**CHAPTER-I**

**INTRODUCTION**

In Bangladesh, livestock is one of the most potential sub-sectors of agriculture which plays an indispensable role in promoting human health and national economy of the country. Livestock is an integral part of farming system which has a better contribution to enhance the economy of Bangladesh. The contribution of Livestock in the magnitude of Gross Domestic Product (GDP) is about 16.23 % in Bangladesh (BBS, 2008). large ruminants (Cattle and Buffalo) and small ruminants (sheep and goat) constitute the major portion of livestock. The present population of livestock is 23.12 million cattle, 1.39 million Buffalo, 24.15 million goat and 3.07 million sheep (DLS, 2010-11). Rapid population growth and urbanization as well as change in diet are the most obvious reasons for increase in the demand for milk and milk products in developing countries (Delgado, 2001). The high-yielding dairy cow produces a lot of milk; and the main emphasis for dairy farmers is to sell as much milk as possible with maximum efficiency, both financially and in terms of animal welfare. In Bangladesh, both intensive and household rearing cattle are suffered from various metabolic diseases during different stage of production. But laboratory investigation is merely done to know the status of hematological and biochemical parameters of dairy cattle related to production stage and diseases. Routine examination of blood and serum is necessary for monitoring the health status of cattle. Gastrointestinal parasitism is a world-wide problem (*Regassa et al.,* 2006). It is thought to be one of the major constraints that hinders the development of livestock population (Kakar *et al.,* 2008 and Jabber and Green, 1983) and it also adversely affect the health and productivity of animals (Irfan, 1984).

The losses caused by parasitic infections are in the form of lowered general health condition, retarded growth rate, diminishing the working efficiency, decrease milk and meat production, abortion, cost associated with preventive measures and reduces the disease resistance capability, which may ultimately lead to higher mortality (Chavhan *et al.,* 2008, Silvestre *et al.,* 2000 and Radostits *et al.,* 1994). But in Bangladesh, there are many constrains in cattle production, among them malnutrition, parasitism and Mastitis are the major limiting factors (Jabber and Green, 1983). The losses due to parasitism and Mastitis take in the form of mortality, lower general health condition, retarded growth, decrease in the production of milk and meat and lower output of work (Faiz, 1972). In ruminants, paramphistomiasis is often associated with diarrhoea, loss of body condition, rough hair coat, dullness, weakness, and loss of appetite, intestinal haemorrhages, anemia, reduced milk production and intermandibular swelling (Chandrasekharan *et al.,* 1982). The flukes are widespread in Bangladesh (Hosain *et al.,* 1987; Rabbani, 1992; Saifuzzaman, 1996). The paramphistomum is normally present in cattle gastro-intestinal tract but over loading has an adverse effect. Milk is considered as an ideal food which is the excellent source of almost all nutrients. However, Bangladesh has an acute shortage of milk. Availability of milk is only 33.95 ml per head per day whereas the maximum requirement per head per day is about 250 ml milk (DLS, 1991). Mastitis is an inflammation of the mammary gland of dairy cows accompanied by physical, pathological and bacteriological changes in milk and glandular tissue. In the present state of knowledge it seems practicable and reasonable to define mastitis as a disease characterized by the presence of significantly increased leucocytes content in the milk from affected gland (Blood *et al.,* 1989). The disease is common high yielding dairy cows. Infection rate is more in successive lactation than the first lactation .Exotic and cross breeds cow are more prone to mastitis .Due to improved breeding the cow udder has to undergo rapid changes in relation to size, position and adjustment for rapid removal of large volume milk and as such it is prone to injury and infection (Gibbons *et al.,* 1970). Mastitis may be clinical or sub-clinical, with subclinical infection preceding clinical manifestation .Yet the relationship between the two within a herd is not predictable (Rolands *et al.,* 1988).The prevalence of clinical mastitis in dairy cows of Baghabari ghat , Sirajgong has been reported to be 16% (Al-Shawabkeh *et al.,*1987) recorded the incidence of mastitis was found to be increased with number of lactation . Mastitis is the one of the most costly disease affecting dairy cows. It has been estimated that mastitis reduced milk yield by approximately 2% and butter fat by 25% in affected cattle as a compared to normal one The milk of the infected cow is unfit for the human consumption. In India financial loss due to incidence of blind teat as a result of mastitis and loss of milk from clinical case of mastitis has been estimated as 92.57 cores of taka every years .(Dhanda *et al.,* 1946).The prevalence of mastitis of clinical (13.3%) and sub clinical(15.8%-19.5% ) in dairy cattle of Bangladesh. The prevalence of sub clinical mastitis in milch cows have been reported to be 16.52%with White slide test (WST) and 15.77% with California mastitis test (CMT) from Baghabarighat , Sirajgong district by Prodhan *et al.,* (1996) and 18.5% with WST from the greater Mymensingh district by Raman *et al.,* (1997).

Considering the above facts, the present study was conducted to fullfil the following objectives:-

I)To detect the prevalence of Paraphistomiasis in milking cows.

II) To detect the prevalence of subclinical mastitis in high yielding cows.

III) To estimate the relationship between blood parameters changes with subclinical mastitis and Paramphistomiasis.

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**CHAPTER-II**

**REVIEW OF LITERATURE**

**2.1. Paramphistomiasis in dairy cows**

Gastro-intestinal parasitosis adversely affect the nutritional status of the hosts and associated with economic losses in terms of lowered fertility, reduced work capacity, involuntary culling, retarded weight gains, lowered milk production, treatment costs and mortality in heavily parasitized animals (Fikru *et al.,* 2006).

The geo-climatic conditions together with the water-logged and low-lying areas in Bangladesh are conducive to parasitic diseases in domestic ruminants. Infact cattle of Bangladesh are affected by various types of helminth parasites (Rahman *et al.,* 1973); (Rahaman *et al.,* 1983).

Out of 80 cow calves, 45 were found positive for gastrointestinal parasites. A higher prevalence of 69.05 % was found in calves between 1 to 6 months age. However, 42.10 % calves having age 7 to 12 months were found positive for worm infestation.

The paramphistomum is normally present in cattle gastro-intestinal tract but over loading has an adverse effect. However, the epidemiological study of Paramphistomiasis has not yet been investigated properly in cattle. Prevalence of GI the wide range of agro-climatic zones in sub Saharan helminthes has been reported ranging from 0.7 to 84.1% in domestic animals from various parts of the world .There are many associated risk factors influencing the Gastrointestinal (GI) parasite infections are a prevalence of GI helminthes including age, sex.

Worldwide problems for both small large scale farmers, weather condition and husbandry or management but their impact is greater in sub Saharan Africa in practices.

The prevalence of 34.48% paramphistomiasis and 1.15% fascioliasis recorded in this study in RCC supports the findings of Siddiki *et al*. (2010) who reported 38% prevalence of paramphistomiasis and 2% fascioliasis in RCC. In addition Siddiki *et al*. (2010) recorded coccidiosis under rural management system whereas it was not recorded in this study but toxocariasis.

The 51.72% prevalence of gastro-intestinal parasitosis recorded in RCC reared under farming system supports the earlier report of Siddiki *et al*. (2010) who reported 53.16% prevalence rate of gastro-intestinal parasitosis in RCC maintained under rural condition.The prevalence of 34.48% paramphistomiasis and 1.15% fascioliasis recorded in this study in RCC supports the findings of Siddiki *et al.* (2010) who reported 38% prevalence of paramphistomiasis and 2% fascioliasis in RCC.

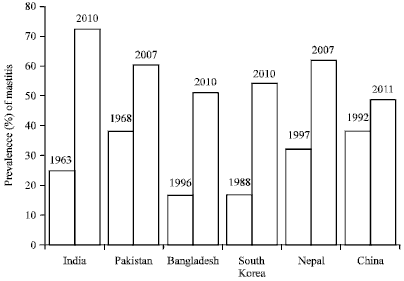
The overall Prevelence of Paramphistomiasis recorded in cattle was very similar with, Saifuzzaman (1996), 52.2%; Sahay *et al*. (1989), 58.3% and slightly lower than the reports of Rahman *et al.,* (1973), 62.6%; and higher than the reports of Rahman *et al.,*(1983), 21.6%; Islam *et al.,*(1989), 46.3%. Saifuzzaman (1996) reported that the percentage of paraphistomum infection in male and female cattle was 45.5% and 55.6%, respectively.

Prevalence of *Paramphistomum spp*. infection of this study was consistent with the observation of Kakar *et al.,* (2008) who recorded 7.82% in Pakistan. Alim *et al.,* (2011) recorded 14.81% and 12.96% of Paramphistomiasis in Holstein Friesian crossbred and indigenous cattle, respectively which was slightly similar with the findings of this study. Higher prevalence of Paramphistomiasis was recorded by Sardar *et al.,* 2006, Raza *et al.,* (2009), Hirani *et al.,* (2006) in different countries of the world. Lower prevalence of *Paramphistomum spp.* infection of this study might be due to geo-climatic conditions (Gupta *et al.,* 1987 and Georgiev *et al.,* 1980) or improved husbandry practices (Alim *et al.,* 2011).

**2.2. Prevalence of sub clinical Mastitis**

Ramachandraiah *et al*;(1990 ) examined milk samples of 80 pure Jersey cows of which 51(63.7%)cows had sub clinical mastitis on California mastitis test (CMT) and cultural examination .The quarter wise incidence was found to be equality distributed among the four quarter, and the percentage of incidence was maximum (56.8%)in single quarter. Sub clinical mastitis due to staphylococcus spp. Recorded the highest (52.9%) followed by Streptococcus spp. (20.3%).

Dhote *et al.,* (1999) reported an overall 20.72% prevalence of sub clinical mastitis in dairy cows. They obtained 331 isolates of which 108 (32.62%) were staphylococci, 138 (41.0%) streptococci and 85 (25.26%) were gram negative bacillus. Majority of the streptococcal and staphylococcal isolates were sensitive to ciprofloxacin and were least sensitive to ampicillin and penicillin, respectively. The gram negative bacteria were highly sensitive to pefloxacin and least to ampicillin and cloxacillin. Studies conducted in different states of India reflecting the high prevalence of bovine mastitis all over India for him past seven decades when the first record of the mastitis was made by Land in 1926.



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| Gra-1: | The increasing trend of bovine mastitis prevalence |

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| Table- 1: | Distribution of mastitis in different geographical regions in Mastitis in Cow. |

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| --- | --- | --- | --- | --- |
| Country | Species | Prevalence of Mastitis | Remarks | References |
| Bangladesh | Cow | 16.5 | LF-30.3 & RH-46.8% | Prodhan *et al*., (1996) |
|  | Cow | 21.20 | Do | Nooruddin *et al*., (1997) |
|  | Cow | 46.60 | Do | Kader *et al*., (2002) |
|  | Cow | 44.80 | Prevalences was high in wet seasons | Rahman *et al*., (2009) |

Neelesh Sharma *et al.,* (2012).

Shike *et al.,* (1998) found pathogenic organisms in milk sample from 15 cows (19quarters) with clinical sings of mastitis and 54 cows (214 quarters) with clinical mastitis. Bacteriological examination revealed pure cultures in 7 (31.82%) and mixed culture in 15 (68.18 %) of those with sub clinical infection , and from (42.86% ) and 12 (57.14 % ) , respectively , from the clinical cases , *Staphylococcus spp.* were the most commonly found pathogen . All of the isolates were sensitive to gentamycin and cephaloridine .

Joyti *et al.,* (1998) reported 77.77% and 41% prevalence of sub clinical mastitis (SCM) in early and late lactation respectively in an organized farm comprising 180 lactating Sahiwal x Holstein cows. Successful treatment of 72 SCM positive cows showed that there was an average increased yield of 1.38 liters of milk / cows per day but the untreated and healthy cows showed a marginally decreased milk yield. If cows were treated during early lactation yields could be increased to about 374 liter / cow per lactation.

Parai *et al.,* (1992) screened 1339 milch cows for the presence of sub clinical mastitis using California Mastitis Test (CMT), of which 19.34%cows had sub clinical mastitis. Breed wise incidence of herd and quarter basis was 29.62% and 16.85% and pure Holstein cows, 23.48% and 13, 40% in J x H cows 22.85% and 10.53% in B x H cows, 20.77% and 10.36% in F x H cows, 13.28% and 6.75% in other crossbred cows respectively.

Roy *et al.,* (1989) reported highest incidence of sub clinical mastitis in Holstein-Friesian xHariana cross-bred (62.80%), followed by Brown swiss x Hariana (44.73%) and Jersy x Hariana (31.85%) crossbreeds. The Jersey x Hariana crossbreeds are least susceptible to mastitis and claims its superiority over the Brown swiss x Hariana and Holstein Friesian crossbreds.

Pal *et al.,* (1988) examined 462 quarter samples of milk by California Mastitis Test (CMT) of which 100% milk samples were the positive for the sub clinical mastitis.

Prodhan *et al.,* (1996) performed CMT and WST to determine the prevalence of sub–clinical mastitis in 805 apparently healthy milch cows at Baghabari ghat of Sirajgong district. They recorded comparatively higher prevalence rate of sub-clinical mastitis with WST (16.52%) than CMT (15.77%).

Karimuribo *et al.,* (2004) reported that percentages of the cows (and quarters)with subclinical mastitis were 75·9 per cent (46·2per cent) when assessed by the CMT and 43·8 per cent (24·3per cent) when assessed by culture. Factors significantly associatedwith an increased risk of a CMT-positive quarter were Boranbreed (odds radio [OR] =3·51), a brought-in cow (rather thanhomebred) (OR=2·39), peak milk yield, and age. The strippingmethod of hand milking was associated with a significantly lower prevalenceof CMT-positive quarters (OR=0·51). The CMT-positivecows were more likely to be culture positive (OR=4·51),as were brought-in (OR=2·10) and older cows.

Significantly higher prevalence of *Paramphistomum spp*. (20.13%) was found in rainy season. *Paramphistomum spp.* infections were more frequent in adult (Alim *et al.,* 2011).

**2.3. Biochemical parameters of blood**

Biochemical analyses of blood serum are very useful to get insight in the metabolic and health status of animals. During diagnostic procedure it is very useful to compare the values obtained from ill animals with normal values in healthy animal.

In Bangladesh, there are many constrains in cattle production, among them malnutrition, Mastitis and parasitism are the major limiting factors (Jabber *et al.,* 1983). The agro-ecological and geo-climatic conditions are favourable for high prevalence of helminthiasis in Bangladesh (Samad, 2000, 2001).

Thomas Colville *et al.,* (2008) reported that blood, anticoagulants, plasma and serum are required to analysis the hematological parameters. Substances that tie up clotting factors and prevent blood from clotting are called anticoagulants. If an anticoagulant is added to a blood sample in a tube or syringe, the blood will not clot. One of the most common anti coagulants is ethlyenediaminetetraacelic acid or EDTA prevents clotting by tying up calcium, clotting factor number IV. No calcium, no clot. If an anticoagulant is added to a blood sample as it is drawn from an animal, the sample will not clot because all the clotting factors are not present. If the blood sample is then centrifuged, the fluid that raises to the top the tube is plasma. If no anticoagulant is added to a blood sample as it is drawn from an animal, t he blood will not clot. If the clotted blood is centrifuged, the fluid that rises to the top of the tube is called serum.

For haematological analysis the important parameter are TEC, TLC, Hb, PCV, ESR and DLC. The changes of these parameters are related with different kinds of diseases. Such as Bacterial diseases, viral diseases, parasitic disease, protozoal diseases and other diseases. (Moore *et al.,* 1904).

In subtropical condition hematological parameters of exotic cows at lactating, pregnant ,and non pregnant is PCV % 31.79±1.65 and 29.75±1.80 respectively ( Sattar *et al.,* 2009 ) that normally ranged between PCV % 24.0-46.0 (Schalm *et al.,* 1975). In cross breed cows the PCV percentage was 29.04 ± 0.89, 31.8 ± 1.06, 28.5 ± 01.06 in early gestation, mid gestation and late gestation period respectively (Manzoor *et al.,* 2008).

In subtropical condition hematological parameters of exotic cows at lactating pregnant and non pregnant is Hb (gm/dl) 9.44 ± 0.34 and 9.24 ± 0.35 respectively, TEC (×106/μl) 5.88 ± 0.46 and 5.30 ± 0.58, respectively, MCV (ſt) 55.16 ± 1.72 and 58.17 ± 2.46, respectively, MCH (pg) 16.77 ± 1.14 and 18.78 ± 1.64, respectively, MCHC (gm/dl) 30.09 ± 1.15 and 18.78 ± 1.64, respectively ( Sattar *et al.,* 2009 ).

In subtropical condition hematological parameters of exotic cows at lactating pregnant and non pregnant is neutrophil % 28.30±3.20 and 23.30±2.97, respectively, lymphocyte % 59.30 ± 3.11 and 65.20 ± 3.00, respectively, monocyte % 7.20 ± 0.74 and 6.40 ± 0.78, respectively, eosinophil % 4.40 ± 1.25 and 4.30 ± 0.54, respectively, basophil % 0.80 ± 0.33 and 0.80 ± 0.20, respectively ( Sattar *et al.,* 2009 ).

Bovine biochemistry reference intervals at 2 wks to 6 month calcium (mmol/lit) 2.35-2.74, but at 2 yrs+ calcium (mmol/lit) 2.10-2.67 ( Lumsden *et al.,* 1980).

Bovine Hematology reference intervals at 1-14 days old is Hb (gm/dl) 5.7 - 15.8 , TEC (×106/μl) 4.9 - 10.9, MCV (ſt) 31.7 - 49.6 MCH (pg) 10.8-16.5, MCHC (gm/dl) 27.4 - 39.8, at 2wks -6 months old is Hb (gm/dl) 8.5-14.1, TEC (×106/μl) 6.5-11.9, MCV (ſt) MCH (pg) 26.6-44.3, MCHC (gm/dl) 31.0 - 32.2, at 6 month -2 years old is Hb (gm/dl) 9.2 - 15.4 , TEC (×106/μl) 6.1-10.6, MCV (ſt) 31.5 - 50.9, MCH (pg) 10.7-19.1, MCHC (gm/dl) 31.0-39.0, at 2 year + old is Hb (gm/dl) 8.5 - 13.2 , TEC (×106/μl) 5 - 7.7, MCV (ſt) 37.8 - 56.0, MCH (pg) 14.2 - 20.1, MCHC (gm/dl) 31.7 - 40.4 ( Lumsden *et al.,* 1980).

In subtropical condition hematological parameters of exotic cows at lactating, pregnant ,and non pregnant is PCV % 31.79±1.65 and 29.75±1.80 respectively ( Sattar *et al.,* 2009 ) that normally ranged between PCV % 24.0-46.0 (Schalm and Jain *et al.,* 1975). In cross breed cows the PCV percentage was 29.04 ± 0.89, 31.8 ± 1.06, 28.5 ± 01.06 in early gestation, mid gestation and late gestation period respectively (Manzoor *et al.,* 2008).

In subtropical condition hematological parameters of exotic cows at lactating pregnant and non pregnant is neutrophil % 28.30±3.20 and 23.30±2.97, respectively, lymphocyte % 59.30 ± 3.11 and 65.20 ± 3.00, respectively, monocyte % 7.20 ± 0.74 and 6.40 ± 0.78, respectively, eosinophil % 4.40 ± 1.25 and 4.30 ± 0.54, respectively, basophil % 0.80 ± 0.33 and 0.80 ± 0.20, respectively ( Sattar *et al.,* 2009 ).

Bovine biochemistry reference intervals at 2 wks to 6 month calcium (mmol/lit) 2.35-2.74, but at 2 yrs+ calcium (mmol/lit) 2.10-2.67 ( Lumsden *et al.,* 1980).

Gavan *et al*., (2010) confirmed the highest count of total leukocytes (14.9×109/L) in dairy cows in 40- 120 days, and lowest (11.68×109/L) in the period of early lactation (0-21 days).

**CHAPTER-III**

**MATERIALS AND METHODS**

**3.1. Study area**

Chittagong Hill Tracts (CHT) the only extensive hill area in Bangladesh lies in southeastern part of the country (21o25'N to 23045'N latitude and 91054'E to 92050'E longitude) bordering Myanmar on the southeast, the Indian state of Tripura on the north, Mizoram on the east and Chittagong district on the west.

**3.2. Study period**

The study was conducted during November , 2013 to January, 2014 covering 5 high yielding varieties of dairy farms.

**3.3. Selection of Farm**

Chittagong area is potential for dairy farm because of its high demand of fluid milk, suitable weather, feeds and fodder availability, available veterinary facilities from Chittagong Veterinary & Animal Sciences University. So there is good communication with university and dairy farms as for giving various technical supports to the farms & for some research work also. Nahar dairy farm, Wahid dairy farm, A.S. dairy farm, Rubel dairy farm, Friend dairy farm was selected for conducting the study because of its suitable location, large population, satisfactory record keeping system, proper feeding & management & also for their kind cooperation.

**3.4. Study population**

Study population was 21 cross bred multiparous (HF X Local, Shahiwal X Local, HF x Shahiwal X Local milking cows. Cows were in different age & production status.

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Fig-1: Sample collection (Feces & Milk)

**3.5. Sample collection**

8ml of Blood sample was collected from the selected dairy cows by puncturing jugular vein with 10ml syringe. Blood was collected by proper aseptic measures. Out of 8ml blood 2 ml was kept in a vial containing EDTA (1 mg/ ml). The rest 6ml of blood in syringe was allowed to stand for about 2 hours at room temperature for proper clotting of blood. After cloting , the fluid portion of blood was serum, used for further analysis in the laboratory .

**3.6. Sample Transportation**

Blood samples were transported to the lab of Physiology & Biochemistry within one hour keeping in a thermo flask with ice and used for hematological (ESR, PCV, TEC, Hb, DLC) and biochemical (Calcium, Magnesium, Phosphorus) estimation.

**3.7. Sample examination**

**3.7.1. Hematological Examination**

Different hematological parameters were studied according to the methods described by Sastri, 1985. For hemoglobin determinationfresh blood mixed with EDTA was kept in the Wintrobe tube and centrifuge at 3000 rpm for 30 minutes. Thus PCV was determined.For hemoglobin determinationN/10 HCl was taken in a graduated hemoglobinometer up to 10 marks and blood was mixed upto 20 cu mm mark**.** Then diluted the acid hematin using water and match the color thus Hb% was determined. TEC determined from freshly collected blood using hemocytometer.DLC determined by making of blood smear on the clear glass slide and then drying of the smear. Then blood smear was stained by the Wright’s stain and dried. Prepared smear was examined under microscope (100xs). Mean corpuscular volume, MCV**=**Hematocrit x 10/RBC count.Mean corpuscular hemoglobin,MCH =hemoglobin x 10/ BRC count. Mean corpuscular hemoglobin concentration, MCHC =hemoglobin x 100/hematocrit value. TEC and TLC were determined by hemocytometer and Hb were Hellige shali method. All differential counts of leukocytes were prepared as thin blood smear stained by Wright’s method. All the above prepared as TEC, TLC, Hb, PCV, ESR and DLC were performed according to the method described by Shastry (1983).

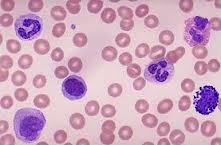
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Fig-2: Blood examination under Microscope.

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Fig-3: Fecal examination under Microscope.

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Fig-4: Detection of subclinical Mastitis by CMT test.

**3.7.2. Biochemical Examination**

Calcium, phosphorus, Magnesium was determined from serum samples by Automated Analyzer established in physiology lab of Chittagong Veterinary and Animal Sciences University.

**3.7.3. Collection of feces**

The feces of the selected animals were collected from rectal content of the animals .The samples were then transported to laboratory to aseptically by using plastic bags .The fecal samples were used for identifying parasitic eggs by Microscopic fecal examination of smear.

**3.7.4. Fecal Examination**

The commonly occurring intestinal parasitic infections of Cattle are efficiently diagnose by microscopic examination of fecal samples by direct smear.

**3.8. Data analysis**

The data obtained were imported, stored and coded according to recorded information in the questionnaire regarding Infectious & non infectious diseases in high yielding variety dairy cow in the data sheet using the Microsoft Excel-2007 program. These data were brought to the SPSS 16.0 for t-test. The level of significance was determined at P<0.05.

**CHAPTER-IV**

**RESULT AND DISCUSSION**

**4.1.Table-2 : Prevalence of sub clinical mastitis in the different dairy farms in Chittagong region**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of the farm** | **Tested of Milk** | **Positive result** | **Prevalence (%)** |
| Nahar Dairy farm | 6 | 3 | 50.0% |
| Wahid dairy farm | 10 | 3 | 30.0% |
| A.S.dairy farm | 9 | 3 | 33.0% |
| Friends dairy farm | 8 | 2 | 25.0% |
| Rubel dairy farm | 9 | 4 | 44.0% |
| Total | 42 | 13 | 30.9% |

Graph-2: Prevalence of sub clinical Mastitis in different dairy farms

From the above table and graph we found that prevalence of subclinical mastitis high in Nahar dairy farms 50.0% where 3 milk samples were positive out of 6 samples. Total prevalence of subclinical mastitis found 30.95%.

**4.2. Table-3: Prevalence of Paramphistomum in the different dairy farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of the farm** | **Tested of feces** | **Positive result of paramphistomum** | **Prevalence (%)** |
| Nahar Dairy farm | 6 | 2 | 33.0% |
| Wahid dairy farm | 10 | 6 | 60.0% |
| A.S.dairy farm | 9 | 3 | 33.0% |
| Friends dairy farm | 8 | 3 | 37.0% |
| Rubel dairy farm | 9 | 4 | 44.0% |
| Total | 42 | 18 | 42.8% |

**Different Dairy farms**

Graph-3: Prevalence of Paramphistomum in the different dairy farms

Prevalence of paramphistomum was high in wahid dairy farm and Rubel dairy farms (60.0% and 44.0%) respectively. The lowest prevalence of Paramphistomum about 33.0%. Overall Prevalence of Paramphistomum in high yielding varieties 42.8%.

**4.3. Table-4: Hematological parameters test in relation to Mastitis of different dairy farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **CMT test** | **Mean±SD** | **P-value** |
| ESR | Positive | 1±0.654 | 0.547 |
| Negative | 1.21±0.425 |
| PCV | Positive | 27.42±5.223 | 0.330 |
| Negative | 28.35±5.32 |
| RBC | Positive | 6.85±1.305 | 0.330 |
| Negative | 7.089±1.332 |
| WBC | Positive | 9.1414±1.741 | 0.329 |
| Negative | 9.450±1.77 |
| Hb | Positive | 8.30±0.4864 | 0.101 |
| Negative | 8.92±0.687 |
| Lymphocyte | Positive | 72±4.727 | 0.067 |
| Negative | 70.50±8.234 |
| Neutrophil | Positive | 15.42±3.4086 | 0.579 |
| Negative | 14.28±6.47 |
| Eosinophil | Positive | 7.71±3.302 | 0.558 |
| Negative | 7.571±2.79 |
| Monocyte | Positive | 4.487±3.387 | 0.943 |
| Negative | 3.928±4.445 |
| Basophil | Positive | 0.4286±o.534 | 0.125 |
| Negative | 0.2143±0.425 |

Total no of 21 blood samples was collected and 5 samples was positive in subclinical mastitis.

**4.3.1.ESR**  
 The Erythocyte sedimentation rate (ESR) of subclinical mastitis affected cows was found lower than normal cows. The variation in ESR of the cow of two groups was statistically Insignificant (P>0.05). The mean ESR in subclinical mastitis affected and normal cows were 1.00 and 1.21, respectively.

**4.3.2. PCV**

The results of the present study showed that higher PCV in normal cows (27.42) then affected cows. The PCV was usually in the upper 20s in adult cattle and slightly higher in calves (Navarre Christine, 2007).

**4.3.3. Hb (%)**

Our study reveals that Hb concentration in normal cows was higher than infected cows (8.30±0.4864). In female Holstein Cattle Hb% was higher in calves ( at 1-14 days old is Hb (gm/dl) 5.7-15.8 , at 2wks -6 months old Hb (gm/dl) 8.5-14.1) than the adult cows ( at 2 year + old is Hb (gm/dl) 8.5-13.2 ) ( Lumsden *et al* ., 1980).

**4.3.4. RBC**

The Red blood corpuscles (RBC) of subclinical mastitis affected cows were found lower than normal cows. The relation in RBC of the cow of two group was statistically insignificant (P>0.05).The average RBC in subclinical mastitis affected and normal cows were 6.89 and 7.09 respectively.

**4.3.5 WBC**

The relation in WBC of the cow of two group was statistically insignificant (P>0.05).The White blood corpuscles (WBC) of subclinical mastitis affected cows were found lower than normal Cows. The average RBC in subclinical mastitis affected and normal cows were 9.14 and 9.450 respectively.

**4.3.6. Differential count**

In this study the total differential count is lower in normal cows than subclinical mastitis cows. Though it was insignificant but it may be due to nutritional deficiency that occurs in starvation or anorexia that cause neutropenia (Spivak *et al.,* 1984). Eosinophil was higher in cows and it was significant. Possible causes of eosinophilia in cows were parasitic infestation (Davidson *et al.,* 1998; Candyce *et al.,* 2003). Differences in Basophil % are not significant.

**4.4. Table-5: Biochemical parameters of blood test in relation to Mastitis of different dairy farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **CMT test** | **Mean±SD** | **P-value** |
| Calcium | Positive | 12.46±4.00 | 0.048 |
| Negative | 11.54±1.87 |
| Magnesium | Positive | 3.37±1.429 | 0.427 |
| Negative | 2.28±1.150 |
| Phosphorus | Positive | 5.78±0.98 | 0.048 |
| Negative | 4.98±0.53 |

**4.4.1.** **Calcium**

Calcium level found in infected cows was 12.46±4.00mg/dl and 11.54±1.87mg/dl in non infected cows. Lower percentages in lactating cows may be due to calcium losses during milk production. In older animal there was a decreased need for calcium (Ca) and Pi for this purpose and this was why lower calcium level in blood levels of cows (Doornenbal *et al.,* 1988). The variation in Calcium of the cow of two group was statistically significant (P<0.05).

**4.4.2. Phosphorus**

There was an increase in phosphorus level in infected cows 5.78±0.98mg/dl .

Other hand the normal cows having 4.98±0.53mg/dl. The variation in phosphorus of the cow of two group was statistically significant (P<0.05).

**4.4.3. Magnesium**

I found the Magnesium level of subclinical mastitis affected cows were found lower than normal cows. The variation in RBC of the cow of two group was statistically insignificant (P>0.05).

**4.5. Table- 6: Hematological parameters test in relation to Paramphistomiasis of different dairy farms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **Paramphistomiasis** | **Mean±SD** | **P-value** |
| ESR | Positive | 1.00±0.707 | O.753 |
| Negative | 1.18±0.44 |
| PCV | Positive | 25.6±4.4 | 0.425 |
| Negative | 28.82±5.28 |
| RBC | Positive | 6.40±1.11 | 0.425 |
| Negative | 7.20±1.3 |
| WBC | Positive | 8.43±1.58 | 0.425 |
| Negative | 9.60±1.76 |
| Hb | Positive | 8.26±0.167 | 0.004 |
| Negative | 8.82±0.726 |
| Lymphocyte | Positive | 72.0±8.21 | 0.928 |
| Negative | 70.78±7.08 |
| Neutrophil | Positive | 15.6±3.36 | 0.629 |
| Negative | 14.37±6.16 |
| Eosinophil | Positive | 5.80±2.164 | 0.385 |
| Negative | 8.18±2.90 |
| Monocyte | Positive | 4.4±0.894 | 0.154 |
| Negative | 4.81±4.532 |
| Basophil | Positive | 0.2±0.447 | 0.301 |
| Negative | 0.4±0.234 |

7 samples were Paramphistomiasis affected found out of 21 blood samples, then go to further analysed**.**

**4.5.1.ESR**  
The variation in ESR of the cow of two groups was statistically Insignificant (P>0.05). The mean ESR in Paramphistomiasis affected and normal cows were 1.00 and 1.18 respectively. The Erythocyte sedimentation rate (ESR) of Paramphistomiasis affected cows was found lower than normal cows.

**4.5.2. PCV**

The results of the present study showed that higher PCV in normal cows then Paramphistomiasis affected cows(25.6%) but the PCV was usually in the upper 20s in adult cattle and slightly higher in calves (Navarre Christine, 2007).

**4.5.3. Hb%**

In female Holstein Cattle Hb% was higher in calves ( at 1-14 days old is Hb (gm/dl) 5.7-15.8 , at 2wks -6 months old Hb (gm/dl) 8.5-14.1) than the adult cows ( at 2 year + old is Hb (gm/dl) 8.5-13.2 ) ( Lumsden *et al* ., 1980). Our study reveals that Hb concentration in normal cows was (8.82%) which was higher than Paramphistomiasis infected cows (8.26%).

**4.5.4. RBC**

The relation in RBC of the cow of two group was statistically insignificant (P>0.05).The average RBC in subclinical mastitis affected and normal cows were 6.40and 7.20 respectively. The Red blood corpuscles (RBC) of Paramphistomiasis affected cows were found lower than normal cows.

**4.5.5 WBC**

The relation in WBC of the cow of two group was statistically insignificant (P>0.05).The White blood corpuscles (WBC) of Paramphistomiasis affected cows were found lower than normal Cows. The average RBC in Paramphistomiasis affected and normal cows were 8.43 and 9.30 respectively.

**4.5.6. Differential count**

Though it was insignificant but it may be due to nutritional deficiency that occurs in starvation or anorexia that cause neutropenia (Spivak *et al.,* 1984). Eosinophil was higher in cows and it was significant. Possible causes of eosinophilia in cows were parasitic infestation (Davidson *et al.,* 1998; Candyce *et al.,* 2003). Differences in Basophil % are not significant. In this study the total differential count is lower in normal cows than In this study the total differential count is lower in normal cows than Paramphistomiasis cows.

**4.6.Table-7: Biochemical parameters of blood test Paramphistomiasis of different dairy farms**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Paramphistomiasis | Mean±SD | P-value |
| Calcium | Positive | 11.90±1.62 | 0.872 |
| Negative | 11.82±3.00 |
| Magnesium | Positive | 3.33±1.20 | 0.278 |
| Negative | 2.44±1.32 |
| Phosphorus | Positive | 4.93±1.53 | 0.970 |
| Negative | 4.41±1.57 |

**4.6.1.** **Calcium**

The variation in Calcium of the cow of two group was statistically insignificant (P>0.05). In older animal there was a decreased need for calcium (Ca) and Pi for this purpose and this was why lower calcium level in blood levels of cows (Doornenbal *et al.,* 1988).Calcium level found in infected cows was 11.90±1.62mg/dl and 11.82±3.00mg/dl in non infected cows. Lower percentages in lactating cows may be due to calcium losses during milk production.

**4.6.2. Phosphorus**

There was an increase in phosphorus level in Paramphistomiasis infected cows 4.93±1.53mg/dl. Other hand the normal cows having 4.41±1.57mg/dl. The variation in phosphorus of the cow of two group was statistically significant (P<0.05).

**4.6.3. Magnesium**

I found the Magnesium level of Paramphistomiasis affected cows were found lower than normal cows. The variation in RBC of the cow of two group was statistically insignificant (P>0.05).

**CHAPTER-V**

**CONCLUSION**

It can be concluded from the study that the Hb, PCV, TEC, Lymphocyte and Eosinophil level of subclinical mastitis infected cows was lower than the non infected dairy cows Sometimes the results were not found similar with standard level due to different procedure, such as automatically and manually. Variation of hematological parameter also occurs due to few no. of cases, breed, age, sex, nutritional status and different conditions.Maximum result is not significant due to study short period, small sample sizes.So it can be go for further study.

**CHAPTER-VI**

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**APPENDIX**

**Survey on haematological changes in sub clinical mastitis and paramphistomiasis affected high yielding dairy cows under chittagong district**

**Questionnaire form**

1. Farm name…………………………………………
2. Mobile no…………………………………………..
3. Address of farm: vill……………….Thana…………….dist……………
4. Total area of farm…………………………………….
5. Farm composition. Adult’s cows………Milking……. Calf: male…………Female……Pregnant…………
6. Types of feed offer: I. Roughages+ Concentrate

II.Mostly concentrates+ Small Roughages

7. Types of breeding system: Natural…% AI….% Both

8. Vaccination: yes /no ………………………………

9. Deworming: Yes /no

10.Different vaccination & anthelmintics are given:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name of vaccine | Age at which given | No of animals | Name of anthelmintics | Admin & dosages | No of animals |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

11. What do you do when your farm animals get sick…….Call vet.../Trained farm employee/others

12. Last year (annual) Frequency of peripartum Events….

a) Abortion……………………………

b)Stillbirth…………………………….

c) Dystocia……………………………..

d) Retained fetal membrane…………………

e)Vaginal prolapsed…………………………

f)Uterine prolapsed…………………………..

g) Endometritis……………………………….

h)Pyometra…………………………………..

13. Any surgical intervention needed in last year: yes /no

14. If yes how many cow………………………….

15. Teat dipping. Yes/no

16. Footbath use: Yes/no

17. Frequency of common diseases & diseases condition in last full year………….

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Diseases type | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| FMD |  |  |  |  |  |  |  |  |  |  |  |  |
| Milk fever |  |  |  |  |  |  |  |  |  |  |  |  |
| Foot rot |  |  |  |  |  |  |  |  |  |  |  |  |
| Mastitis |  |  |  |  |  |  |  |  |  |  |  |  |
| Parasitic infestation |  |  |  |  |  |  |  |  |  |  |  |  |
| Arthritis |  |  |  |  |  |  |  |  |  |  |  |  |

18. Type of services wants to get from various intuitions ……………....

**Thanks a lot for your kind cooperation**