixed on the table near the gel/apparatus, and took 5ul of bromophenol blue dye with micropipette and dropped them on the Parafilm and added 10µl PCR reaction mixture in each per tubes similarly, took 5ul &6ul DNA loaded in two separate well for optimization in gel. Each sample was loaded in a separate well and the current supplied to the gel electrophoresis apparatus and kept at voltage of 160 and stopped the supply after 45 minutes by observing the dye front. The gel was removed, and placed on transilluminator to observe the bands and for storage via the gel documentation apparatus.

Prevalence rate-

The prevalence rate of Giardiasis amongst the cows, buffaloes and calves was determined as per the formula described by Threshold et al. (1997).

\[
\% = \frac{\text{No. of infected animal at particular point}}{\text{Total no. of animal present at that particular point}} \times 100
\]

Similarly, the month, age, sex and seasonal prevalence of disease was also determined.
Materials and Methods

Meteorological Data

The meteorological data regarding temperature, humidity, and rainfall was collected from the meteorological station at Lahore. Monthly averages were calculated and their possible relationship to disease prevalence was determined.

Experiment Design for chemotherapy

One hundred and fifty cattle age ranging from of 2-3 years were used in the experiments. The management and hygiene measures were maintained at each farm. Experimental animals were dewormed. These animals were randomly divided into five group’s i.e. A, B, C, D and E, each group having 30 animals. Animals in group A were further sub-divided into three sub groups i.e. A1, A2, and A3, with ten animals in each sub-group. Animals in group A1, A2, and A3 were given Albendazole at 20, 15, and 10mg/kg body weight respectively by oral route. All animals were treated daily for five days. Animals in group B were sub-divided into three groups i.e. B1, B2 and B3, each having 10 animals and were respectively given metronidazole (flagyl) at 150, 100 and 50mg/kg, body weight orally. Similarly, all animals were treated daily for five days.

Animals (30) in group C were sub-divided into three groups i.e. C1, C2 and C3. Animals in group C1, C2, and C3 were respectively given mebendazole at 20, 10, and 7mg/kg, body weight orally for five days. The Giardia infection was given orally to four groups of animals i.e. A, B, C, and D at 200 cyst/gm of feces which was isolated during epidemiological study. After 10 days, the cyst /gm of feces of each group of animals was recorded and was assumed as zero days during launching the
chemotherapeutic trials. The three drugs at different dose level were administered orally for five consecutive days and the counting of cyst / gram of faeces was recorded to evaluate the efficacy of the drugs.

Animals (30) in group D were kept as positive control. *Giardia* infection was given at same rate i.e. 200 Cysts/gram of faeces. After 10 days cyst counting was started and continued up to the end of experiment. The counts were on the 6\textsuperscript{th}, 13\textsuperscript{th}, 20\textsuperscript{th} and 27\textsuperscript{th} day post treatment. Animals (30) in-group E served as negative controls.

**CYST COUNTING**

Faecal samples were collected directly from the rectum of animals and kept in labeled plastic bags, which were placed in an ice cold container and transported to postgraduate laboratory of Parasitology department, University of Veterinary and Animal Sciences, Lahore. The cysts were isolated and stained with Iron Haematoxylin and examined under a microscope at 10x, 40x and 100x. Cysts were counted by using the formula as adopted by Handley *et al.*, (1999) and were mentioned earlier.

**EFFICACY OF THE DRUGS**

The efficacy of the different dose levels of the drugs i.e. albendazole, metronidazole and mebendazole was assessed on the basis of shedding of cysts in faeces after treatment. The cysts were counted on days zero, 06, 13, 20 and 27 day post treatment. The cysts were isolated by floatation and sedimentation techniques from faeces, and smears were prepared from each animal and stained with iron
Materials and Methods

Haematoxylin the cysts were counted under the microscope at 10x, 40x and 100x magnification as described earlier. The alternate method for counting was that number of cysts seen in the stained slide was multiplied by 125. Thus achieving cyst/gram of faeces. Efficacy of the drugs was calculated as per the formula, described by Moskey and Hardwood, (1941).

\[
\frac{\text{(Total no. cysts before treatment – Total cysts after treatment)}}{\text{Total no. Cysts before treatment.}} \times 100
\]

**Effect of Treatment**

Side effects of drugs, if any, were also recorded i.e. shivering, sweating, salivation and diarrhoea. Effects of treatment on body weight and feed intake were also noted in randomly selected animals from each group/sub group.

**Haematological data**

The blood samples were obtained from each group of animals before *Giardia* infection by sterilized vacutainer having a capacity of 5ml. Blood samples were mixed through a haem shaker before processing then vacutainer having 5ml blood was subjected to haemanalyser, which sucked 1 μl of blood and reports were obtained and recorded. The Hb and leukocyte counts were also ensured.

After the *Giardia* infection and appearance of the cysts in faeces. Blood was obtained in a similar way and processed the blood through haemanalyser, and thus a complete haemogram was obtained. The leukocyte count particularly eosinophils was carried out on blood films stained by Giemsa for establishing the percentage of each cell types (Ferrera et al., 1981).
The comparison of haemoglobin levels and leukocyte counts with *Giardia* infected cattle and normal cattle were made.

**Physiological & clinical examination**

The clinical examination was carried out in animals, which were selected for the chemotherapeutic trails before and after the *Giardia* infection. The weight of each animal was recorded on alternate days and pulse rate, rectal temperature, heart rate, respiration rate palpation of lymph node and abdomen, were also recorded.

The consistency of the faeces was also recorded throughout the experiment. All these parameters were recorded regularly before and after the *Giardia* infection as well as after the treatment.

**Protein Profile of Giardia Isolates using SDS-PAGE**

For protein analysis of *Giardia* isolated from the bovine faeces sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method was used (Laemmli, 1970).

**Reagent preparation**

**Acrylamide- Bisacrylamide Monomer Solution (30%)**

An amount of 29.2g of acrylamide (Sigma, USA) and 0.8g of bisacrylamide (Sigma, USA) was weighed and dissolved in 50 ml deionized distilled water separately, using a magnetic stirrer and stored both of the solutions, combined in a brown bottle wrapped with aluminum foil at 4°C in a refrigerator to avoid any degradation of solution.
Materials and Methods

1. **Resolving gel buffer (pH 8.8)**

An amount of 63.6 grams of Tris base (Hydroxymethyl amino methane, Sigma, USA) was measured and dissolved in 200 ml with deionized water. The pH was adjusted to 8.8 and the buffer was stored at 4°C in a refrigerator.

2. **Stacking gel buffer (pH 6.8)**

Six grams of Tris base (Tris Hydroxymethyl amino methane, Sigma, USA) was weighed in a cylinder and dissolved in 100 ml with deionized water on a magnetic stirrer. The pH was adjusted to 6.8 with hydrochloric acid (1N) or sodium hydroxide (1N) solutions. The solution was stored at 4°C in a refrigerator.

3. **SDS 10%**

Ten grams of SDS (Sodium Dodecyl Sulphate, Sigma, USA) was weighed in a beaker and added with 80 ml of deionized water. The solution was shaken gently on the magnetic stirrer with low speed to avoid the bubble formation. After dissolving SDS completely, the volume was made to 100 ml and the solution stored at room temperature.

4. **Bromophenol Blue (1%)**

One gram of bromophenol blue (Sigma, U.S.A.) was dissolved in 100 ml deionized water. The dye thus prepared was stored at room temperature in a dark bottle.

5. **Ammonium persulphate (APS; 10%)**
Ammonium persulphate (10% solution) was freshly prepared by dissolving 1 ml ammonium persulphate (Sigma, U.S.A.) in 10 ml deionized water.

6. **Electrophoresis (tris-glycine-SDS) buffer**

Three grams of Tris base (tris hydroxymethyl amino methane, Sigma, U.S.A.); 14.4 grams of glycine (Mack, Germany) and 1 gram of SDS (Sigma, U.S.A.) were dissolved in 500 ml deionized water in a beaker. The final volume was made up to 1 liter with deionized water. The buffer was stored at 4°C.

7. **Loading Dye (2x Denaturing Buffer)**

An amount of 1.54 gram of dithiothreitol (DTT) or 1.5 ml of β2-mercaptoethanol (Sigma, U.S.A.) and 2 gram of SDS were dissolved in 8 ml of Tris 1M (pH 6.8). The solution was shaken on the magnetic stirrer with low speed to avoid bubble formation. Then 10 ml of glycerol and 20 mg of bromophenol blue dye was added to the solution. After mixing, the buffer was stored in a dark bottle at 4°C in a refrigerator.

8. **Staining Solution**

An amount of 0.125 grams of Coomassie Blue R 250 (Sigma, U.S.A.) was weighed in a stopper flask followed by 112.5 ml of methanol, 22.5 ml of acetic acid and were dissolved in 112.5 ml of deionized water and stored in a dark bottle at room temperature to avoid the chances of denaturation.
9. Destaining Solution

Fifty ml of methanol and 70 ml of acetic acid was mixed in 100 ml of distilled water and stored at room temperature until used.

Preparation of Protein Samples for Electrophoresis

Extracted total protein samples of Giardia isolates were diluted with 2x sample buffer (loading dye) with the ratio of 1:1 to prepare working solution for loading on the gel. For this purpose, 100µl of protein samples was added to a separate eppendorff and then 100µl of loading dye was added to each of these tubes. The samples were mixed well and heated in a boiling water bath for 3-5 minutes to denature the proteins. Then 30µl of each of these samples loaded on the gel.

Preparation of Protein Marker for loading

Lyophilized mixture of 9 protein (Invitrogen, USA) including myosin 250 kDa, bovine serum albumen 98 kDa, glutamine dehydrogenase 64 kDa, alcohol dehydrogenase 50 kDa, carbonic anhydrase 36 kDa, myoglobin 30 kDa, lysozyme 16 kDa, aprotinin 6 kDa and insulin b chain 4 kDa, were reconstituted in Tris-glycine-SDS buffer to achieve the final concentration of 1µg/µl and stored at 4°C.

Sonication of Giardia Cysts

Take 1ml of resuspended pellet of purified cysts with concentrations of 200-400 cysts/ml was prepared. The ependroff containing 1ml purified cysts were sonicated (Branson Sonifier250) at 20 kHz/second in a ice jar for two minutes. Then
the sonicated solution was centrifuged at 500 x g for 10 minutes. The supernatant were separated by pipette into a clean ependorff and stored at 4°C for further use while the sediment was discarded.

**Determination of Protein Concentration**

Protein samples were extracted by sonication as described by Jordi et al (1994) Clear supernatants, stored at 4°C were used for total protein estimation of the samples. Protein concentrations of Giardia isolates were determined by the UV Spectrophotometer (Shamardzo Japan) at 280nm wave length using bovine serum albumen (BSA) as standard according to the method described by Whitaker, (1980) For this purpose, a standard curve was created and the solution of BSA was prepared at the concentration of 300mg/3ml as a stock solution and then used at of 100mg/ml.

This stock was diluted to concentrations of 10, 20, 40, 60 and 80 mg/ml. A blank of solvent alone was also prepared The spectrophotometer was allowed to warm up for 30 minutes and then set 280nm wavelength with the solvent blank. The spectrophotometer was set to zero and the absorbance measure 0.069, 0.074, 0.079, 0.083, 0.085 and 0.088. The concentration of samples was also determined by the spectrophotometer at the blank 0 and absorbance at 280 nm wavelength.

<table>
<thead>
<tr>
<th>Concentration Cysts/ml</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cysts.</td>
<td>200</td>
<td>250</td>
<td>300</td>
<td>350</td>
<td>400</td>
</tr>
<tr>
<td>OD value</td>
<td>0.110</td>
<td>0.112</td>
<td>0.114</td>
<td>0.116</td>
<td>0.118</td>
</tr>
</tbody>
</table>
**SDS GEL Preparation**

A typical gel of 10% acrylamide composition was used to separate polypeptides to analyze the protein profile of the isolates. Casting stand V10.SET Scie-plas (Max Fill) U.K was used to prepare the mini-slab gels. Two clean plates with two Teflon spacers were assembled as a single cassette. The cassette was stacked upright in the stand with the bottom of the cassettes tight to the bottom of the stand, using rubber pads to seal and to make a water-tight chamber. Using a well-former (comb) as a template, a fill line about a centimeter below the bottom of the comb, was marked for the height of the first (separating) gel solution.

**Recipe for Separating Gel**

The total volume between the plates of cassettes, for separating gels was 9 ml, so 10 ml of gel-mix was prepared.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer stock</td>
<td>3.33 ml</td>
</tr>
<tr>
<td>Resolving gel buffer</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>Water</td>
<td>4 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>10% Ammonium persulphate</td>
<td>50 µl</td>
</tr>
<tr>
<td>TEMED</td>
<td>4 µl</td>
</tr>
</tbody>
</table>

The acrylamide monomer mixture, resolving gel buffer and 10% SDS were brought to final volumes, using distilled water to prepare a 10% separating gel. Rapid polymerization was achieved by adding freshly prepared 10% ammonium persulphate (APS) to the mix followed by N, N, N', N'-tetramethylethylenediamine
(TEMED). After swirling the mix, the solution was poured into the space occupied by the cassettes. Immediately after pouring the gel mix, the water-saturated butanol was overlaid to an additional height of 0.5cm. The butanol was added to produce a smooth, completely leveled surface on top of the separating gel, so that bands were straight and uniform. Polymerization was confirmed by pulling some of the remaining gel mix into the pipette, allowing it to stand and checked after 10 minutes. When squeezing the bulb can no longer expel the gel mix, the separating gel was assumed to be set.

**Stacking gel preparation**

Four ml of stacking gel was mixed as sufficient for one cassette. However, for the sake of accuracy it was preferable to make five ml giving allowance for any wastage.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer stock</td>
<td>0.665 ml</td>
</tr>
<tr>
<td>Staking gel buffer</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Water</td>
<td>3.05 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>50 µl</td>
</tr>
<tr>
<td>10% Ammonium persulphate</td>
<td>25 µl</td>
</tr>
<tr>
<td>TEMED</td>
<td>2.5 µl</td>
</tr>
</tbody>
</table>

Before adding the final two components, which start polymerization, the butanol was poured off the separating gels into a sink either with running tap water or removed from the surface with a pipette. A 4 % of stacking gel was poured on the resolving gel. The comb was inserted at once taking care not to catch bubbles under the teeth of the comb.
Assembling, Loading and Running Gels

The comb was removed from the gel with care before filling the upper buffer compartment. The upper and lower buffer compartments were filled with an electrode buffer (running buffer). An amount of 30 µl of denatured protein sample was loaded per well. A typical mini-gel well holds 30µl easily and perhaps 35 µl or little more if the well was in good shape. The anode (+ electrode) was connected to the bottom chamber and the cathode to the top chamber. The negatively charged proteins were move toward the anode. Gels were usually run at a voltage 4.54V/cm and the tracking dye was run to the bottom of the gel without overheating.

Disassembly

When the dye front was nearly at the bottom of the gel, the power was turned off and cables were removed. Gel was removed from the cassette taking care not to tear the gel. The plates were separated and the gel was dropped into a staining dish containing deionized water. After a quick rinse, the water was poured off and stain was added.

Staining protein gels

Coomassie Blue dye (0.1%) in 50% methanol and 10% glacial acetic acid was used to stain for detecting protein bands acrylamide. The gel was usually stained for 2-3 hour with agitation. The agitation circulates the dye, facilitating penetration and ensuring uniformity of staining.
Materials and Methods

Destaining protein gels

Destaining with acetic acid and methanol washed out excess dye. It was most efficient to destain in two steps, starting with 50% methanol, 10% acetic acid for 1-2 hours, then using 7% methanol, 10% acetic acid to finish. The first solution shrinks the gel, squeezing out much of the liquid component, which needs to be followed with gel swelling and clearing by the second solution in order to regain the normal texture of the gel. Ideally, properly stained and destained gels display a pattern of blue protein bands against a clear background.

Photography and Image Sorting

After destaining, the gel was photographed and its image was saved on a photographic film as a permanent record.

Calculation of molecular weights of *Giardia* proteins

**Relative mobility (Rf)** is the distance, migrated by a band, divided by the distance, covered by the dye front. Relative mobility is useful because it can be used to compare the migration of a protein from gel to gel, regardless of the physical length of the gel or duration of electrophoresis.

Relative molecular weight (MW) of each protein fraction was determined by plotting a standard curve of relative mobility of a standard protein band verses log_{10} MW of ladder (Pre-stained Marker). The molecular weights of the protein bands of the sample were read off the log MW of the standard curve. To reach the actual
molecular weights of protein bands, the log values were converted into corresponding antilog figures.

Relative mobility was calculated by the following formula:

\[
\text{Relative mobility (R}_f\text{ value) = \frac{\text{Distance covered by a protein band}}{\text{Total distance of the dye front}}}
\]

Statistical Analysis of the data;

Data was analyzed statistically by using the computer software, SPSS 16 and Duncun test, Tukey test and chi square test and ANOVA. P<0.05 was considered significant.
RESULTS

A total of 2160 fecal samples were collected from buffaloes, cattle and calves located in the Military dairy farm, Gawala colonies, Government dairy farm and Household animals at Lahore during the period August 2007-July 2008. The seasonal and climatic condition of the above cited farm was the same because of their location in same jurisdiction of municipality, however differences were observed in managemental and hygienic condition of the four areas. The prevalence (%) of *Giardia* infection in each category of animals was determined.

1. *Giardiasis in Calves*

1.1 Overall Prevalence (%)

An overall prevalence (%) of *Giardiasis* in calves was found to be 50.27% (362/720) in Lahore from August 2007- July 2008, It was significantly higher (P<0.05) when analyzed by Tukey’s test (Table. 1, Fig. 1.1).

1.2 Monthly Prevalence (%)

The data was analyzed monthly for the purpose of tracing out the specific period of the year with the highest prevalence. The highest prevalence was recorded in August (65%), while the lowest prevalence was recorded in December (30%). The studies in the different farm/ household areas reflect the differences and revealed significantly higher (P<0.05), prevalence from August, September and October as compared to the lowest prevalence in December (Table.1 Fig. 1.2, Fig. 1.3 and Fig. 1.4).
1.3 Area wise prevalence (%) in degree of prevalence

The highest degree of prevalence was recorded in Government Dairy Farm (63.33 %) followed by Gawala colonies (55%), Military Dairy Farm (43.33 %) and the lowest in Household Dairies (34.44%). The prevalence was significantly higher (P<0.05) when analyzed statistically (Table.1, Fig. 1.1).

1.4 Seasonal Prevalence (%)

The highest prevalence was recorded in Autumn (72.5%), followed by spring (60.83%), Summer (59.16%) and the lowest in winter season (34.16 %). Prevalence was significantly higher (P<0.05) during autumn, spring and summer as compared to winter (Table.1, Fig.1.6).

1.5 by sex Prevalence (%)

The overall prevalence in Calves showed that females (56.74%) were more susceptible to Giardia infection than males (35.18%). A statistical (P<0.05) difference was noted between the females and males analyzed. (Table1, Fig.1.7).

1.6 by age Prevalence (%)

It was evident from the table.1 and fig.1.7 that prevalence was higher in calves (71.52 %) of age group (0-6 months) than over 6 months (7-12months of age) (36.11%) (Fig 1.5).
### Table No. 1: OVERALL PREVALENCE (%) OF GIARDIASIS IN CALVES AT LAHORE FROM AUGUST -07 TO JUL-08

<table>
<thead>
<tr>
<th>Factors</th>
<th>Military D. Farm</th>
<th>Gawala Colony</th>
<th>G. Dairy Farm</th>
<th>Household Dairies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected / Total</td>
<td>Prevalence (%)</td>
<td>Infected / Total</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>Aug-07</td>
<td>9/15</td>
<td>60%♣</td>
<td>11/15</td>
<td>73%♣</td>
</tr>
<tr>
<td>Sep-07</td>
<td>8/15</td>
<td>53.33%♣</td>
<td>10/15</td>
<td>66.66%♣</td>
</tr>
<tr>
<td>Oct-07</td>
<td>8/15</td>
<td>53.33%♣</td>
<td>11/15</td>
<td>73%♣</td>
</tr>
<tr>
<td>Nov-07</td>
<td>4/15</td>
<td>26.66%♣</td>
<td>6/15</td>
<td>40%♣</td>
</tr>
<tr>
<td>Dec-07</td>
<td>3/15</td>
<td>20%♣</td>
<td>5/15</td>
<td>33.33%♣</td>
</tr>
<tr>
<td>Jan-08</td>
<td>5/15</td>
<td>33.33%♣</td>
<td>6/15</td>
<td>40%♣</td>
</tr>
<tr>
<td>Feb-08</td>
<td>5/15</td>
<td>33.33%♣</td>
<td>6/15</td>
<td>40%♣</td>
</tr>
<tr>
<td>Mar-08</td>
<td>7/15</td>
<td>46.66%♣</td>
<td>10/15</td>
<td>66.66%♣</td>
</tr>
<tr>
<td>Apr-08</td>
<td>9/15</td>
<td>60%♣</td>
<td>10/15</td>
<td>66.66%♣</td>
</tr>
<tr>
<td>May-08</td>
<td>7/15</td>
<td>46.66%♣</td>
<td>9/15</td>
<td>60%♣</td>
</tr>
<tr>
<td>Jun-08</td>
<td>6/15</td>
<td>40%♣</td>
<td>7/15</td>
<td>46.66%♣</td>
</tr>
<tr>
<td>Jul-08</td>
<td>7/15</td>
<td>46.66%♣</td>
<td>8/15</td>
<td>53.33%♣</td>
</tr>
<tr>
<td>Spring</td>
<td>16/30</td>
<td>53.33%♣</td>
<td>21/30</td>
<td>66.66%♣</td>
</tr>
<tr>
<td>Summer</td>
<td>29/60</td>
<td>48.33%♣</td>
<td>35/60</td>
<td>58.33%♣</td>
</tr>
<tr>
<td>Autumn</td>
<td>16/30</td>
<td>53.33%♣</td>
<td>21/30</td>
<td>70%♣</td>
</tr>
<tr>
<td>Winter</td>
<td>17/60</td>
<td>28.33%♣</td>
<td>23/60</td>
<td>38.33%♣</td>
</tr>
<tr>
<td>0-6 month</td>
<td>54/72</td>
<td>75%♣</td>
<td>56/72</td>
<td>77.77%♣</td>
</tr>
<tr>
<td>6-12 month</td>
<td>24/108</td>
<td>22.22%</td>
<td>43/108</td>
<td>39.81%♣</td>
</tr>
<tr>
<td>Male</td>
<td>16/54</td>
<td>29.62%</td>
<td>23/54</td>
<td>42.29%♣</td>
</tr>
<tr>
<td>Female</td>
<td>61/126</td>
<td>49.20%</td>
<td>77/126</td>
<td>60.31%♣</td>
</tr>
<tr>
<td>Total</td>
<td>78/180</td>
<td>43.33%%</td>
<td>99/180</td>
<td>55%♣</td>
</tr>
</tbody>
</table>

Statistical analysis: Tukey’s test
Significance (P< 0.05) ♣ and
Non significant (P>0.05) ♠ and for season applied the chi square test

Summer: May, June, July and August
Winter: Nov., Dec., January and February
Spring: March and April
Autumn: September and October

* It is clear that season in Pakistan occur in different months is differ to the season in other parts of the world.
Results

Prevalence of Giardiasis in Calves at Lahore from Aug 2007 to July 2008

Fig. 1.1 Statistical analysis Tukey’s test; significant (P< 0.05).
1.6 **Prevalence by area and month (%)**

The highest prevalence (%) was recorded during August in all the areas *i.e.* 73.3%, 86.66%, 93.33 % and 60 % at Military dairy Farm, Gawala Colonies, Govt. Dairy Farm and Household dairies respectively, while the lowest during December *i.e.* 20 %, 33.33 %, 46.66 % and 20 % respectively. When data was analyzed it was significantly higher (P<0.05).

1.7 **Prevalence by area and season (%)**

When the data arranged by season it was observed that all the areas showed insignificant difference (P>0.05). The highest prevalence was noted during autumn, followed by spring, then summer and the lowest (30%) during winter season (Fig.1.6)

1.5 **Prevalence by area and sex (%)**

During the study period, it was observed that the prevalence was highest in females at Government dairy farm, Gawala dairy colonies, Military dairy farm and Household dairy animals *i.e.* 77.77%, 61.11%, 48.41% and 39.68% respectively, as females were more susceptible than males. In males, prevalence was 46.29 %, 42.59%, 29.62% and 22.22% respectively .A statistically insignificant difference (P >0.05) was noted.

1.6 **Prevalence by area and age**

It was noted that prevalence (%) was higher in calves of 0-6 months in all areas than those between 7-12 months. Statistical analysis revealed nonsignificant difference (P> 0.05) among both age groups of animals (Fig.1.5).
Prevalence of *Giardiasis* in Calves at Gawala Colonies, Lahore

**Fig. 1.2**

Prevalence of *Giardiasis* in Calves at Govt. Dairy Farm Lahore

**Fig. 1.3**
Prevalence of Giardiasis in Calves at Household dairies, Lahore

Fig. 1.4
Table No.2  BY SEASON, SEX AND AGE PREVALENCE % GIARDIASIS OF CALVES IN DIFFERENT FORM/AREAS OF LAHORE FROM AUGUST 2007-JULY 2008.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Military D. Farm</th>
<th>Gwala Colony</th>
<th>G.D. Farm</th>
<th>House H. Dairies</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected/Total %</td>
<td>Infected/Total %</td>
<td>Infected/Total %</td>
<td>Infected/Total %</td>
<td></td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>16/30 (53.33)</td>
<td>20/30 (66.66)</td>
<td>24/30 (80)</td>
<td>13/30 (43.33)</td>
<td>73/120</td>
</tr>
<tr>
<td>Summer</td>
<td>29/60 (48.33)</td>
<td>35/60 (58.33)</td>
<td>45/60 (75)</td>
<td>24/60 (40)</td>
<td>133/240</td>
</tr>
<tr>
<td>Autumn</td>
<td>16/30 (53.33)</td>
<td>21/30 (70)</td>
<td>24/30 (80)</td>
<td>13/30 (43.33)</td>
<td>74/120</td>
</tr>
<tr>
<td>Winter</td>
<td>17/60 (28.33)</td>
<td>23/60 (38.33)</td>
<td>30/60 (50)</td>
<td>12/60 (34.16)</td>
<td>82/240</td>
</tr>
<tr>
<td>Male</td>
<td>16/54 (29.62)</td>
<td>23/54 (42.29)</td>
<td>25/54 (46.29)</td>
<td>12/54 (22.22)</td>
<td>76/216</td>
</tr>
<tr>
<td>Female</td>
<td>62/126 (49.20)</td>
<td>76/126 (60.31)</td>
<td>98/126 (77.77)</td>
<td>50/126 (39.68)</td>
<td>286/504</td>
</tr>
<tr>
<td>2-3 years</td>
<td>54/72 (75)</td>
<td>56/72 (77.77)</td>
<td>60/72 (83.33)</td>
<td>36/72 (50)</td>
<td>206/288</td>
</tr>
<tr>
<td>3-7 years</td>
<td>24/108 (22.22)</td>
<td>43/108 (39.81)</td>
<td>63/108 (58.38)</td>
<td>26/108 (24.07)</td>
<td>156/432</td>
</tr>
</tbody>
</table>

**Notes:**
- GIARDIASIS: Trichomonas foetus, a parasite that can cause gastrointestinal disease in calves.
- The table details the prevalence of GIARDIASIS during different seasons, sexes, and ages.
- The data is from August 2007 to July 2008 in different areas of Lahore.
Results

Fig. 1.5

Fig. 1.6

Summer: May, June, July and August
Winter: Nov., Dec., January and February
Spring: March and April
Autumn: September and October
Results

Prevalence of Giardiasis by sex group in Calves at Lahore

% age

Area

Military D.Farm  Gwala Colony  G.D.Fam  House H. Dairies

Male  Female

Fig. 1.7
Results

2. *Giardiasis in Cattle*

2.6 **Overall Prevalence (%)**

The overall prevalence (%) of *Giardiasis* in cattle was found to be 28.05% (202/720) at Lahore from August 2007-July 2008. A statistically significant difference (P< 0.05) was noted when data was analyzed by Duncan test (Table 3, Fig.2.1).

2.2 **Monthly Prevalence (%)**

The highest (35%) month wise prevalence was recorded during August (35%), while, the lowest (21%) during January (21%). A statistical analysis gave significant differences P<0.05 (Table 3, Fig.2.1).

2.3 **Area wise Prevalence (%) in degree of prevalence**

The highest (41.67 %) prevalence was recorded at Government Dairy Farm followed by Gawala colonies (32.78%), then Military Dairy Farm (22.78%) and whereas, the lowest (15%) at Household Dairies (15%). The prevalence difference was P>0.05 when analyzed statistically (Table 3, Fig. 2.2).

2.4 **Seasonal Prevalence (%)**

Overall the highest (31.16%) prevalence was reported during autumn (September and October), followed by summer, may, June, July and august, (30.41%), then spring, March and April, (29.16%) while the lowest in winter, November, December, January and February, (23.33%). A statistically non-significant difference (P>0.05) was noted, when data was analyzed by Duncan test (Table 3, Fig. 2.4).
2.5 Sex Wise Prevalence (%)

The overall prevalence in Cattle showed that females (34.62%) were more susceptible to *Giardia* infection than males (18.75%). A statistically non-significant (P>0.05) difference was noted between the prevalence of females and males cattle (Table 3, Fig. 2.6).

2.6 Age Wise Prevalence (%)

From the table 3 it is revealed that overall prevalence in younger animals was higher (38.88%) than in older animals *i.e.* above three years.
Table No.3  Month Wise Prevalence of *Giardiasis* in Cattle at Lahore From August 2007 to July 2008.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Month</th>
<th>Parameter</th>
<th>Military form</th>
<th>Gawala Colonies</th>
<th>G. Dairy Form</th>
<th>Household Dairies</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aug. 07</td>
<td>No. Infected</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>46.67</td>
<td>53.34</td>
<td>13.34</td>
<td>35%</td>
</tr>
<tr>
<td>2</td>
<td>Sept. 07</td>
<td>No. Infected</td>
<td>4</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>40</td>
<td>46.67</td>
<td>20</td>
<td>33.34%</td>
</tr>
<tr>
<td>3</td>
<td>Oct. 07</td>
<td>No. Infected</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>33.34</td>
<td>40</td>
<td>20</td>
<td>30%</td>
</tr>
<tr>
<td>4</td>
<td>Nov. 07</td>
<td>No. Infected</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>20</td>
<td>26.67</td>
<td>40</td>
<td>13.34</td>
<td>25%</td>
</tr>
<tr>
<td>5</td>
<td>Dec.07</td>
<td>No. Infected</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>20</td>
<td>33.34</td>
<td>20</td>
<td>23.34%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Jan. 08</td>
<td>No. Infected</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>13.34</td>
<td>26.67</td>
<td>40</td>
<td>6.67</td>
<td>21.67%</td>
</tr>
<tr>
<td>7</td>
<td>Feb. 08</td>
<td>No. Infected</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>20</td>
<td>26.67</td>
<td>33.34</td>
<td>13.34</td>
<td>23.34%</td>
</tr>
<tr>
<td>8</td>
<td>Mar. 08</td>
<td>No. Infected</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>33.34</td>
<td>40</td>
<td>13.34</td>
<td>28.34%</td>
</tr>
<tr>
<td>9</td>
<td>Apr.08</td>
<td>No. Infected</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>33.34</td>
<td>40</td>
<td>18</td>
<td>30%</td>
</tr>
<tr>
<td>10</td>
<td>May.08</td>
<td>No. Infected</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>20</td>
<td>40</td>
<td>46.67</td>
<td>6.67</td>
<td>28.34%</td>
</tr>
<tr>
<td>11</td>
<td>June. 08</td>
<td>No. Infected</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>26.67</td>
<td>26.67</td>
<td>33.34</td>
<td>20</td>
<td>26.67%</td>
</tr>
<tr>
<td>12</td>
<td>July.08</td>
<td>No. Infected</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>20</td>
<td>40</td>
<td>53.34</td>
<td>15.34</td>
<td>31.67%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td>41</td>
<td>59</td>
<td>75</td>
<td>27</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%age</td>
<td>22.78</td>
<td>32.78</td>
<td>41.67</td>
<td>15</td>
<td>28.05%</td>
</tr>
</tbody>
</table>

Statistical analysis; Duncan test significant (P<0.05) in month wise
Non significant (P>0.05) in-group wise.
Results

Prevalence of *Giardiasis* in Cattle at Lahore from August 2007 to July 2008

![Graph showing prevalence of Giardiasis in cattle by month and area.]

**Fig. 2.1**

Prevalence of Giardiasis in Cattle at Lahore

![Bar chart showing prevalence by area.]

**Fig. 2.2**

2.8 Prevalence by area and month (%)

The highest prevalence (%) was recorded during August in all the areas *i.e.* 53.34%, 46.67%, 26.67% and 13.34% at Govt. dairy Farm, Gawala dairy Colonies,
Military dairy Farm and Household dairies animals respectively, while the lowest prevalence was recorded during January i.e. 40%, 26.67%, 13.34% and 6.67%. The significant difference (P<0.05) was noted when the data was analyzed statistically (Table 3, Fig. 2.1)

2.9 Prevalence by area and season (%)

Overall the highest (31.66%) season wise prevalence was noted during autumn, followed by summer (30.41%), then spring (29.16%) and the lowest was recorded during winter (23.33%). A statistically non-significant difference (P>0.05) was noted by Duncan test (Table 4, Fig.2.5).

2.10 Prevalence by area and sex

During the study, higher prevalence was noted in females at Govt. dairy farm, Gawala dairy colonies, Military dairy farm and Household dairy animals i.e. 43.12 %, 33.75 %, 23.75% and 16.25% respectively, whereas in males it was 30%, 25%, 15% and 5% respectively, in all locations. A statistically non-significant difference (P>0.05) was noted by Duncan test (Table 4, Fig. 2.6).

2.11 Prevalence by area and age

From the Table 4, it was evident that animals of age group 2-3 year had a higher prevalence than animals above 3years of age. A statistical analysis revealed that non-significant difference (P>0.05) was noted when data was analyzed by Duncan test (Table 4).
Table No. 4. Prevalence of *Giardiasis* by season, sex and age in cattle at Lahore from August 2007- July 2008

<table>
<thead>
<tr>
<th>Factors</th>
<th>Military D. Farm Infected/ Total %</th>
<th>Gawala Colony Infected/ Total %</th>
<th>G.D. Farm Infected/ Total %</th>
<th>Household Dairies Infected/ Total %</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected/ Total %</td>
<td>Infected/ Total %</td>
<td>Infected/ Total %</td>
<td>Infected/ Total %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(26.66)</td>
<td>(33.33)</td>
<td>(40)</td>
<td>(16.66)</td>
<td>29.16%</td>
</tr>
<tr>
<td>Season</td>
<td>Spring</td>
<td>8/30 (26.66)</td>
<td>10/30 (33.33)</td>
<td>12/30 (40)</td>
<td>5/30 (16.66)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>14/60 (23.34)</td>
<td>23/60 (38.34)</td>
<td>28/60 (46.67)</td>
<td>8/60 (13.34)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>8/30 (26.66)</td>
<td>11/30 (36.66)</td>
<td>13/30 (43.38)</td>
<td>6/30 (20)</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>11/60 (18.34)</td>
<td>15/60 (25)</td>
<td>22/60 (36.67)</td>
<td>8/60 (13.34)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>38/160 (23.75)</td>
<td>54/160 (33.75)</td>
<td>69/160 (43.15)</td>
<td>26/160 (16.25)</td>
</tr>
<tr>
<td></td>
<td>2-3 years</td>
<td>16/45 (35.55)</td>
<td>19/45 (42.22)</td>
<td>22/45 (48.88)</td>
<td>13/45 (28.88)</td>
</tr>
<tr>
<td></td>
<td>3-7 years</td>
<td>25/135 (18.51)</td>
<td>40/135 (29.62)</td>
<td>53/135 (39.25)</td>
<td>14/135 (10.25)</td>
</tr>
</tbody>
</table>

Statistical analysis; Duncan test,( P>0.05) in group wise.
Results

Monthly Prevalence of Giardiasis in Cattle at Lahore

Fig. 2.3

Overall Prevalence of Giardiasis in Cattle at Lahore

Fig. 2.4
Results

Prevalence of Giardiasis by Season in Cattle at Lahore

Fig. 2.5

Prevalence of Giardiasis by sex in Cattle at Lahore

Fig. 2.6

Summer: May, June, July and August
Winter: Nov., Dec., January and February
Spring: March and April
Autumn: September and October
3. *Giardiasis* in Buffaloes

3.1 **Overall Prevalence (%)**.

The overall prevalence (%) of *Giardiasis* in buffaloes was 26.11% (188/720) at Lahore from August 2007- July 2008. A non-significant difference (P>0.05) was noted when analyzed by Chi square test (Table 5, Fig. 3.1).

3.2 **Monthly Prevalence (%)**

The highest (46.66 %) month wise prevalence was noted during August, while lowest (6.66%) prevalence was noted during months of November and December. A non-significant difference (P>0.05) was noted when data was analyzed statistically by using Chi square test.

3.3 **Prevalence by Age (%)**

From the Table 6 and Fig. 3.1, it was evident that overall higher prevalence was noted in younger animals below 3 years (29%) and than above 3 years (25%). A non-significant difference (P>0.05) was noted by Duncan test.

3.4 **Prevalence by Season (%)**

Overall the highest (30.80%) prevalence was recorded during Autumn, followed by Summer (27.91%), then spring (25.83%) and the lowest (22.08%) during winter. A non-significant difference (P>0.05) was noted when data was analyzed by Duncan test (Table 5, Fig. 3.4).

3.5 **Prevalence by Sex (%)**

A higher prevalence was noted in females (26.66%) than males (20%), when data was analyzed statistically by Duncan test (P>0.05.) non-significant difference was observed.
Table No. 5 Overall prevalence (%) Of *Giardiasis* in Buffaloes at Lahore From August 07 to July 08.

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>Military D. Farm Prevalence</th>
<th>Gawala Coloney Prevalence</th>
<th>G. Dairy Farm Prevalence</th>
<th>Household Dairies Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected / Total</td>
<td>(%)</td>
<td>Infected / Total</td>
<td>(%)</td>
</tr>
<tr>
<td>Aug-07</td>
<td>5/15 33.33</td>
<td>6/15 40.00</td>
<td>7/15 46.66</td>
<td>3/15 20.00</td>
</tr>
<tr>
<td>Oct-07</td>
<td>3/15 20.00</td>
<td>6/15 40.00</td>
<td>7/15 46.66</td>
<td>2/15 13.33</td>
</tr>
<tr>
<td>Nov-07</td>
<td>2/15 13.33</td>
<td>5/15 33.33</td>
<td>6/15 40.00</td>
<td>1/15 6.66</td>
</tr>
<tr>
<td>Feb-08</td>
<td>3/15 20.00</td>
<td>3/15 20.00</td>
<td>6/15 40.00</td>
<td>2/15 13.33</td>
</tr>
<tr>
<td>Apr-08</td>
<td>3/15 20.00</td>
<td>4/15 26.66</td>
<td>6/15 40.00</td>
<td>3/15 20.00</td>
</tr>
<tr>
<td>May-08</td>
<td>3/15 20.00</td>
<td>4/15 26.66</td>
<td>6/15 40.00</td>
<td>2/15 13.33</td>
</tr>
<tr>
<td>Jul-08</td>
<td>3/15 20.00</td>
<td>5/15 33.33</td>
<td>6/15 40.00</td>
<td>2/15 13.33</td>
</tr>
<tr>
<td>Summer</td>
<td>15/60 25.00</td>
<td>19/60 31.66</td>
<td>25/60 41.66</td>
<td>8/30 13.33</td>
</tr>
<tr>
<td>Autumn</td>
<td>7/30 23.33</td>
<td>11/30 36.66</td>
<td>15/30 50.00</td>
<td>4/30 13.33</td>
</tr>
<tr>
<td>Winter</td>
<td>9/60 15.00</td>
<td>16/60 26.66</td>
<td>22/60 36.66</td>
<td>6/60 10.00</td>
</tr>
<tr>
<td>2-3 yrs</td>
<td>12/50 24.00</td>
<td>16/50 32.00</td>
<td>22/50 44.00</td>
<td>8/50 16.00</td>
</tr>
<tr>
<td>3-5yrs</td>
<td>26/130 20.00</td>
<td>38/130 29.23</td>
<td>51/130 39.23</td>
<td>15/130 11.30</td>
</tr>
<tr>
<td>Female</td>
<td>36/165 21.81</td>
<td>50/165 30.30</td>
<td>68/165 41.20</td>
<td>22/165 13.33</td>
</tr>
<tr>
<td>Overall</td>
<td>38/180 21.11</td>
<td>54/180 30.00</td>
<td>73/180 40.55</td>
<td>23/180 12.77</td>
</tr>
</tbody>
</table>

Statistical analysis; *Chi* square test. P >0.05 NS
Results

Month Wise Prevalence Of Giardiasis in buffalo,s in Lahore (From August 07-July 08).

Fig. 3.1
Results

Overall Prevalence of Giardiasis in buffaloes at Lahore from August 07- July 08

![Bar chart showing prevalence of Giardiasis across different sites in Lahore from August 2007 to July 2008.](image)

**Fig. 3.2**
3.7 Prevalence by area and month (%)

Highest prevalence (%) was recorded during August in all the study areas *i.e.* 46.66%, 40%, 33.33 % and 20% at Govt. dairy Farm, Gawala dairy Colonies, Military dairy Farm and Household dairies animals respectively, while the lowest was recorded during December *i.e.* 33.33%, 26.66%, 13.33% and 6.66% respectively. When the data was analyzed a non-significant difference was noted by Chi test.

3.8 Prevalence by area and season (%)

Overall the highest (30.80%) prevalence was noted during Autumn, followed by Summer (27.91%), then spring (25.83%) and the lowest (22.08%) during winter. A non-significant difference (P>0.05) was noted by using Chi square test. (Table 5).

3.9 Prevalence by area and sex (%)

During the study, it was observed that females were more frequently affected than males. The highest prevalence at Govt. dairy farm, Gawala colonies, Military farm and Household dairy *i.e.* 41.2%, 30.30%, 21.81% and 13.33% respectively, whereas, males it was 33.33%, 26.66%, 13.33% and 6.66% respectively. Statistically non significant difference (P>0.05) was noted by Chi square test. (Table 5, Fig. 3.3).

3.10 Prevalence by area and age (%)

It was noted that prevalence (%) was higher in animals below 3 years of age in all areas than above 3 years of age. Statistical analysis revealed that non significant difference (P>0.05) was noted by Chi square test. (Table 5).
Results

Fig. 3.3

Prevalence of Giardiasis by sex in buffaloes at Lahore from August 07-July 08

Fig. 3.4

Prevalence of Giardiasis by Season in buffaloes at Lahore

Summer: May, June, July and August
Winter: Nov., Dec., January and February
Spring: March and April
Autumn: September and October
4. RELATION OF GIARDIA INFECTION WITH STOOL CONSISTANCY

4.1 Calves

A total of 720 stool samples of calves below 1 year of age were collected and examined. Of these, Giardia cysts were found in 223 (30.97%) normal and 139 (19.30%) abnormal stool samples. This indicated that Giardia cysts were more commonly found in normal stools than abnormal stools. Statistical analysis of the data revealed that a non-significant difference (P>0.05) was noted by Chi square test (Table.6 Fig.4.1).

4.2 Cattle.

A total of 720 stool samples of cattle from 2-7 years were examined. Of these 127 (17.63%) were normal stools and 75 (10.41%) were abnormal stools. Giardia cysts were more commonly found in normal stools than abnormal stools. Statistically difference of (P>0.05) was noted by using the Chi square test (Table.6, Fig.4.1).

4.3 Buffaloes

A total of 720 stool samples of buffaloes from 2-5 years of age were examined. Amongst these 118 (16.38%) were normal stools and 70 (9.72%) abnormal stools. Giardia cysts were more commonly found in normal stools than abnormal stools. Statistically insignificant difference (P>0.05) was noted by Chi square test (Table. 6 Fig.4.1)
### Table No. 6 RELATION OF GIARDIA INFECTION WITH STOOL CONSISTENCY.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Normal stools</th>
<th>Abnormal stools</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive/total</td>
<td>Prevalence %</td>
<td>Positive/total</td>
</tr>
<tr>
<td>1.</td>
<td>Calves</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>223/720</td>
<td>30.97 %</td>
<td>139/720</td>
</tr>
<tr>
<td>2.</td>
<td>Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>127/720</td>
<td>17.63 %</td>
<td>75/720</td>
</tr>
<tr>
<td>3.</td>
<td>Buffaloes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>118/720</td>
<td>16.38 %</td>
<td>70/720</td>
</tr>
</tbody>
</table>

Statistical analysis: Chi square test (P>0.05) NS (non significant)
Relation of Giardia infection with stool consistency

![Bar chart showing the relation of Giardia infection with stool consistency among Calves, Cattle, and Buffaloes. The chart indicates the percentage of normal and abnormal stools for each group.]

Fig. 4.1
5. Molecular diagnosis of *Giardiasis* by Polymerase chain reaction (PCR)

A total of 720 fecal samples were analysed with GF-1 tissue DNA extraction kit (Vivantis), amongst these 224 DNA were amplified through PCR of the cattle in different areas / farms in Lahore. So the molecular prevalence rate was 31.11% (224/720) in cattle. A statistically significant difference (P<0.05) was noted by Chi square test (Table. 7)

5.1 By area

Area wise molecular prevalence (%) was the highest at Government dairy farm (46.11%), followed by Gawala colonies (36.11%), then Military dairy farm (25.55%), while the lowest in the Household dairies (16.66%). Statistical analysis by using chi square test, indicated a significant difference was observed (P<0.05.) Table 7, Fig.5.1).

5.2 By Season

The highest Season wise molecular prevalence (%) was noted during Autumn (September and October) 36.66%, followed by summer (May, June, July and August) 34.58%, then spring (March and April) 30.83% and the lowest during winter (November and December) 25% Statistical analysis by using chi square test, significantly difference (P<0.05) (Table. 7, Fig.5.1) was noted.
5.3 Prevalence by age.

The higher prevalence in younger (41.66%) animals below 3 years of age was noted than above 3 years (27.59%). Significant difference (P<0.05) was noted by Chi square test (Table. 7, Fig. 5.3)

5.4 Prevalence by sex.

Females showed slightly higher (31.71%) prevalence than males (26.25%). A significant difference (P<0.05) was noted by using the Chi square test. (Table 7, Fig. 5.4).
Table. No.7; MOLECULAR DIAGNOSIS OF *GIARDIASIS* IN CATTLE AT LAHORE FROM AUGST 07-JULY 08

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. of positive/ Total samples</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Military dairy Farm</td>
<td>46/180</td>
<td>25.55♣</td>
</tr>
<tr>
<td>Gawala Colonies</td>
<td>65/180</td>
<td>36.11♣</td>
</tr>
<tr>
<td>Govt. dairy Farm</td>
<td>83/180</td>
<td>46.11♣</td>
</tr>
<tr>
<td>Household dairies.</td>
<td>30/180</td>
<td>16.66♣</td>
</tr>
<tr>
<td>Spring</td>
<td>37/120</td>
<td>30.83♣</td>
</tr>
<tr>
<td>Summer</td>
<td>83/240</td>
<td>34.58♣</td>
</tr>
<tr>
<td>Autumn</td>
<td>44/120</td>
<td>36.66♣</td>
</tr>
<tr>
<td>Winter</td>
<td>60/240</td>
<td>25♣</td>
</tr>
<tr>
<td>2-3 years</td>
<td>75/180</td>
<td>41.66♣</td>
</tr>
<tr>
<td>3-7 years</td>
<td>149/540</td>
<td>27.59♣</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21/80</td>
<td>26.25♣</td>
</tr>
<tr>
<td>Female</td>
<td>203/640</td>
<td>31.71♣</td>
</tr>
</tbody>
</table>

Statistical analysis. Chi square test. Significant $P<0.05$♣, non-significant $P>0.05$♣.
Results

Molecular diagnosis by Season in cattle at Lahore from August 07- July 08

Fig. 5.1

Comparison of PCR based detection of Giardiasis in cattle in different Farms of Lahore

Fig. 5.2
Molecular prevalence of Giardiasis by age group in cattle at Lahore

Fig. 5.3

Overall molecular prevalence of Giardiasis in cattle at Lahore from August 07-July 08

Fig. 5.4
Results

Fig. A. Gel showing DNA of *Giardia*

Fig. B

<table>
<thead>
<tr>
<th>M</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B. Giardia DNA amplification by PCR**

**Result**

Lane M. 1Kb DNA Ladder Marker (Gene Ruler™ Fermentas USA)
Lane 2-7, 11 & 12: Negative samples
Lane 8-10: Positive sample (753bp product)

6.1 Temperature (C°)

Maximum mean temperature (37.7 °C) was recorded during May, while the lowest mean temperature (6.9 °C) was recorded during January. Maximum and minimum temperatures were recorded daily and a monthly average was calculated (Table. 8).

6.2 Relative Humidity (%).

Mean highest relative humidity (87.08 %) was observed during October while, the lowest mean humidity was recorded (47.36) during May. The relative humidity was recorded morning and evening daily and the monthly averages were calculated (Table.8, fig.12.1).

6.3 Rainfall (mm)

Overall highest (3.78 mm) rainfall was recorded during July, while, the lowest (0.00 mm) was recorded during October (Table.8).
Table No. 8 Mean Month wise Temperature, Humidity and Rain Fall at Lahore During 2007-2008

<table>
<thead>
<tr>
<th>Season</th>
<th>Time (Months)</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>Morning</td>
</tr>
<tr>
<td>August 2007</td>
<td>2aaw7.6</td>
<td>35.37</td>
<td>31.48</td>
<td>76.19</td>
</tr>
<tr>
<td>Autumn 2007</td>
<td>September</td>
<td>25.4</td>
<td>33.85</td>
<td>29.62</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>19.20</td>
<td>32.50</td>
<td>25.85</td>
</tr>
<tr>
<td>Winter 2007</td>
<td>November</td>
<td>14.44</td>
<td>26.39</td>
<td>20.41</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>08.50</td>
<td>20.83</td>
<td>14.66</td>
</tr>
<tr>
<td></td>
<td>January</td>
<td>06.09</td>
<td>17.80</td>
<td>11.94</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>09.40</td>
<td>21.17</td>
<td>15.28</td>
</tr>
<tr>
<td>Spring 2008</td>
<td>March</td>
<td>19.10</td>
<td>30.90</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>28.38</td>
<td>33.30</td>
<td>30.84</td>
</tr>
<tr>
<td></td>
<td>May 2008</td>
<td>25.40</td>
<td>37.70</td>
<td>31.55</td>
</tr>
<tr>
<td>Summer 2008</td>
<td>June</td>
<td>27.15</td>
<td>35.82</td>
<td>31.48</td>
</tr>
<tr>
<td></td>
<td>July 2008</td>
<td>28.36</td>
<td>35.49</td>
<td>31.92</td>
</tr>
<tr>
<td>Mean</td>
<td>19.98</td>
<td>30.09</td>
<td>25.03</td>
<td>70.77</td>
</tr>
</tbody>
</table>
**Results**

**Association between *Giardiasis* and meteorological factors**

It was observed that the highest (78.34%) prevalence of *Giardiasis* in Calves was noted in August when the average temperature was 31.48 °C. However, the maximum and minimum temperatures were 35.37 °C and 27.6 °C respectively. The average relative humidity was 71.28%, whereas it ranged from 66.38 to 76.19% and rainfall at 3.2 mm was noted.

Similarly, the highest (35%) prevalence of *Giardiasis* in cattle was associated with temperature 27.6 °C (minimum) and 35.37 °C (maximum) during August. The mean temperature was (31.48 °C). Relative humidity (71.28%) and the rainfall (3.2mm) were recorded during this month.

The highest 35% prevalence of *Giardiasis* in buffaloes was also correlated with the same temperature 27.6 (minimum) and 35.37 °C (maximum) in August, whereas, the mean temperature was (31.48 °C). Average relative humidity (71.28%) and the rainfall (3.2mm) were reported.

It was observed that prevalence of *Giardiasis* is greatly concerned with high temperature, humidity and rainfall. During the month of August and despite the ranges of these parameters high prevalence of *Giardiasis* present (Table 8, Fig. 6.1).
Mean Month Wise Temperature (°C), Humidity (%) and Rainfall(mm) at Lahore during August 07-July08

<table>
<thead>
<tr>
<th>Months</th>
<th>Temperature</th>
<th>Humidity</th>
<th>Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 07</td>
<td>78</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Sept 07</td>
<td>80</td>
<td>85</td>
<td>30</td>
</tr>
<tr>
<td>Oct 07</td>
<td>85</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>Nov 07</td>
<td>90</td>
<td>95</td>
<td>70</td>
</tr>
<tr>
<td>Dec 07</td>
<td>95</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>Jan 08</td>
<td>100</td>
<td>95</td>
<td>80</td>
</tr>
<tr>
<td>Feb 08</td>
<td>90</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>Mar 08</td>
<td>80</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>Apr 08</td>
<td>70</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>May 08</td>
<td>60</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Jun 08</td>
<td>50</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Jul 08</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. 6.1
7. Comparison of PCR with Coprological diagnosis

7.1 In Cattle

Overall prevalence of *Giardiasis* diagnosed through Coprological examination and PCR test was observed as 28.05 % and 31.12 % respectively (Table 9). When the data was analyzed by using the *T*-test, a statistically non-significant difference (P>0.05) was noted.

7.2 Comparison of PCR with Coprological diagnosis by month

In month wise data same pattern of prevalence was observed by both the diagnostic methods. Prevalence was highest during August (35 %) and (40 %) by microscopic examination and PCR respectively, while lowest during January (21.67%) and (23.33%) (Table.14).

7.3 Comparison of PCR with Coprological diagnosis by season

When the data was formulated on the basis of season, it was noted that the highest prevalence was associated with autumn (31.66%), (36.66%), followed by summer (30.41%), (34.58%), spring (29.16%), (30.83%) and lowest in winter (23.33%), (25%) by coprological examination and PCR.

7.4 Comparison of PCR with Coprological diagnosis by age

Younger animals up to 3 years showed higher rate of prevalence (38.88%), (41.66%) than above 3 years (24.44%), (27.59%) by microscopic examination and PCR respectively (Table 9).
7.5  **Comparison of PCR with Coprological diagnosis by sex**

Female cows showed higher susceptibility, as (34.64%), (31.71%) than males as (18.75%), (26.25%) respectively by Microscopic examination and PCR (Table 9).

7.6  **Comparison of PCR with Coprological diagnosis by area**

The higher prevalence was recorded in the Govt. dairy Farm (41.67 %), (46.11%) followed by Gawala dairy Colonies (32.78%), (36.11%), then Military dairy Farm (22.78%), (25.55%), while the lowest in Household dairies (15%), (16.66%) by Coprological examination and PCR respectively (Table 9).
Table No. 9  COMPARISON OF MICROSCOPIC AND PCR BASED DIAGNOSIS OF GIARDIASIS IN CATTLE AT LAHORE

<table>
<thead>
<tr>
<th>Factors</th>
<th>Total Sample Observed</th>
<th>Microscopic</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Positive</td>
<td>Prevalence %</td>
</tr>
<tr>
<td>Time (Monthly)</td>
<td>Aug-07</td>
<td>60</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Sep-07</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Oct-07</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Nov-07</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Dec-07</td>
<td>60</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Jan-08</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Feb-08</td>
<td>60</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Mar-08</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Apr-08</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>May-08</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Jun-08</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Jul-08</td>
<td>60</td>
<td>19</td>
</tr>
<tr>
<td>Season</td>
<td>Spring</td>
<td>120</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>240</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>120</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>240</td>
<td>56</td>
</tr>
<tr>
<td>Age</td>
<td>2-3 yrs</td>
<td>180</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>3-7 yrs</td>
<td>540</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>640</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>720</td>
<td>202</td>
</tr>
</tbody>
</table>

Statistical analysis \( t\)-test: \( P>0.05 \) (Non significant)

(The stool samples of the cattle were collected during the study and same were examined by PCR and coprological method of examination and than compare its prevalence)
Table No. 10  Microscopic and PCR based by area prevalence of *Giardiasis* in cattle.

<table>
<thead>
<tr>
<th>Factors (Areas)</th>
<th>Total sample observed</th>
<th>Microscopic</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Positive</td>
<td>% age</td>
</tr>
<tr>
<td>Military Dairy Farm</td>
<td>180</td>
<td>41</td>
<td>22.78</td>
</tr>
<tr>
<td>Gawala Colonies</td>
<td>180</td>
<td>59</td>
<td>32.78</td>
</tr>
<tr>
<td>G. Dairy Farm</td>
<td>180</td>
<td>75</td>
<td>41.67</td>
</tr>
<tr>
<td>Household Dairies</td>
<td>180</td>
<td>27</td>
<td>15.00</td>
</tr>
</tbody>
</table>
Results

Prevalence of Giardiasis in cattle by PCR & Microscopy based techniques

Fig. 7.1
Results

Prevalence of *Giardiasis* in Cattle by PCR & Microscopic based techniques

Fig. 7.2
8. Comparative efficacy (%) of Albendazole, Metronidazole and Mebendazole against Giardiasis in infected Cattle.

The efficacy (%) of the different drugs was calculated on the basis of a reduction in the numbers of cyst per gram of faeces after treatment. The mean % of the Giardia cyst number of each treated group was compared with each other and with control group.

8.1 Efficacy of Albendazole (Group A)

Cysts per gram of faeces (CPG) of Giardiasis in cattle treated with albendazole are shown in Table 11. CPG count showed an increasing trend in control (untreated) animals. A dose of 20mg/kg body weight of albendazole caused a significant decrease in CPG count from 6 day post treatment onward (P<0.05). The efficacy of Albendazole was 77.2% at 13 day post treatment, 92% on 20 day post treatment and 98.5% at 27 day post treatment were noted during the experiment. Statistical analysis by using the Duncan test showed a significant difference (P<0.05) decrease in the CPG count after treatment, when the data was analyzed (Table No. 12).

At 15mg/kg body weight of Albendazole caused a significant decrease in CPG count from 06 day post treatment onward (P<0.05). The efficacy of albendazole was 88.5% at 13 day post treatment, 97.1% on 20 day post treatment and 100% at 27 day post treatment were noted during the experiment. Duncan test showed the significant
difference (P<0.05) decrease in the CPG count after treatment, when the data was analyzed (Table No.12).

At 10 mg/kg body weight of albendazole caused a significant decrease in CPG count from 6 day post treatment onward (P<0.05). The efficacy of Albendazole was 56% at 13 day post treatment, 75% on 20 day post treatment and 86.3% at 27 day post treatment. Duncan test showed a significant difference (P<0.05) in the decrease in the CPG count after treatment, when the data was analyzed (Table No.12).

8.2 Efficacy of Metronidazole (Group B)

A dose of 50 mg/kg body weight of metronidazole caused a significant decrease in CPG count from 6 day post treatment onward (P<0.05). The drugs caused 57.7% reduction in CPG count on 13 day post treatment (P<0.05). The efficacy of metronidazole was 73.7% at 20 day post treatment and 85.4% on 27 day post treatment respectively. Duncan test showed the significant (P<0.05) decrease in the CPG count after treatment in all days (Table No.12).

At 100mg/kg body weight of metronidazole caused a significant decrease in CPG count from 06 day post treatment onward (P<0.05). The drugs caused a 54.5% reduction in CPG count on 13 day post treatment (P<0.05). The efficacy of metronidazole was 72.7% at 20 day post treatment and 87.8% on 27 day post treatment were noted respectively (Table 13).

At 150mg/kg.body weight of metronidazole caused a significant decrease in CPG count from 6 day post treatment onward (P<0.05). The drugs caused 68.6%
Results

87

reduction in CPG count on 13 day post treatment (P<0.05) The efficacy of
metronidazole 85.0% at 20 day post treatment and 94.02% on 27 day post treatment
were noted respectively. When the data was analyzed using the Duncan test, a
significant difference (P<0.05) in decrease in the CPG count after treatment in all
days was observed (Table No.13).

8.3 Efficacy of Mebendazole (Group C)

CPG count showed an increasing trend in untreated control animals. A dose of
15mg/kg body weight of mebendazole caused a significant decrease in CPG count
from 6 day post treatment onward (P<0.05). The drugs caused 55% reduction in CPG
count on 13 day post treatment (P<0.05). The efficacy of mebendazole was 79.4% at
20 day post treatment and 90.4% on 27 day post treatment were noted respectively.
When the data was analyzed, the significant difference (P<0.05) in decrease in the
CPG count after treatment on all days was observed (Table No.12).

At 10mg/kg body weight of mebendazole caused a significant decrease in
CPG count from 6 days post- treatment onward (P<0.05). The drugs caused 53%
reduction in CPG count on 13 day post treatment (P<0.05). The efficacy of
mebendazole was 77.1% at 20 days post- treatment and 87.3% on 27 day post

At 7.5mg/kg body weight of mebendazole there was a significant decrease in
CPG count from 6 day post treatment onward (P<0.05). The drugs caused a 40.2%
reduction in CPG count on 13 day post treatment (P<0.05). The efficacy of
mebendazole was 63.7% at 20 days post treatment and 81.15% on 27 days post
Results

treatment. When the data was analyzed using the Duncan test, a significant decrease (P<0.05) in the CPG count after treatment on all days was observed (Table No.12).

From the Table 11, 12 and fig.8.1 showed that all three drugs i.e. albendazole, metronidazole and mebendazole were effective against Giardiasis in regarding the reducing the CPG count at the different dose level. Of these, the efficacy of albendazole at dose of 15mg/kg body weight was greatest in reducing the CPG count.
### Table No.11.  Cyst per gram of faeces in Cattle treated with different drugs on different days

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Subgroups (n=10)</th>
<th>Dose Mg/kg.body.wt</th>
<th>Cyst per gram of faeces on different days.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>Albendazole</td>
<td>A1</td>
<td>20mg/kg.b.wt</td>
<td>878</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>15mg/kg.b.wt</td>
<td>875</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A3</td>
<td>10mg/kg.b.wt</td>
<td>825</td>
</tr>
<tr>
<td>B</td>
<td>Metronidazole</td>
<td>B1</td>
<td>150mg/kg.b.wt</td>
<td>837</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B2</td>
<td>100mg/kg.b.wt</td>
<td>825</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B3</td>
<td>50mg/kg.b.wt</td>
<td>857</td>
</tr>
<tr>
<td>C</td>
<td>Mebendazole</td>
<td>C1</td>
<td>15mg/kg.b.wt</td>
<td>912</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2</td>
<td>10mg/kg.b.wt</td>
<td>887</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3</td>
<td>7.5mg/kg.b.wt</td>
<td>862</td>
</tr>
<tr>
<td>D</td>
<td>Group</td>
<td></td>
<td></td>
<td>878</td>
</tr>
<tr>
<td>E</td>
<td>Group</td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Statistical analysis: Univariate ANOVA, Duncan test. Significant (P<0.05).
Table No. 12. Comparative efficacy (%) of different drugs against *Giardiasis* in cattle

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Subgroups (n=10)</th>
<th>Dose Mg/kg.b.wt</th>
<th>Efficacy %</th>
<th>06day</th>
<th>13day</th>
<th>20day</th>
<th>27day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Albendazole</td>
<td>A1 20mg/kg.b.wt</td>
<td>31.6</td>
<td>77.2</td>
<td>92</td>
<td>98.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2 15mg/kg.b.wt</td>
<td>58.7</td>
<td>88.5</td>
<td>97.1</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A3 10mg/kg.b.wt</td>
<td>32.1</td>
<td>56</td>
<td>75</td>
<td>86.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Metronidazole</td>
<td>B1 150mg/kg.b.wt</td>
<td>41.7</td>
<td>68.6</td>
<td>85.0</td>
<td>94.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B2 100mg/kg.b.wt</td>
<td>36.3</td>
<td>54.5</td>
<td>72.7</td>
<td>87.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B3 50mg/kg.b.wt</td>
<td>28.5</td>
<td>57.7</td>
<td>73.7</td>
<td>85.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Mebendazole</td>
<td>C1 15mg/kg.b.wt</td>
<td>21.19</td>
<td>55</td>
<td>79.4</td>
<td>90.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2 10mg/kg.b.wt</td>
<td>21.12</td>
<td>53</td>
<td>77.1</td>
<td>87.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3 7.5mg/kg.b.wt</td>
<td>17.1</td>
<td>40.2</td>
<td>63.7</td>
<td>81.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean %; Univariate ANOVA comparison within group by Tukey’s test;

A=P<0.05, B=P<0.05 and C=P<0.05.
Comparative Efficacy of Different Drugs (Medium Dose) Against Giardiasis in Cattle

Fig. 8.1
8.4 Side effects

On the administration of the higher doses, sweating was observed in the six cattle. Of these, 3 were in group A and 2 were in group B, while, one in group C. Diarrhea was also noted in the sub group of A and B, which became normal by day 13. No other side effect was recorded during experiments.

8.5 Effect on body weight

In all the groups, an increase in body weight was noted except group D (infected control). Statistically no significant difference was noted by using student’s T-test and significant increase in the feed intake was noted. (Table No.14).

9. Haematology

A significant difference was observed in the value of leukocytes and eosinophil of infected cattle post inoculation at day 6 and day 13. The leukocytes/lymphocytes count of *Giardia* infected cattle was 58.09% and eosinophil 9.69% respectively. A significant difference (P<0.05) was noted by using the chi square test, while, the non-significant difference (P>0.05) was noted in the value of haemoglobin (Hb) 11.06gm/100ml (Table No.15).

10. Zoonotic transmission

The suspected positive samples were collected from the diagnostic laboratories of Mayo Hospital, Lahore during the month of July and August 2007. The samples were stained with Iron haematoxylin and then examined. The positive samples were compared with the bovine sample and it was found that both *Giardia* cysts were indistinguishable and very similar morphologically and in size.
### Results

Table 14. Comparison of Body Weight (Kg) and Feed Intake (Kg/day) Before and After Treatment of Cattle

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sub Groups</th>
<th>Body weight (Kg) n=5</th>
<th>Feed (Kg/day) n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td>Increase/Decrease (%)</td>
</tr>
<tr>
<td>A1</td>
<td>188.32±15</td>
<td>224.98±19</td>
<td>19.14↑</td>
</tr>
<tr>
<td>A2</td>
<td>191.93±19</td>
<td>205.24±21</td>
<td>6.93↑</td>
</tr>
<tr>
<td>A3</td>
<td>189.11±14</td>
<td>196.73±15</td>
<td>4.02↑</td>
</tr>
<tr>
<td>B1</td>
<td>186.03±10</td>
<td>207.78±15</td>
<td>11.6↑</td>
</tr>
<tr>
<td>B2</td>
<td>186.74±15</td>
<td>205.61±7</td>
<td>10.10↑</td>
</tr>
<tr>
<td>B3</td>
<td>203.22±10</td>
<td>211.16±11</td>
<td>3.90↑</td>
</tr>
<tr>
<td>C1</td>
<td>184.19±12</td>
<td>190.52±13</td>
<td>3.43↑</td>
</tr>
<tr>
<td>C2</td>
<td>188.81±22</td>
<td>195.41±23</td>
<td>3.49↑</td>
</tr>
<tr>
<td>C3</td>
<td>204.18±8</td>
<td>212.17±8</td>
<td>3.91↑</td>
</tr>
<tr>
<td>D</td>
<td>186.89±12</td>
<td>155.18±12</td>
<td>16.96Ψ</td>
</tr>
<tr>
<td>E</td>
<td>183.07±25</td>
<td>213.46±13</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Mean ± Standard Error. (X ± S.E); Student’s t test p<0.05 Sig *, p>0.05 NS†
Table No. 15. Mean Comparative Hematology of infected and non-infected cattle (n=30)

<table>
<thead>
<tr>
<th>Group</th>
<th>Hb gm/100ml</th>
<th>Lymphocytes %</th>
<th>Monocyte %</th>
<th>Eosinophil %</th>
<th>Basophil %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non infected</td>
<td>11.04</td>
<td>54.38</td>
<td>5.67</td>
<td>5.33</td>
<td>0.592</td>
</tr>
<tr>
<td>Infected</td>
<td>11.06 ▼</td>
<td>58.09 ♣</td>
<td>5.63</td>
<td>9.69 ♣</td>
<td>0.58</td>
</tr>
<tr>
<td>Normal</td>
<td>10.8-11</td>
<td>54.3</td>
<td>5.7</td>
<td>5.2</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Statistical analysis: chi square test.
Significant (P<0.01) ♣,
Non significant (P>0.05) ▼
10. Protein profile of *Giardia* isolates

Proteins, being a major constituent of any living cell serve many functions. In comparative protein analysis for epidemiological or taxonomic purposes living cells are grouped according to the protein profiles, displayed by the use of SDS-PAGE method. Molecular size of *Giardia* protein was estimated by SDS-PAGE method, with marker protein of known molecular size was included in one of the lanes of the gel. However, results were cautiously interpreted because some amino acid substitutions can affect the migration rate of protein. The results of 10% acrallyamide SDS PAGE of *Giardia* proteins are shown in fig.9.1

Total protein extraction of samples of different cysts concentration was sonicated. The sonication efficiency increased with the increase of sonication power and with the decrease of the suspension volume and cell concentration. Spectrophotometer determination of protein concentration of the samples was used with the known protein samples (bovine serum albumen was commonly used standard for this method) to standardize protein concentration at wave length 280nm absorbance and blanks at 0 nm were used to determine protein concentration. The calibration curve was created by plotting the concentration of the standards and absorbance of the samples was used to determine the concentration from the calibration curve by using the formula.
Concentration = Standard Factor* Dilution Factor* Optical Density (Fig. 9.2).

Fig. 9.1. SDS PAGE electrophoresis of *Giardia* protein extracts of different cysts/ml concentrated sonicated samples. Lane M is the molecular size (K Da) marker while lane 1, 2, 3, 4, and 5 indicates the different sample run.)
Fig. 9.2. Standard Curve of known protein samples. This curve was made by plotting the absorbance at 280 nm wavelength versus concentration of the bovine serum albumen standard. A stock solution containing 300 mg / 3 ml of BSA was made in normal saline which was further diluted to prepare concentrations of 10 %, 20%, 40%, 60%, 80% and 100% mg/ml.
Chapter 5

DISCUSSION

In this study faecal samples were obtained from bovines over a 12 month period from August 2007 to July 2008 from four different sites. This is the first longitudinal study in Pakistan, particularly at Lahore to report prevalence, microscopic diagnosis and molecular characterization data of Giardia spp in bovine from Military dairy farm, Gawala colonies, Government dairy farm and Household dairy.

The present study showed some epidemiological aspects of Giardiasis in bovines. The occurrence of Giardiasis at Military dairy farm, Gawala colonies, Government dairy farm and Household dairies is influenced by a variety of factors including of host, parasites transmission processes and environmental effects.

In the present study, the overall prevalence of Giardiasis in calves was found to be 50.27%. Similar findings were reported by (Deshpande and Shastri, 1981; Wade, et al., 2000; Nikitin, et al., 1991; McAllister, et al., 2005).

In this study, an overall prevalence of Giardiasis in cattle was found to be 28.05 % and similar findings were reported by (Olson, et al., 1997b; Wade, et al., 2000). In the present study, the highest seasonal wise prevalence 31.66% was noted during Autumn followed by summer then Spring whereas
the lowest 23.33 percent during winter. Similar results were also reported by (Wade, et al., 2000; Ralston, et al., 2003; McAllister, et al., 2005). In the present study, the highest overall age wise prevalence was 38.88%. Similar results were also reported by Fabienne, et al., (2006) Prevalence reported in the present study was 34.62% in females and 18.75% in males similar to that reported by McAllister, et al.,(2005) of all the sites examined. The highest prevalence(41.67%) was noted in Government dairy farm followed by Gawala dairy colonies (32.72%), the Military dairy farm (22.78%), while, the lowest (15%) in Household dairies Results of the present study are similar to those of (Trout, et al., 2005; Fabienne, et al., 2006; Wade, et al., 2000; Ralston, et al., 2003; McAllister, et al., 2005 ). Minor differences in the prevalence may be due to difference in the management and environmental conditions.

In the present study, the overall prevalence of Giardiasis in buffaloes was 26.11%. Studies conducted throughout the world have indicated the widespread distribution of Giardiasis in farm animals, particularly in bovines reported by Deshpande and Shastri, 1981; Nikitin, et al., 1991; Xiao, 1994; Fanthum, 1921). This difference in prevalence may be due to different geographical regions and their environments.

Studies in different areas showed a variation in degree of prevalence in calves. The highest prevalence was recorded in the Government dairy farm 68.33%, followed by Gawala colonies 55% then Military dairy farm 43.3%, while, the lowest was in Household dairies 34.44%. Statistical analysis showed significant differences (P<0.05) amongst them. In British Columbia
75-83% of calves from 2-24 weeks of age on 20 different farms were positive for *Giardia* cyst (*Olson, et al., 1997a).

In the present study, *Giardia* infection was more frequently associated with normal stools than with abnormal stools. A non significant difference (P>0.05) was noted. Some authors have reported of *Giardia* infections associated with diarrhoea (*Deshpande and Shastri, 1981; Jean, et al., 1987*). Our results are in agreement with similar findings from other studies, which reported no association between giardia infection and diarrhoea (*Gasser, et al., 1987; Olson, et al., 1997*). However, the high rates of detection in normal stools and long duration of infection was observed in four different areas of Lahore in bovines, which suggests that *Giardia* infection is not associated with chronic diarrhoea in calves, cattle and buffaloes. Moreover, the findings of *Giardia* cysts in stools are helpful for diagnostic purposes.

In the present study, significant difference (P<0.05) was observed in increase % of eosinophil and lymphocytes in *Giardia* infected cattle. (*Jain, 1986*) reported that eosinophils are present in the blood and tissue and predominantly the skin, lungs, intestine and urogenital tract, their number generally proportional to the degree of antigenic stimulation and resulting principally from presence of parasites. A similar study was reported by *Bordeau, (1993)* who described that dog with Giardiasis present with eosinophilia. A study reported by (*Jimenez, et al., 2004*) that *Giardia duodenalis* in the intestinal tract was associated with the development of...
inflammatory processes in the epithelium, which could influence in the
dynamics of lymphocytes/neutrophil, observed in the present study.

In the present research study, high prevalences were recorded in the
young animals as compared to the adults. Similarly, 71.52% prevalence was
recorded in the 0-6 month age group and 36.11% in 7-12 months of age. The
higher Prevalence in younger calves was highly significant (P<0.05). Likewise in cattle and buffaloes, the younger animals were more susceptible
than the adults. It was observed during the study that the age was an important
factor in determining the prevalence of the infection and shedding of the cysts
in faeces. The steady drop of the infection rate from younger animal towards
older animals showed this relationship of age and infection. This age effect
may be attributed to the development of the immunity, age related resistance
and movement of the animals to a less contaminated environment after
weaning. Similar results were also reported by (Markovics and Pipano, 1987;
Xio and Hard, 1994). In the present study, the lowest rate of infection of
giardia was observed in the winter season than other seasons, whereas, the
highest prevalence was observed during summer. Freezing and thawing were
shown to reduce cyst viability. Similar results were also reported by
(Bingham, et al., 1979; Dickerson, et al., 1991). It was observed that the cold
weather of the year 2007 contributed to the lower prevalence of giardia
infection in bovines. Similar finding were reported by (Taminelli. V and J.
Eckert, 1989).
Other studies on *Giardia* in humans and bovines were based on the microscopy examination, other methods for detection of the cyst in stool specimen like enzyme immunoassay for stool antigen and *Giardia* CELISA (CEUABAS pty Ltd). These assays are not sensitive enough to detect low levels of infection (Johnston, *et al.*, 2003).

In the present study, PCR diagnosis indicated higher prevalences than microscopic detection of cysts of Giardia. The PCR based prevalence was 31.11% in cattle as compared to direct microscopic examination 28.05%. The statistical analysis showed that the difference was significant (P<0.05). The sensitivity of detection by PCR was greater than that of microscopy, making it of great use for detection of low numbers of parasites in stool samples (McGlade, *et al.*, 2003; Mathis, *et al.*, 1996).

Considering the role of the meteorological factors may have the spread of Giardiasis in calves, cattle and buffaloes, it was noted that the monthly distribution pattern of Giardiasis in bovines was related to the temperature, humidity and the rainfall. Gradual increases in average temperature was from 19.98 to 30.09°C. Humidity and rainfall were associated with increased incidence of the disease. The highest prevalence of Giardiasis in calves, cattle and buffaloes was noted during August. The mean temperature was 31.48°C, humidity 71.28% and rainfall at 3.2mm was noted. It was noted that with an increase of temperature, humidity and rainfall. That was an increase of % prevalence of Giardiasis in calves, cattle and buffaloes. During the months of November and January, where temperature is lower i.e. 20.41°C and 14.66°C
and humidity was noted 67.09% and 65.32% respectively, the prevalence of Giardiasis was very low. This shows that temperature and humidity may play a major role in determining prevalence. A statistically significant difference ($P<0.05$) was noted with Giardia infection more frequently diagnosed during the rainy season in diarrhoeic patients at Bangladesh (Alam, et al., personal communication) whereas in Saudi Arabia higher prevalences in humans were reported during Autumn season (Kasim and Elhelu, 1983). It is important to note that Autumn, Spring, Summer and Winter all occur at different times of year in different parts of the world.

In the present study, three different antigiardial drugs, a albendazole (Valbazen – Pfizer), metronidazole (Flagyl) and mebendazole (Zentel) at three different dose levels, were evaluated. Albendazole at 10mg/kg.b.wt in cattle gave 86.36% reduction in cpg on 27th day respectively. At 15mg/kg.b.wt. and 20mg/kg.b.wt. efficacy was 98.5% and 100% respectively. This was significant ($P<0.05$) by a Duncan test when compared with the untreated control group. Similar findings of albendazole at 20mg/kg.b.wt in calves with efficacy 98.5, 97.6 and 90 percent were noted on 1, 2 and 6 weeks by (Xiao, et al., 1996). Albendazole can attached the cytoskeletal structure of ventral disk of Giardia and disrupt the glucose intake and causes parasite death (Oxberty, et al., 1994; Xiao, et al., 1996).

Metronidazole (Flagyl) group B at dose rate of 50mg/kg.b.wt reduced the cyst per gram of faeces 85.88% on 27th day post treatment. At the dosage level of 100mg/kg and 150mg/kg.body weight the efficacy was 87.88% and
94.02% on 27th day respectively. A significant difference (P<0.05) was noted with Duncan test when compared with the controls. Similar findings were reported by (Timothy, et al., 2001; Oxberty, et al., 1994). The efficacy of mebendazole at the dose of 7mg/kg.b.wt was 81.15% on the 27th day post treatment. Similarly at the dose rates of 10mg and 20mg/kg body weight were 87.3% and 90.4% on 27th day respectively. Duncan test showed that statistically significant different was noted when compared with infected untreated control group. Similar results were also reported by (Katiyar, et al., 1994). All the three drugs used in the present study i.e. albendazole, metronidazole and mebendazole were appeared to be effective in suppressing and eliminating the cyst of Giardia from infected cattle.

In the present study, the Giardia cysts from humans and bovines were morphologically indistinguishable and very similar in structure and size of supporting the hypothesis of transmission between the bovines and humans. Similar findings were reported by Thompson, R.C.A, (2004).

Proteins, being a major constituent of any living cell, serve many functions. In comparative protein analysis for epidemiological or taxonomic purposes living cells are grouped according to the protein profiles, displayed by the use of SDS-PAGE, (Koebink, et al., 2000). Molecular size of Giardia protein was estimated by SDS-PAGE method, as described by Laemmli, (1970) with marker protein of known molecular size is included in one of the lanes of the gel. However, results were cautiously interpreted because some amino acid substitutions can affect the migration rate of protein (Lugtenberg,
Discussion

The results of 10% Acralyamide SDS PAGE of Giardia protein bands were 20kb, 24 kb, 35kb, 46kb, 47kb and 66kb respectively. Similar results were also reported by (Stranden, et al., 1990).

Recommendations

It is recommended that

1. Filtered water should be used for drinking purposes both for animals and humans.

2. Albendazole (15mg/kg body weight) should be used due to its safe and effective elimination of *Giardia* infection in cattle.

3. Further studies are needed to determine the zoonotic strains of Giardia in Pakistan.

4. The proteins bands of Giardia isolates may be under taken for study to detect its immune responses for possible future vaccine production.
Chapter 6

SUMMARY

*Giardia* is a protozoan parasite of the small intestine that causes extensive morbidity worldwide. Dairy calves can excrete high numbers of the cysts of *Giardia* and the disease in cattle is clinically important and can reduce the growth performance of the ruminants. *Giardia* is the cause of non-viral diarrhoea in humans and is responsible for epidemics in the developed and developing countries. The cyst is the infectious form, is ingested in contaminated water or food or directly from faecal-oral contact. *Giardia duodenal* is the only species, which is found in both humans and animals including dogs, cats, bovines, pigs, sheep and equine.

The present study was conducted to determine the prevalence in bovines at Military dairy farm, Gawala dairy colonies, the Government dairy farm and Household dairies in Lahore. The effect of season, sex, and age on infection rate and shedding of the cysts were also noted, and association of the Giardia infection with normal and abnormal stools was also studied.

Overall 2160 bovine faecal samples (720 buffaloes, 720 cattle and 720 calves) were examined during the study period from August 2007 to July 2008, amongst calves 362/720 (50.27%) were found to be positive. The highest prevalence was recorded in the Government. Dairy farm (68.33%) followed by Gawala colonies (55%), then the Military dairy farm (44.33%) and the lowest (34.44%) was recorded in Household dairies. Overall, highest (61.6%) seasonal prevalence was recorded during autumn, followed by spring (60.83%), then summer (53.4%) and the lowest
(34.1%) was recorded during winter. The highest (65%) prevalence was reported during August and the lowest (30%) during December.

Females were found to be more susceptible (56.74%) than males (35.1%). The prevalence was significantly higher (71.52%) in younger calves than the adults (36.11%) (P<0.05).

Overall prevalence in cattle was 28.05%. The highest (41.67%) prevalence was recorded at the Government dairy farm, followed by Gawala colonies (32.72%), then the Military dairy farm (22.72%) and the lowest (15%) was recorded in Household dairies. The highest (35%) prevalence was found during August and the lowest (21%) during January. A significant difference (P<0.05) was noted. Females were found to be more susceptible (29.21%) than males (18.75%). The young calves had significantly higher (38.88%) prevalence as compared to the adults (24.44%).

Similarly, the overall prevalence in buffaloes was found to be 20.11% percent. The highest (40.55 %), prevalence was recorded at the Government Dairy Farm, followed by Gawala colonies (30%) then Military Dairy Farm (21.11%) and the lowest prevalence i.e. 12.77% was reported in Household Dairies. A non significant difference was recorded (P>0.05). The highest (46.66 %) prevalence was recorded during August, while, the lowest (6.66%) during November and December. Females were found to be more susceptible than males. Where as the prevalence in a younger buffalo was significantly higher as compared to the adults.

Comparison of direct microscopic examination and PCR based methods was made at the Government dairy Farm, Gawala colonies; Military Dairy Farm and
Household Dairies. By direct Microscopic examination prevalence was found to be 28.05% (202/720) in cattle whereas by PCR it was 31.11%. Statistically analysis showed that the prevalence by PCR was significantly (P<0.05) higher than the microscopic examination.

It was observed that the highest prevalence of Giardiasis in bovines (Calves, Cattle and buffalo) was noted during August when the average temperature was 31.48°C. However the maximum and minimum temperatures were 35.37°C and 27.6°C, relative humidity 71.28% and rainfall was 3.2mm. The results of therapeutic trials by using albendazole, metronidazole, and mebendazole in cattle were calculated on the basis of reduction in the cysts count in the faeces after treatment.

Efficacy of albendazole at three dose levels i.e.10mg/kg.b.wt, 15mg/kg.b.wt, 20mg/kg.b.wt was 86.33%, 98.5% and 100% respectively, on day 27 after treatment. Efficacy of the metronidazole at 50mg/kg.b.wt, 100mg/kg.b.wt, and 150mg/kg.b.wt. Was 85.42%, 87.8% and 94.02% respectively on day 27. Efficacy of mebendazole at three dosage level i.e. 7.5mg/kg.b.wt, 10mg/kg.b.wt and 20mg/kg.b.wt was 81.15 %, 87.32%, and 90.4% on day 27 after treatment. Among these drugs, albendazole at 15mg/kg.body.weight was found to be most effective drug in the elimination Giardia infection. The significant (P<0.05) decrease in the CPG count after treatment in all the three groups and dose levels was noted. A significant difference (P<0.05) was observed in the level of leukocytes and of eosinophils of infected cattle at day 06 and day 13 post inoculation. The leukocytes/lymphocytes count of Giardia infected cattle was 58.09%. Whereas, eosinophils constituted of leukocytes 9.69%. The total proteins of the sample were studied by sodium doedocyl sulphate polyacrylamide gel
Summary

electrophoresis (SDS PAGE). The result indicated that 8 different molecular weight peptide bands were identified with size ranges from 20 to 70 KDa and common bands reported at 20, 24 and 35 KDa.
LITERATURE CITED


Maddox-Hyttel, C., R. B. Langkjaer, Heidi, L. Enemark and Hakan vigre (2006). Cryptoспорidium and Giardia in different age groups of Danish cattle and


Nizeyi, J. B, M. R. Cranfield and T. K. Graczyk (2002). Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of Cryptosporidium


O'Handley, R. M., C. Cockwill, M. Jelinski, T. A. MeAllister, D. W. Morck and M. E. Olson (1999). Duration of naturally acquired giardiosis and


Department of Parasitology  
University of Veterinary & Animal Sciences, Lahore.  
Questionnaire Proforma for “Epidemiology, Molecular diagnosis and chemotherapy of Giardiasis in Bovine.”

1. Name of owner with address__________________________
2. Animal species.____________________________________
3. Bread: Cow________ Buff.________ Calf.______________
4. No of cow/Buff.: Cow_____ Buff.____ Calf.____________
5. No of animals having Diarrhea. Cow._______ Buff.____ Calf____
6. No. of animal having dysentery. Cow___ Buff.____ Calf____
7. No of mortality due to diarrhea. Cow.____ Buff.____ Calf____
8. Occurance of disease(Giardiasis) having diarrhea. 
   Winter. Cow.____ Buff.____ Calves____________________
   Summer. Cow.____ Buff.____ Calves____________________
   Autumn. Cow.____ Buff.____ Calves____________________
   Spring. Cow.____ Buff.____ Calves____________________
9. Clinical symptom of disease observed in. 
   Cow.____ Buff.____ Calf______________________________
10. Have you knowledge about the disease Giardiasis occurrence season: yes________ No.___________________
11. Have you knowledge about the Giardia detection. 
    Yes__________No_______________________________
12. Have you knowledge about the Giardia transmits to human. 
    Yes__________No_______________________________
13. Any test perform for Giardia detection during the 
    Last year. Yes._____No._________________________
14. Was the calves most affected with Giardia? 
    Yes.______No._____________________________
15. Was the adult Cow/Buff. Mostly affected with the 
    Giardiasis. Yes.______No._____________________
16. Was the Buffaleo mostly affected with Giardiasis? 
    Yes.______No._____________________________
17. Was the old Cow/Buff mostly affected with Giardiasis? 
    Yes.______No._____________________________
18. Were the both Cow/Buff. Mostly affected. 
    Yes.______No._____________________________
19. Is vet. Lab services available in the area. 
    Yes__________No____________________________
20. Were the vet. lab. Services utilized. 
    Yes__________No____________________________
21. Was vet. Hospital services available in the area.
   Yes.______ No._______

22. Was vet. Hospital services utilized?
   Yes.______ No._______

23. Have you got any Giardiasis control advices from the Deptt.
   Yes.______ No._______

24. Was the animal treated by home made treatment and cure.
   Yes.______ No._______

25. Was the animal treated by non technical person? and cure.
   Yes.______ No._______

26. Was the animal treated by the religious person and cure.
   Yes.______ No._______

27. Was the animal treated and not cured.
   Yes.______ No._______

28. Is the general condition of the farm/Household? dairy shed & animal satisfactory.
   Yes.______ No._______

29. Is the overall feeding programme satisfactory?
   Yes.______ No._______

30. Was the floor of the shed cemented?
   Yes.______ No._______

31. Was the shed cemented? Yes.______ No._______

32. Was calves allowed to suck the dam? Yes.______ No._______

33. Are the flies observed at the farm? Yes.______ No._______

34. Were the animal suffering from Giardiasis? Yes.______ No._______

35. Were all type of animals affected with Giardiasis?
   Yes.______ No._______
36. No. of animals suffering from *Giardiasis* detected by lab.test.____________

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy:</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>PCR:</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>ELIZA:</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>Immunoflourence Techniques.</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Sultan Ayaz  
Ph.D Scholar  
Deptt.of Parasitlogy  
UVAS Lahore.  
(investigator)