بسم الله الرحمن الرحيم

Effect Of Bovine Theileriosis On Haematology And Serum Constituents In Calves

BY

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Dedication

To
My father and mother,
all my brothers and sisters,
all my friends,
and my daughter
Nada

Acknowledgement

I introduce my thankfulness to all people who provided the materials of this thesis . I'm specially grateful to ***Dr. Ahmed Gubara A/ Rahim****, the supervisor,* ***Dr. Barakat ALhussien*** *and the staff of the Department of Biochemistry, University of Khartoum for their lending hands to bring out this work and enabling me to obtain information necessary to write this thesis .*

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ملخص الدراسة

الغرض من هذه الدراسة البحث في كيمياء مصل الدم والصور الدموية في العجول الهجين (فريزيان ـ كنانه) المصابة بداء الثايليرا والتي تتراوح أعمارها ما بين 1-3 أشهر من الجنسين الذكور والإناث .

تم تحديد 31 عجل مصاب بداء الثايليرا وتم تحديد العامل المسبب للمرض وذلك باخذ مسحات دموية من وريد الأذن وكذلك أخذ خزعات من العقد اللمفاوية .

كذلك لقد تم الإهتمام بالعلامات السريرية المميزة للمرض ، وأخذ عينات برازية لإستبعاد وجود طفيليات داخلية .

تمت مقارنة نتائج مصل العجول المصابة بالمرض مع نتائج مصل عجول أخرى غير مصابة بالمرض تم إستجلابا من مزارع الهيئة العربية للإستثمار والإنماء الزراعي بالباقير .

العجول المصابة التي لم تظهر أجسام كوخ (الشايزونت) **Schizont** في مسحات الخذعات من العقد اللمفاوية تم إستبعادها من الدراسة.

العينات الحقلية للعجول المصابة في هذه الدراسة تم جمعها من مزرعة جامعة الخرطوم بشمبات ، كذلك شملت الدراسة جمع عينات من مزارع بالسامراب والحلفايا وكذلك مزرعة السلطة القضائية بالحاج يوسف .

أوضحت نتائج الصور الدموية أن العجول المصابة بالمرض تعطي معدلات منخفضة في الـ **Packed Cell Volume (PCV)** وتركيز الهيمغلوبين **Haemoglobin** وتركيز الجلكوز مقارنة بالعجول السليمة .

أما في نتائج فحوصات مصل الدم في العجول المصابة مقارنة بالعجول السليمة أظهرت أن البروتين الكلي والالبيومين منخفضة مقارنة مع العجول السليمة .

هنالك إذدياد في اليوريا **Urea** والـ **Total triglycerides** في العجول المصابة مقارنة مع العجول السليمة . كذلك هنالك إذدياد في معدل إنزيمي الـ **Aspartate transaminase** (**AST**) و الـ **Alanine transaminase**((**ALT** في العجول المصابة مقارنة مع العجول السليمة .

أما بالنسبة لعنصري الصوديوم والبوتاسيوم في مصل العجول المصـابة أعطـى الأول إنخفاض طفيف ، ولا يوجد تغيير يذكر في الثاني في العجول المصابة مقارنـة مـع العجـول السليمة.

أما بالنسبة للسيريلوبلازمين **Ceruloplasmin** في مصل العجول المصابة أعطى تراكيز متدنية في مصل العجول المصابة مقارنة مع مصل العجول السليمة .

أما المعادن مثل النحاس والزنك أعطت معدلات منخفضة في العجول المصابة مقارنة مع العجول السليمة .

Abstract

The aim of this study is to evaluate serum chemistry and haematological pictures in cross-bred calves (Friesian, kenana) of both sex naturally infected with theileriosis. Their ages ranged between 1-3 month.

Thirty one naturally infected calves with theileriosis were selected, and the causative agent of the disease was determined, by blood smears from the ear vein and lymph node biopsy smears. The clinical signs of the disease were also observed. Faecal samples were taken from infected calves and control calves to exclude the presence of internal parasites. Comparison between the results of haemology and serum of the infected calves and the results of haemology and serum of the healthy calves was made.

The samples of the healthy calves were brought from the farm of the Arabic Corporation For Investment And Agricultural Development in Al bagair. The infected calves which did not reveal theilerial schizonts in their lymph node biopsy smears were not included in the study.

Field samples of the infected calves were collected from the farm of University of Khartoum in Shambat , farms in Alhalfia, Alsamrab and Judge service administration farm in Alhag yousif.

The haematological results of the infected calves showed low levels of Packed cell volume (PCV), haemoglobin concentration and glucose concentration compared to healthy calves.

Serum chemistry of the infected calves showed low levels of total protein and albumin compared to healthy calves. Serum urea and triglycerides of the infected calves showed high levels compared to

healthy calves. Serum Alanine transaminase (ALT) and Aspartate transaminase (AST) were increased in the infected calves compared to healthy calves.

Serum sodium and potassium showed slightly decrease in the first and no changes in the latter in the infected calves compared to healthy calves.

Serum ceruloplasmin concentration gave low levels in the infected calves compared to healthy calves.

Minerals copper and zinc gave low levels in the infected calves serum compared to healthy calves.

Chapter One

Introduction

Theilerioses are group of tick-borne diseases of cattle, sheep, goats, buffalo , and occasionally of wild ruminants caused by species of protozoa in the genus theileria (losos , 1986). Theileriosis annulata is an infectious , virulent , inoculabe , non contagious disease that affect cattle , caused by Theileria annulata , and transmitted by ticks of genus Hyalomma.

Theileriosis infection is characterized by pyrexia, anorexia, enlargement of superficial lymph nodes, nasal discharges, lacrymation, bilateral exophthalmia, pale and occasionally icteric mucous membranes, diarrhoea and depression (Mira and Ralph, 1989).

Distribution of bovine tropical Theileriosis extends in wide belt of tropical and subtropical zones, and the incidence of the vector tick was determined in these zones (Purnell , 1978). H. a. anatolicum is the efficient tick vector in Sudan.

Theileriosis infection causes severe economic losses due to the high incidence of mortality in infected animals particularly calves of small ages, drop of milk production in the infected cows, expensive measurement of prevention and control, expensive of antitheilerial drugs and the infected animals remain as healthy carriers and potential sources of theileria infection.

The aim of this study is to evaluate the effect of theileriosis infection on haematological parameters such as packed cell volume (PCV),haemoglobin concentration and glucose concentration. Also serum chemistry such as total protein, albumin, urea, triglycerides, the

enzymes aspartate transaminase (AST) and alanine transaminase (ALT), ceruloplasmin, sodium, potassium, copper, zinc, of the infected calves.

Chapter Two

Literature Review

Theilierioses are group of tick-borne diseases of cattle, sheep, goats, water buffalo, and occasionally of wild ruminants caused by species of protozoa in genus theileria (losos, J. George 1986).

2.1 Aetiology of tropical theileriosis:

Bovine tropical theileriosis, or theileriosis annulata is an infectious, virulent, inoculable, non contagious disease that affect cattle and caused by the protozoon Theileria annulata. Transmission occurs only after cyclical development in the tick vector. The disease is characterized by generalized febrile adenitis. The disease is also known as tropical piroplasmosis theileriosis annulata and mediterranean coast fever (Robinson, 1982).

Piroplasmosis in cattle was not known to occur in Sudan up to 1905 when Balfour (1905) found a small piroplasm in the blood cell of Sudanese cattle. In 1939 the name Theileria annulata of tropical theileriosis was then realized and became of common occurrence all over the country.

According to Fischer and Say, (1989). T. parva is the causative agent of upland tropical African theileriosis of cattle. It is distribution coincides with that of it is vector tick Rhipicephaulus appendiculatus in Equatorial East African and high altitude Southern African, and Rh. duloni on Angola Plateau. T. annulata is the causative agent of tropico- mediteranean basin theileriosis with the predominance of circular or piriform trophozoites.

2.2 Geographical distribution:

According to Purnell, (1978) the disease is prevalent throughout tropical and subtropical zones, North Africa, South wards down to Sudan and Eriterria and east wards up to South East Europe, the Near East and across the Indian subcontinent to China and the far east. T.annulata has not been reported in East Africa and the southern limit of its distribution is the Sudan and this limit does not overlap with that of T.parva and T. lawrenci (Neitz, 1957).

Distribution of T.annulata has been shown to coincide with that of it is vector Hyalomma.d.detritum in the Mediterranean basin and H.a.anatolicum in Central Western Asia, Egypt and Northern Sudan (Fischer and Say, 1989).

2.3 Vectors:

The disease is transmitted by species of the tick of genus Hyalomma (Koch, 1844, 1847). The distribution of the disease coincides with the distribution of the vector species and it seems likely that in some areas where the vector ticks are present, but the disease has not been reported. Careful investigation may show the parasite to be present (Purnell, 1978).

According to Robinson, (1982) many species of Hyalomma have been shown to transmit T,annulata either naturally or experimentally. The transmission may occur by bite or by being inoculated in the form of crushed infected ticks suspension.

H.anatolicum is reported as a transmitter of the disease in north Sudan (Hoogstral, 1950 and Jonge Jan et al., 1983). Egypt (Hassan, 1986). Kwait (Ahmed et al., 1987). Turkey (Dumnali, 1987), India (Sastry et al., 1980). Bhattacharyulu, and Samntry, (1989). Afghanistan (Bulman et al., 1979) and Russia (Dubovy, 1982).

2.4 Species Involved:

According to Soulsby (1982), bovine, zebu, and water buffalo are the most susceptible to infection. Bos.taurus and Bos. indicus were found by Dhar et al., (1973) to be the commonly affected species. Robinson, (1982) reported that the exotic breeds are generally more susceptible to infection than indigenous breeds. Sheep were shown to be refractory to infection (Bernett, 1977) and no wild mammals reservoirs were reported (Uilenberg, 1976).

T.annulata was found to be highly pathogenic to susceptible friesian and cross friesian with kenana in Sudan causing mortality over 80% (F.A.O, 1983). Serological surveys showed the parasite to be endemic in the North Sudan, Khartoum state and Gezera area extending as far as south Sinnar district (F.A.O, 1983).

2.5 Clinical signs of theileria infection:

As reviewed by Fischer and Say, (1989), theileria inoculation being 3-days after attachment of adult Hyalomma and Rhipicephalus. Incubation requires 1-3 weeks depending on the initial schizonic cycle in the lymph node corresponding to the site of the infected tick bite. This primary adenitis occurs before hyperthermia. The spread of the merozoites through out the lymphatic system where the schizogony occurs, is accompanied by the rise in the temperature up to 41-42ºC. The fever continued for 1-3 weeks until death or recovery of the animal. The appearance of the lymphoblast schizonts coincides with the attach of fever. Erythrocytes trophozoites are found in the periferal blood after several days in the case of recovery. The lymphoblast schizonts became scarce and disappear, only the erythrocytes trophozoites remain until the end of an acute attack.

The clinical signs associated with hyperthermia is generalized adenitis, invasion and tugescence of the small submucosal lymph nodes inducing mucosal congestion revealed by epistaxis and lacrymation (with swelling of the eyelids and petichiae on the conjunctiva).

Obstruction of the reticuloendothelial system coincides with the destruction of the parasitized erythrocytes by phagocytosis. This condition induces anaemia, but haemolysis is not usually followed by subicterus may some times be observed. Other general signs are pulmonary oedema, ruminal atony and alternative constipation, diarrhoea, emaciation and depressions. In addition to that the enlargement of the superficial lymph nodes due to the multiplication of the parasite, bilateral exophthalmia and nasal discharges.

Death occurs within one week in the peracute form, and in the acute form during the second week. The subacute form lasts 2-3 weeks and ends with recovery (corridor diseases). Recovery of cattle is only possible when the disease is mild.

2.6 Postmortem findings:

The carcass shows evidence of considerable emaciation amounting to cachexia. The muscles are pale and degenerated. All the lymph nodes are hypetrophic, fleshy oedematous and sometimes bloody on pulby. The spleen is hypetrophic but remains firm. The liver is enlarged, light brown in colour possibly with subcapsular haemorrhages, icterus is inapparent or not very pronounced. The kidneys are oedematous, congested with red and white infarction. The heart shows petichial haemorrhages. The lungs showed oedema. The digestive tract represents haemorrhages and necrotic patches (Soulsby, 1982).

2.7 Diagnosis of bovine theileriosis:

Despite the fact that clinical picture of the disease is not pathonomic (Lossos, 1986) diagnosis is presumptive on the basis of acute febrile

onset with swollen of the peripheral lymph nodes in cattle in areas known to be endemic (Siegmund, 1979).

The disease can be confirmed by detecting the schizont form in the lymphocytes obtained from lymph nodes or by spleen puncture or by detecting the piroplasms in the red blood cells (Soulsby, 1982).

Some serology can be obtained such as enzyme linkage immuno sorbent assay (ELISA) and complement fixation test.

Chapter Three

Materials and Methods

Determinations of the theilerial parasites either the piroplasms in the blood smears or schizonts in lymph nodes biopsies, and the faecal samples for exclusion of the internal parasites of the infected calves was done. The haematology of both infected and healthy calves was also done. Serum total protein, albumin, urea, triglycerides, aspartate transaminase (AST) and alanine transaminase (ALT), ceruloplasmin, sodium and potassium, copper and zinc of both infected and healthy calves were determined.

3.1 Animals:

Thirty one Gross-bred calves, their ages ranged 1-3 months of both sex were used in this study. They were kept in zero grazing system. This thirty one calves showed typical clinical signs of the theileriosis infection which are characterized by pyrexia, increased plus rate, increased respiration rate, tachycardia, enlargement of the superficial lymph nodes, nasal discharges, lacrymation, diarrhoea, bilateral exophthalmia, pale or icteric mucous membranes and depression.

Blood smears and lymph nodes biopsies smears were taken and stained by 10% Giemsa's stain. Ten samples from healthy calves were brought from the Arabic Corporation for Investment and Agricultural Development farm in Albagair and considered as the negative control. Calves of which lymph nodes biopsies smears did not show theilerial schizonts were excluded.

3.2 General clinical examination:

The clinical examination was conducted following standard method of Blood et al., (1985). Physical condition, posture, gait, abnormal behavior were examine as well. Other important parameters such as auscultation and percussion of the lung, respiration rate, pulse rate, body temperature were measured.

3.3 Area of Investigation:

Field samples of naturally infected calves were collected from the farm of University of Khartoum in Shambat, also Alsamrab, Alhalfia, and

Judge Service Administration farm in Alhagyousif. Farms localized in these areas showed very poor hygiene and traditional management. Tick control in these areas did not follow the suitable way, that facilitate the abundance of many clinical cases.

3.4 Sampling:

3.4.1 Blood Smears:

The area on the outer surface of the pinna of the ear near the margin of each calf was shaved. After wiping the exposed skin with ethanol, the margin ear vein was punctured with a sharp-pointed needle, then quickly a drop of blood was put onto a slide margin and drawn by the aid of another slide, allowed to dry and fixed with absolute methanol for 5-minutes.

3.4.2 Lymph node biopsy smears:

The site of the parotid or prescapular lymph node was shaved. A sterile disposable syringe was inserted into the lymph node and the lymph node materials was drown out by negative pressure. A drop from these materials was dropped onto a slide margin and drawn by the aid of another slide, allowed to dry and fixed with absolute methanol for 5-minutes. Then kept until examined.

3.4.3 Staining:

10% of Giemsa's stain was used to examine the blood smears and lymph node biopsy smears slides. These slides were put in an absolute methanol for 5-minutes, then allowed to dry. These slides were put into 10% Giemsa's stain for 30minutes, washed with distilled water for 30 seconds, dried and examined under the light microscope at (100x). When ever examining a smear a drop of emersion oil was dropped onto it.

3.4.4 Faecal samples:

3.4.4.1 Sedimentation:

Small amount of faeces was placed in a centrifuge tube. Water was added and mixed thoroughly with a glass rod. The tube was filled with water and centrifuged at 1500 rpm for 5-minutes. Then the supernant was decanted. By using the pipette a drop from the top layer of the sediment was placed onto the slide, another from the middle layer and a third from the bottom layer. The three slides were covered with cover slides and examined systematically under the lower power (100X).

3.4.4.2 Flotation:

Small amount of faeces was taken in a cartney bottle. The faeces was covered with saturated salt solution, mixed well, the bottle was filled to the rim with saturated salts solution and few drops of salts solution was added in excess. The bottle was covered with cover slide. The cover slide was removed after one hour and examined systematically using the lower magnification (100x).

3.4.5. Whole blood samples:

The area of the jugular vein was cleaned with ethanol. The vein was raised using a tourniquet round the neck. The skin over the vein was tested before inserting the needle. A heparinized glass vacutainer with tube holder and double ended needle was used. Then the blood was gently distributed inside the vacutainer and kept at 4ºC in a plastic blood container until examined.

3.4.6 Serum samples:

It is the same as the procedure above except that the glass contained no anticoagulant. The vacutainer tubes were placed away from direct light for at least one hour, centrifuged, then the clear serum was replaced into sterile serum container using sterile Pasteur pipette. The serum collected was freezed until examined.

3.5 Haematological parameters:

3.5.1 Packed cell volume "PCV":

A sample of heparinized blood was drawn in the capillary tube, sealed with crystaseal centrifuged for 5-minutes. Then read at the haematocrit reader.

3.5.2 Haemoglobin concentration:

Principle:

Ferrous ions of haemoglobin are oxidized to the ferric state by potassium ferricyanide to form hemoglobin (methemoglobin). Haemoglobin reacts with cyanide to form hemoglobin cyanide (cyanmethemoglobin) that can be measured by colorimeter.

Reagents:

- Haemoglobin standard solution 15 gm/100 ml.
- Cyanide reagent (Drabkin's solution).

This was made by dissolving 150 mg of potassium dihydrogen orthophosphate, 50 mg of potassium ferricyande and 200 mg of potassium cyanide in one liter of distilled water.

Technique:

Two test tubes were prepared for the sample and standard as follows:

4.0ml of Drabkin's solution were placed in both, sample and standard test tubes. In the sample test tube 20 µL of the blood sample was added. In standard test tube 20 µL of the standard haemoglobin was added. The tubes were shaken well and let to stand for 15 minutes, then read at 540 nm using colorimeter. The Drabkin's solution was used as a blank.

Calculation:

Haemoglobin concentration g/100ml

$$= \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \text{concentration of the standard}$$

3.5.3 Glucose concentration:

Glucose concentration in the plasma was determined according to Trinder P. Ann, (1969) method.

Principle:

Enzymatic color test on the basis of Trinder reaction.

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconate} + H_2O$$

$$2\,H_2O_2 + \text{Phenol} + 4 - \text{aminoantipyrine} \xrightarrow{\text{Peroxidased}} \text{chinonimin} + 4H_2O.$$

Reagents concentration:

Reagent one "R_1" buffer reagent was composed of phosphate buffer, PH 7.5 150 mmol/L and phenol 7.5 mmol/L.

- Reagent Two "R_2" enzyme reagent composed of GOD 12000 u/L, POD 660U/L and 4-aminoantipyrine 0.40 mmol/L.
- Reagent for "R_4" was the standard and it was composed of glucose standard 100mg/dl.
- Preparation of the working reagent:

The contents of the enzyme reagent (R_2) was diluted with the corresponding volume of buffer (R_1).

Technique:

Three test tubes were prepared as the blank, standard and the unknown respectively. 1000 µL of the working reagent was added in the tube of the blank. 1000 µL of the working reagent and 10 µL of the standard (R_4) were added in the standard tube. 1000 µL of the working reagent and 10 µL from the sample (Plasma) were added in the unknown test tube. Then incubated for 15 minutes at 37°C. Within 60 minutes the absorbance were taken using the colorimeter at 550 nm.

Calculation:

Glucose concentration mg/dl =

$$\frac{\text{Optical density of the unknown}}{\text{Optical density of the standard}} \times \text{standard concentration}$$

3.6 Serum chemistry:

3.6.1 Determination of serum total proteins:

Determination of serum total proteins was performed following Reinhold (1953) method.

Principle:

The determination of total protein by Biuret method depends on the precipitation of globulins by a mixed sodium sulphate and sulphite solution in proportions of 3:1.

Reagents:

Sulphate-sulphite solution (278g/L) :

208g of anhydrous sodium sulphate and 70g of anhydrous sodium sulphite were dissolved in 900ml of distilled water to which 2 ml concentrated sulphuric acid was added in 2-liter beaker. Then the pH was set above 7.0, and the solution was kept at 37°C in the incubator.

Stock Biuret reagent:

45g of Rochelle salt was dissolved in 400ml of 200 mmol/L sodium hydroxide, then 15g of copper sulphate was added and followed by 5g of potassium iodide. Thereafter, the solution was made up to a liter with 200 mmol/L of sodium hydroxide.

Biuret reagent for use:

200 ml of the stock reagent was diluted to a liter with 200 mmol/L of sodium hydroxide containing 5g of potassium iodide per liter.

Tartarate-iodide solution:

9g of Rochelle salt was dissolved in 200 mmol/L of sodium hydroxide containing 5g potassium iodide/L.

Standard serum:

Bovine serum g/100 ml.

Technique:

6.0 ml of sulphate-sulphite solution were pipetted into centrifuge tube and into it 0.4 ml of the serum were added, and mixed well, then 2ml of the mixture was removed and added to 5 ml of biuret reagent in test tube.

Serum blank: 2ml serum-sulphate-sulphite mixture was added to 5ml tartrate-iodide solution and was mixed well.

Biuret blank: 2ml of sulphate-sulphite solution was added to 5 ml of the biuret reagent.

Standard: 0.4 ml of the standard serum was pipetted into 6.0 ml of the sulphate-sulphite solution as above, and 2ml of the mixture was transferred into 5ml of biuret reagent in a test tube.

Standard serum blank: It was prepared as described for test serum blank.

After shaking, all tubes were placed in a water bath at 37ºC for 10 minutes. Then they were allowed to cool for 5 minutes at room temperature. Reading by (Jenway 6105, u.v/Vis. Spectrophotometer) was at 540-560nm wave length. Serum blank was read against the tartrate-iodide solution, and the test and standard against the biuret blank.

Calculation:

Serum total protein g/L =

$$\frac{\text{Reading of unknown} - \text{Reading of unknown serum blank}}{\text{Reading of standard} - \text{Reading of standard serum blank}} \times \text{concentration of standard}$$

3.6.2 The determination of serum albumin:

The determination of serum albumin was performed using Modified Spencer and Prince, (1977) method.

Principle:

This method depends on the binding of bromocresol green (BCG) with albumin at pH 4.1 using succinate buffer.

Reagents:

Stock succinate buffer, pH 4.1, 0.5 mol/L:

10g of sodium hydroxide and 56g succinic acid were dissolved in 800 ml of distilled water. The pH was adjusted to 4.1 at 20ºC with molar solution of sodium hydroxide. The volume was made up to one liter with distilled water and stored at 4ºC.

Stock BCG dye solution, 10 mmol/L:

1.75 g BCG was dissolved in 5 ml of one mole/ L of sodium hydroxide and was made up to 250 with distilled water. When diluted 1/1000 with succinic buffer, pH 5.3, 0.2 mole/L, and reading was against distilled water at 632 nm, the solution had an extinction of 0.315 $\pm$ 0.015.

Stock sodium a zide:

40g of sodium a zide were dissolved in one liter of distilled water.

Stock Brij 35: 250 g/l:

25g of solid Brij 35 was warmed in distilled water to dissolve and made up to 100 ml with distilled water.

Working dye solution:

500ml of the stock succinate buffer, 40 ml of the stock dye solution, 12.5 ml of the stock sodium a zide, and 12.5 ml of the stock Brig 35were mixed in a 5 liter volumetric flask and kept at 20ºC.

Stock standard albumin solution, 100g / L:

10g of boving serum albumin and 50 mg sodium a zide were dissolved in distilled water and the mixture was made up to 100ml with distilled water.

Working albumin standards:

20, 30, 40, 50 and 60g/L were prepared by diluting the stock standard with a 500mg/L solution of sodium a zide in distilled water, and was kept at 4ºC.

Technique:

20 µL of the serum were added to 4 ml of the working dye solution at 25ºC, then, after 10 minutes, in a cuvette the test was read in the spectrophotometer at 632nm against the blank of the working dye solution. For standard 20 µL of the stock standard albumin solution was added to 4ml of the working dye solution.

Calculation:

Serum albumin (g/l) =

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times \text{concentration of the standard}$$

Where :

40 is the concentration of the standard.

3.6.3 Determination of serum urea:

Determination of serum urea was performed by "Fawcett, & Scott" which is considered as a simple colorimetric test.

Principle:

Urea is hydrolyzed into ammonia and carbon dioxide Ammonia reacts with salicylate and hypochlorite to form green indophenol. The colour intensity is proportional to the concentration of urea.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

Reagents:

Three reagents were considered in this test. Reagent one (R_1) which is composed of phosphate pH 6.7, EDTA 2mmol/L, sodium, salicylate 60mmol/L, sodium nitroprusiate 3.2 mmol/L. Reagent two (R_2) composed of sodium hypochlorite 140mmol/L and sodium hydroxide 150mmol/L. Reagent three (R_3) composed of urease 30000 U/L.

Standard:

The standard was urea 50mg/dl in the form of tablets.

Preparation:

One tablet of (R_3) was dissolved in the bottle of reagent one (R_1+R_3). Reagent two was ready for use.

Technique:

Three test tubes were named as the unknown, standard and blank respectively. In the blank tube 1ml of the solution (R_1+R_3) was added. In the standard test tube 1ml of the solution (R_1+R_3) and 10 µL of standard were added. In the unknown test tube 1 ml of the solution (R_1+R_3) and 10 µL of serum were added then incubated for 5 minutes at 37ºC. 1ml of (R_2) was added to each tube mixed well and incubated for 5 minutes at 37ºC. Readings were taken using colorimeter at 580nm against blank reagent.

Calculation:

Urea mg/dl =

$$\frac{\text{Optical density of the unknown}}{\text{Optical density of the standard}} \times \text{concentration of the standard}$$

3.6.4 Determination of serum triglycerides:

Ttriglycerides were determined after enzymatic hydrolysis with lipase. The indicator is aquinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Principle:

- Triglyceride + H_2O $\xrightarrow{\text{Lipase}}$ glycerol + Fatty acids

- Glycerol + ATP $\xrightarrow{\text{G.K}}$ glycerol – 3 – phoshate + ADP

- glycerol – 3-phosphate + O_2 $\xrightarrow{\text{GPO}}$ dihydroxy acetone phosphate + H_2O_2

- $2H_2O_2$ + 4 – aminophenazone + 4 – chlorophenol $\xrightarrow{\text{POD}}$

quinoneimine + HC1 + 4 H_2O

Reagents:

- Buffer reagent composed of pipes buffer 40 mmol/L, pH 7.6, 4 chlorophenol 5.5 mmol/L, magnesium ions 17.5 mmol/L.
- Enzyme reagent composed of 4 – aminophenazone 0.5 mmol/L. ATP 1.0 mmol/L, lipase $\geq$ 150 U/ml, glycerol – kinase $\geq$ 0.4 U/ml, Glycerol-3- phosphate oxidase $\geq$ 1.5 U/ml, peroxidase $\geq$ 0.5 U/ml.
- Standard reagent 2.29 mmol/L

Preparation of solution:

- Buffer contents ready for use.
- Reagent enzyme: 15ml of buffer reconstituted one vial of the enzyme reagent, and stored at 25ºC.
- Standard reagent: read for use.

Technique:

10 µL of the sample and 1000 µL of the reagent were placed in the test tube. 10 µL of the standard and 1000 µL of the reagent were placed in the standard test tube. Only the reagent was placed the blank test tube. Then the tubes were mixed well and incubated for 5 minute at 37ºC then read at 500 nm using colorimeter.

Calculation:

serum triglycerides mg/dl

Optical density of sample X concentration of the standard 200 mg/dl
Optical density of standard

3.6.5 Determination of serum aspartate transaminase "AST" and alanine transaminase "ALT":

AST and ALT values were determined using Reitman and Frankel (1957) method.

Principle:

The principle of this assay depends on the intermolecular transfer of an amino group from a donor alpha amino acid to an acceptor alpha keto acid with out intermediate formation of ammonia.

Reagents:

Dipotassium hydrogen orthosphosphate	14.61 gram
Potassium dihydrogen orthophosphate	2.17 gram
L-aspartic acid	2.66 gram
L – alanine	1.78 gram
Alpha ketoglutaric acid	1.46 gram
2, 4 dinitro phenol hydrazine	0.0495 gram
Sodium pyruvate	0.022 gram

Buffer phosphate:

14.6 gram of dipotassium hydrogen orthophosphate and 2.17 gram of potassium dihydrogen orthophosphate were dissolved in 800 ml distilled water. The pH was adjusted to 7.5 using sodium hydroxide and hydrochloric acid. Then the volume was made up to one liter and stored at 4ºC.

Colour reagent:

2, 4 dinitrophenyl hydrazine, 0.0495 gram was dissolved in small amount of N hydrochloric acid and shaken well while heating until it

dissolved. Then the volume was made up to 250ml with N hydrochloric acid and kept at room temperature.

Alpha ketoglutaric acid:

1.46 gram of alpha-ketoglutaric acid was dissolved in 50ml distilled water, the pH was adjusted to 7.5 using N sodium hydroxide and N hydrochloric acid.

Alanine transaminase substrate:

1.78 gram of L – alanine was dissolved in 50ml phosphate buffer, the pH adjsuted to 7.5 using N sodium hydroxide and N hydrochloric acid. 1ml of alpha-ketoglutaric acid was added, the volume was made up to 100 ml with phosphate buffer, few drops of chloroform was added and stored at 4ºC.

Aspartate transaminase substrate:

2.66 gram of L – aspartate was dissolved in 60 ml of phosphate buffer, and 22ml of N sodium hydroxide was added, warmed until dissolved, then cooled the pH was adjusted to 7.5. Then transferred in aconical graduated flask. 1ml of alpha-ketoglutaric acid was added and the volume was made to 100 ml with phosphate buffer. Few drops of chloroform were added and stored at 4ºC.

Sodium pyruvate standard solution:

0.02 gram of sodium pyruvate was dissolved in phosphate buffer and made up to 100ml.

Technique:

1 ml of AST substrate was placed in the tube and placed in a water bath at 37ºC to warm for three minutes. 0.2 ml of serum was added, shaken gently and left in the water bath at 37ºC for 60 minutes. 1 ml of colour reagent was added and left at room temperature for 20 minutes. 10 ml of 0.4 N sodium hydroxide was added and waited for 5-minutes. The readings were at 515 nm using spectrophotometer.

Notice:

For ALT the same above mentioned procedure should be followed except that ALT substrate, is used and the incubation period should be for 30 minutes not for 60 minutes as for AST.

Standard AST and ALT:

Set 6 tubes:

Blank

1	2	3	4	5	6	Tubes
1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	Buffer

Standard

0 ml	0.1 ml	0.2 ml	0.3 ml	0.4 ml	0.5 ml	Sod. pyruvate
1 ml	0.9 ml	0.8 ml	0.7 ml	0.6 ml	0.5 ml	Buffer

1ml of colour reagent was added to all tubes, waited for 20 minutes. Then 10 ml of 0.4 N sodium hydroxide was added, waited for 5 – minutes. The readings were taken at 515 nm using spectrophotometer. For each estimation standard carve was drawn.

3.6.6 Determination of serum sodium and potassium:

Serum sodium and potassium were determined using Flamephotometer according to varley et al, (1967 and 1980).

Principle:

In flamephotometer, a solution containing the substance to be determined, was passed under carefully controlled conditions as a very fine spray into the air supply of a burner. In the flame the solution was evaporated and the substance was first converted to the atomic state. As the temperature was raised the thermal energy of the flame made the electrons able to be absorbed. Light of characteristic wave length is emitted and passes through specific filter for sodium or potassium onto a selenium cell, and the amount was read on a gelvanometer.

Reagents:

Stock standard of sodium:

58.5 gram of sodium chloride was dissolved in one liter. of distilled water.

Working sodium standard:

High standard : 8 ml of the stock standard in one liter distilled water.

Low standard : 7 ml of stock standard in one liter distilled water.

Stock standard of potassium:

7.5 gram of potassium chloride was dissolved in one liter distilled water.

Working potassium standard:

High standard : 7ml of stock potassium chloride in one liter distilled water.

Low standard : 5ml of stock potassium chloride in one liter distilled water.

High standard of sodium and potassium:

8ml of high sodium standard and 7ml of high potassium standard, then made up to one liter with distilled water.

Low standard of sodium and potassium:

7ml of low sodium standard and 5ml of low potassium standard, the volume was made up to one liter with distilled water.

Techniques:

The emission flamephotometer was used as follow: the butane, burner, air compressor were adjusted. The zero point of the gelvanometer was adjusted against distilled water, and the 100 point of sodium or potassium was adjusted against the high standard of each, after selecting the wave length key of sodium or potassium. For either sodium or potassium 0.1ml serum sample was diluted in 9.9 ml of distilled water. The concentration of sodium was 140 mEqu/L and of potassium was 5mEqu/L. The diluted sample was read against the low standard of both sodium and potassium.

Calculation:

- Serum sodium (mEqu/L) :

$$\frac{\text{Reading of the unknown X 140 mEqu/L}}{\text{Reading of the low standard of sodium}}$$

- Serum potassium (mEqu/L) :

$$\frac{\text{Reading of the unknown X 5 mEqu/L}}{\text{Reading of the low standard of potassium}}$$

mEqu/L = milliequivalent per liter.

3.6.7 Determination of serum ceruloplasmin:

Serum ceruloplasmin was determined according to Boyd Houchin, C. (1958) method.

Reagents:

- Buffer: This was made by adding of 163 gram of sodium acetate to 20ml of glacial acetic acid and diluted to one liter with distilled water ionic strength 1-2, pH 5-2.
- Paraphenylenediamine “PPD” this was made by dissolving of 0.7g PPD in 100ml of distilled water.
- Sodium azide: this was made by dissolving of 0.02 gram of sodium azide in 100 ml distilled water.

Techniques:

0.1 ml of serum was added to 1.0 ml of freshly prepared 0.7% PPD in acetate buffer at 37ºC. Then incubated for 10 minutes at 37ºC. Then 5ml of sodium azide was added to stop the reaction.The blank was distilled water and the optical densities were determined within 30 minutes using spectrophotometer at 525nm.

When optical density for the oxidase activity was plotted against ceruloplasmin concentration a straight line was obtained fig(3-1),fig (3-2).

Calculation:

Calculations was made by using the formula:

$$y = a + bx$$

Where:

y = ceruloplasmin concentration in mg/100ml

x = copper oxidase activity measured in optical density

a = - 1.7

b = 150

3.6.8 Determination of serum copper and zinc:

Serum copper and zinc were determined according to Dawson, (1968) and Butrimovitz and Purdy, (1977).

Scope:

Serum copper and zinc were determined in serum or plasma using atomic absorption spectrophotometer. Samples were diluted with deionized water. The analysis was performed against standards prepared in glycerol to approximate the viscosity characteristic of the diluted samples.

Sample preparation:

For determination of serum copper the sample was diluted with an equal volume of deionized water. For determination of serum zinc the sample was diluted 1:5 with deionized water.

Analysis:

Serum copper concentration and serum zinc concentration were determined using the conditions listed in the "Standard Condition" Section. Copper standards were prepared by diluting the copper stock standard solution with 10% (v/v) glycerol. A 10% of (v/v) glycerol solution was used as a blank solution.

Zinc standards were prepared by diluting the stock standard solution with 5% (v/v) glycerol. A 5% (v/v) glycerol solution was used as a blank.

Fig (3-1)

Copper oxidase activity expressed in optical density plotted against ceruloplasmin concentration of the control calves

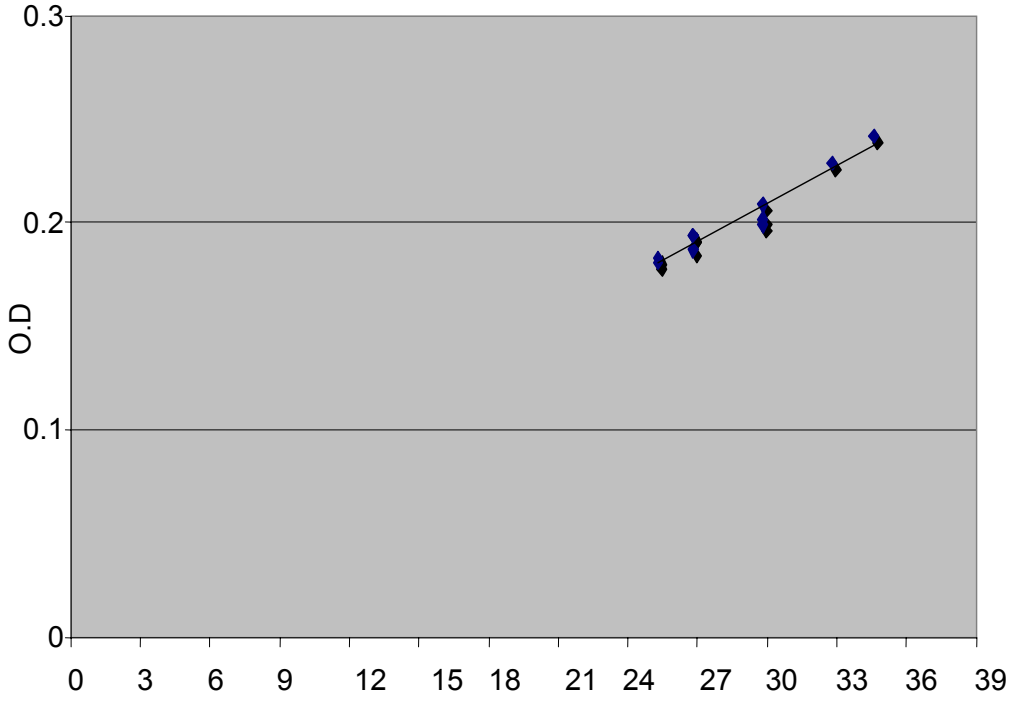


Table (3-1): correlation between copper oxidase activity and ceruloplasmin content of the control calves.

No	Ceruloplasmin mg/dl	copper oxidase optical density
1	34.6	0.242
2	26.8	0.187
3	25.3	0.181
4	26.8	0.194
5	25.3	0.183
6	32.8	0.229
7	29.8	0.209
8	29.8	0.199
9	26.8	0.194
10	29.8	0.202

Fig (3-2)

Copper oxidase activity expressed in optical density plotted against ceruloplasmin concentration of the infected calves:

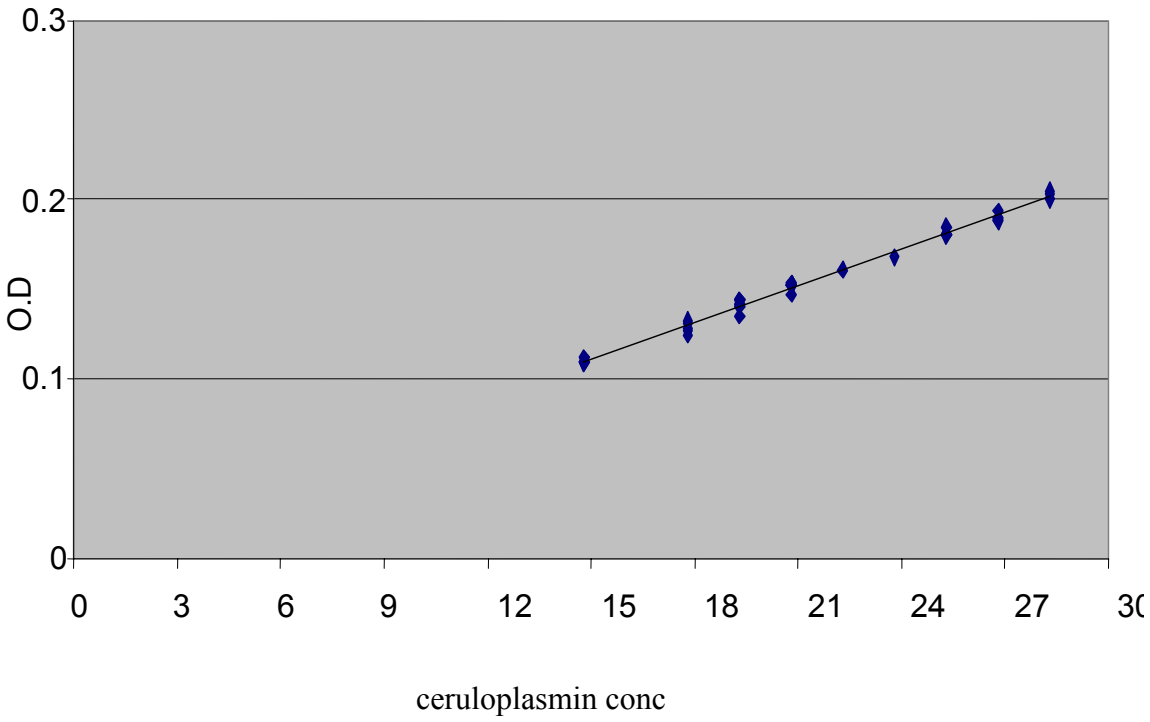


Table (3-2): correlation between copper oxidase activity and ceruloplasmin content of the infected calves

NO	CERULO-PLASMIN mg/dl	COPPER OXIDASE OPTICAL DENSITY	NO	CERULO-PLASMIN mg/dl	COPPER OXIDASE OPTICAL DENSITY
1	28.3	0.203	17	20.8	0.152
2	28.3	0.210	18	17.8	0.125
3	20.8	0.154	19	22.3	0.161
4	19.3	0.144	20	14.8	0.109
5	17.8	0.128	21	25.3	0.180
6	17.8	0.133	22	14.8	0.112
7	19.3	0.141	23	25.3	0.181
8	17.8	0.131	24	23.8	0.168
9	19.3	0.144	25	19.3	0.142
10	20.8	0.153	26	19.3	0.135
11	25.3	0.185	27	28.3	0.200
12	20.8	0.147	28	26.8	0.188
13	14.8	0.109	29	26.8	0.194
14	17.8	0.127	30	20.3	0.153
15	26.8	0.190	31	25.3	0.181
16	28.3	0.205			

Chapter Four

Results

Theileriosis infected calves results were compared with the results of the healthy calves. The infected calves showed typical clinical signs of the disease which characterized by pyrexia, anorexia, pale mucous membranes and, in some, icteric mucous membranes, enlargement of the superficial lymph nodes such as parotid and prescapular lymph nodes, lacrymation and nasal discharges.

The vector of the disease was also observed in the areas of the infected calves.

4-1 Haematological parameters findings

4-1-1 Packed cell volume (PCV):

Table (4-1) and figure (4-1-1) showed that theileriosis naturally infected calves have significantly lower (PCV) values ($P< 0.05$) than the control calves.

4-1-2 Haemoglobin concentration:

As seen in table (4-1) and figure (4-1-2) theileriosis naturally infected calves have significantly lower haemoglobin concentration values ($P< 0.05$) than the control calves.

4-1-3 Glucose concentration:

Table (4-1) and figure (4-1-3) showed that theileriosis naturally infected calves have significantly lower glucose concentration values ($P< 0.05$) than the control calves.

4-2 Serum chemistry Findings:

4-2-1 Serum total protein:

As seen in table (4-2) and figure (4-2-1) theileriosis naturally infected calves have significantly lower serum total protein concentration values ($P< 0.05$) than the control calves.

4-2-2 Serum albumin:

As seen in table (4-2) and figure (4-2-1) theileriosis naturally infected calves have significantly lower serum albumin concentration values ($P< 0.05$) than the control calves.

4-2-3 Serum urea:

Table (4-2) and figure (4-2-2) showed that theileriosis naturally infected calves have significantly higher serum urea concentration values ($P< 0.05$) than the control calves.

4-2-4 Serum triglycerides:

Table (4-2) and figure (4-2-3) showed that theileriosis naturally infected calves have significantly higher serum triglycerides concentration values ($P< 0.05$) than the control calves.

4-2-5 Serum aspartate transaminase: (AST)

Table (4-2) and figure (4-2-4) showed that theileriosis naturally infected calves have higher serum (AST) mean than the control calves with out any statistical significance.

4-2-6 Serum alamine transaminase: (ALT)

Table (4-2) and figure (4-2-4) showed that theileriosis naturally infected calves have higher serum (ALT) mean than the control calves with out any statistical significance.

4-2-7 Serum ceruloplasmin (CP) :

Table (4-2) and figure (4-2-5) showed that theileriosis naturally infected calves have significantly lower serum ceruloplasmin concentration values ($P< 0.05$) than the control calves.

4-2-8 Serum sodium:

Table (4-2) and figure (4-2-6) showed that theileriosis naturally infected calves have significantly lower serum sodium concentration values ($P< 0.05$) than the control calves.

4-2-9 Serum potassium:

Table (4-2) and figure (4-2-7) showed that theileriosis naturally infected calves have higher serum potassium mean than the control calves with out any statistical significance.

4-2-10 Serum copper:

Table (4-2) and figure (4-2-8) showed that theileriosis naturally infected calves have significantly lower serum copper concentration values ($P< 0.05$) than the control calves.

4-2-11 Serum zinc:

Table (4-2) and figure (4-2-8) showed that theileriosis naturally infected calves have significantly lower serum zinc concentration values ($P< 0.05$) than the control calves.

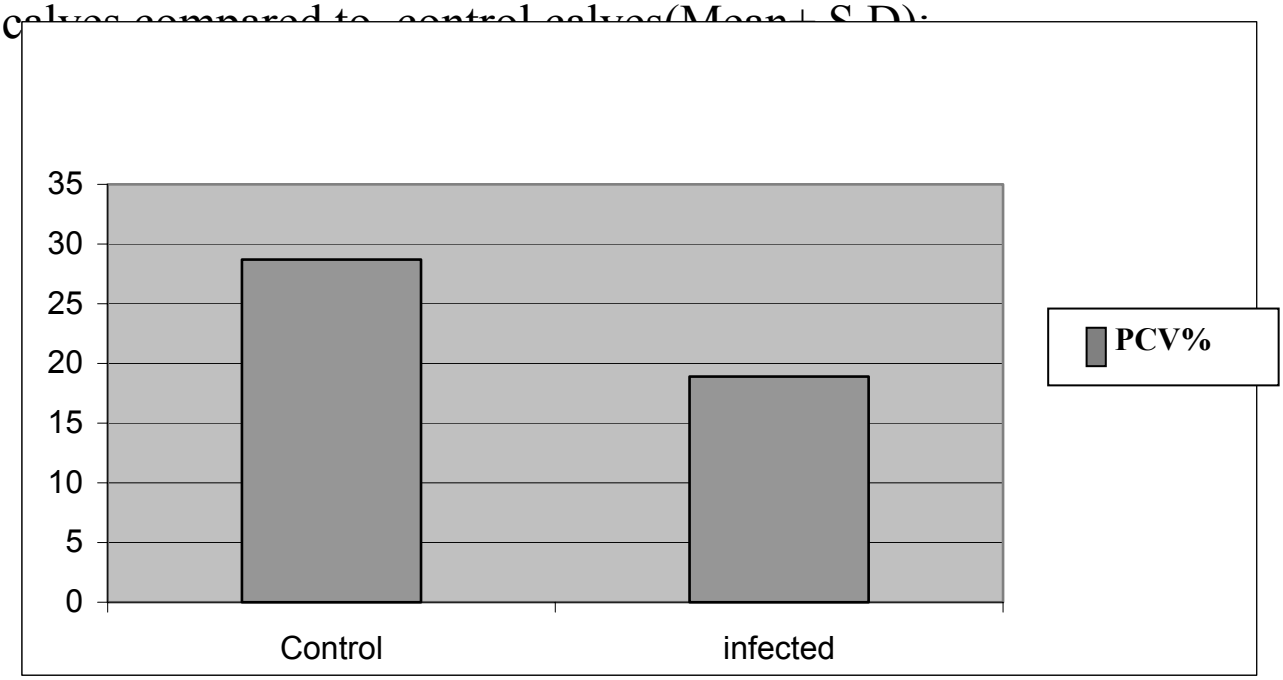
Table (4-1) Mean and ranges of haemological values of theileria infected calves compared to control calves

	Range of control calves	**Mean of control calves**	**Range of infected calves**	**Mean of infected calves**
PCV%	26 - 31	28.7	11 - 22	18.9
Hb g/dl	11.9 - 15	13.15	5.5 - 8.7	7.72
Glucose mg/dl	54.2 – 69.2	71.75	48.2 - 69.2	57.19

Table (4-2) Mean and ranges of serum chemistry values of theileria infected calves compared to control calves

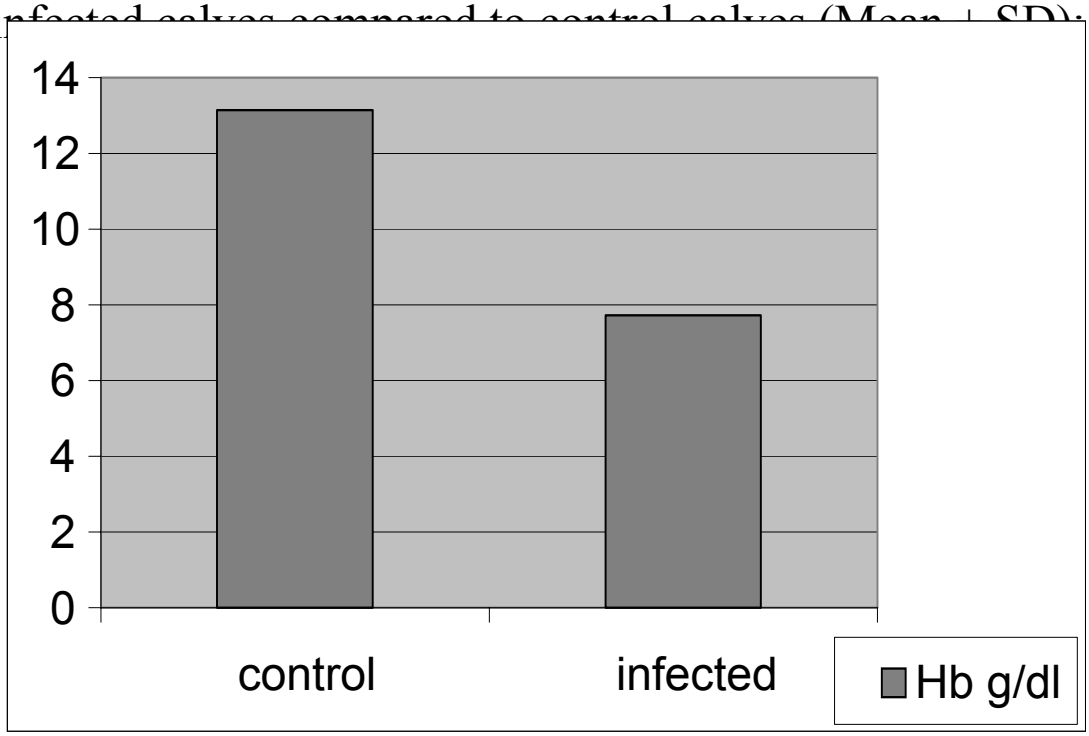
	Range of control calves	Mean of control calves	Range of infected calves	Mean of infected calves
Serum total protein g/l	6.8-8.8	7.59	3.9-7.1	6.23
Serum albumin g/l	3.5-5.1	4.04	1.4-3.7	2.89
Serum urea mg/dl	19.8-44	32.28	22.0-101.2	47.30
Serum triglycerides mg/dl	130.4-183.6	157	144.4-259.7	198.14
AST U/L	51-129	91.10	56-148	104.8
ALT U/L	66-166	90.3	71-128	98.7
Ceruloplasmin mg/dl	34.6-25.3	28.8	28.3-14.8	21.8
Serum sodium mEqu/L	128.2-144.1	137.33	112.2-129.9	121.99
Serum potassium mEqu/L	3.1-4.9	3.97	3.2-5.5	4.02
Serum copper mg/l	0.566-0.791	0.688	0.154-0.511	0.330
Serum zinc mg/L	0.337-0.811	0.628	0.217-0.585	0.333

Fig(4-1-1) PCV% values of the theileriosis infected calves compared to control calves(Mean ± S.D):

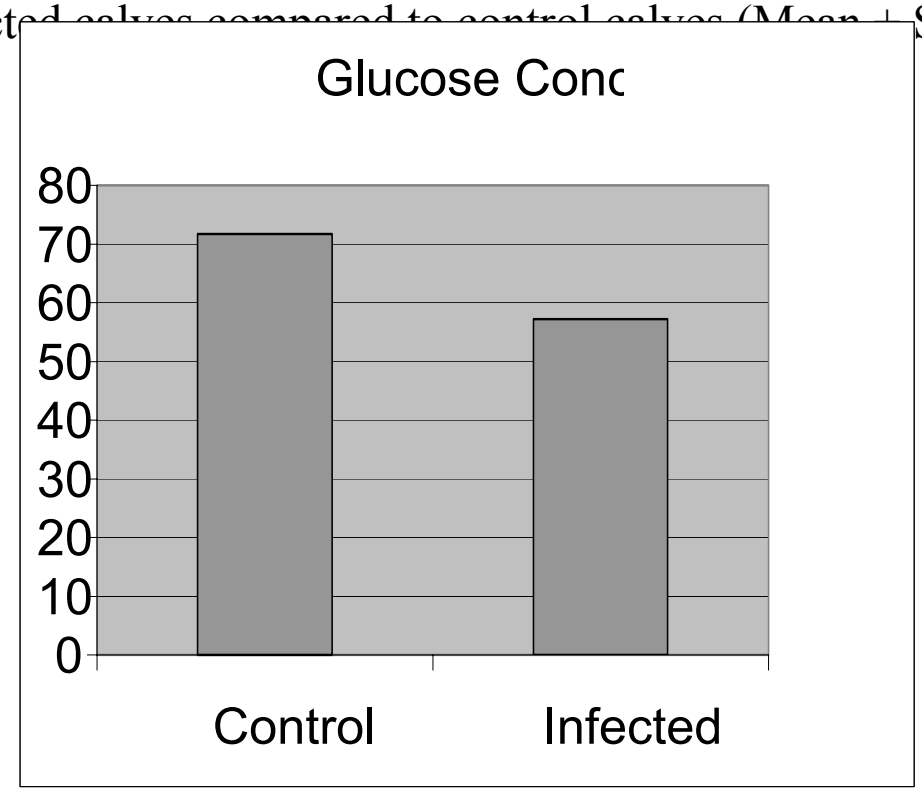


Fig(4-1-2) Haemoglobin concentration g/dl of theileriosis

infected calves compared to control calves (Mean ± SD):



Fig(4-1-3) Glucose concentration mg/dL of theileriosis infected calves compared to control calves (Mean ± SD)



Fig(4-2-1) Serum total protein and albumin g/L of theileriosis infected calves compared to control calves (Mean ± SD)

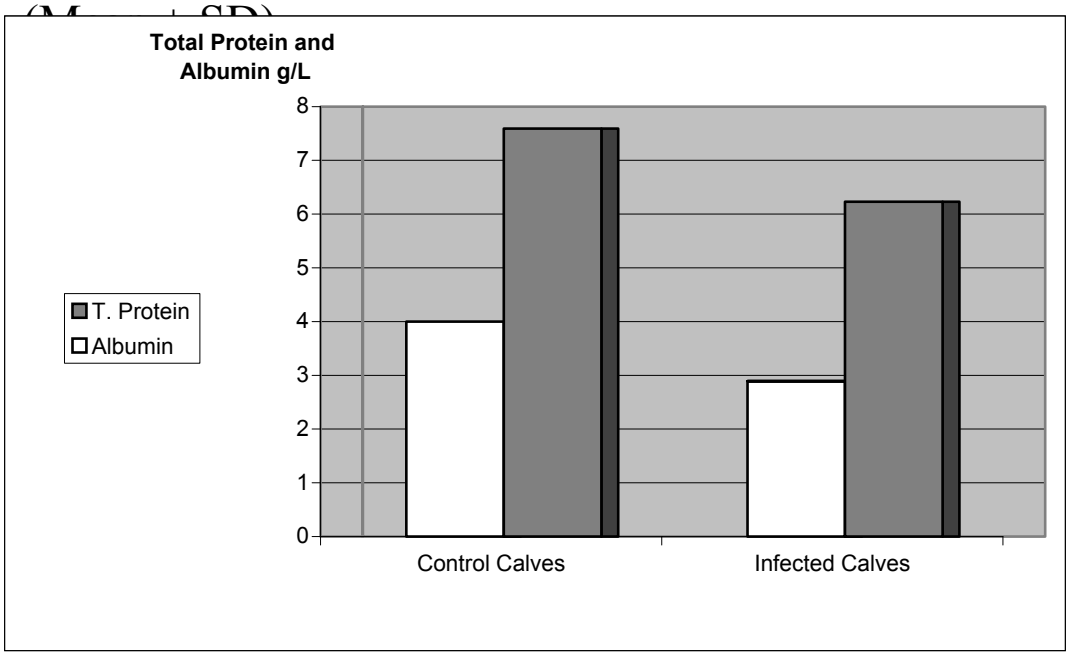


Fig (4-2-2) Serum urea concentration mg/dL of theileriosis infected calves compared to control calves (Mean $\pm$ SD):

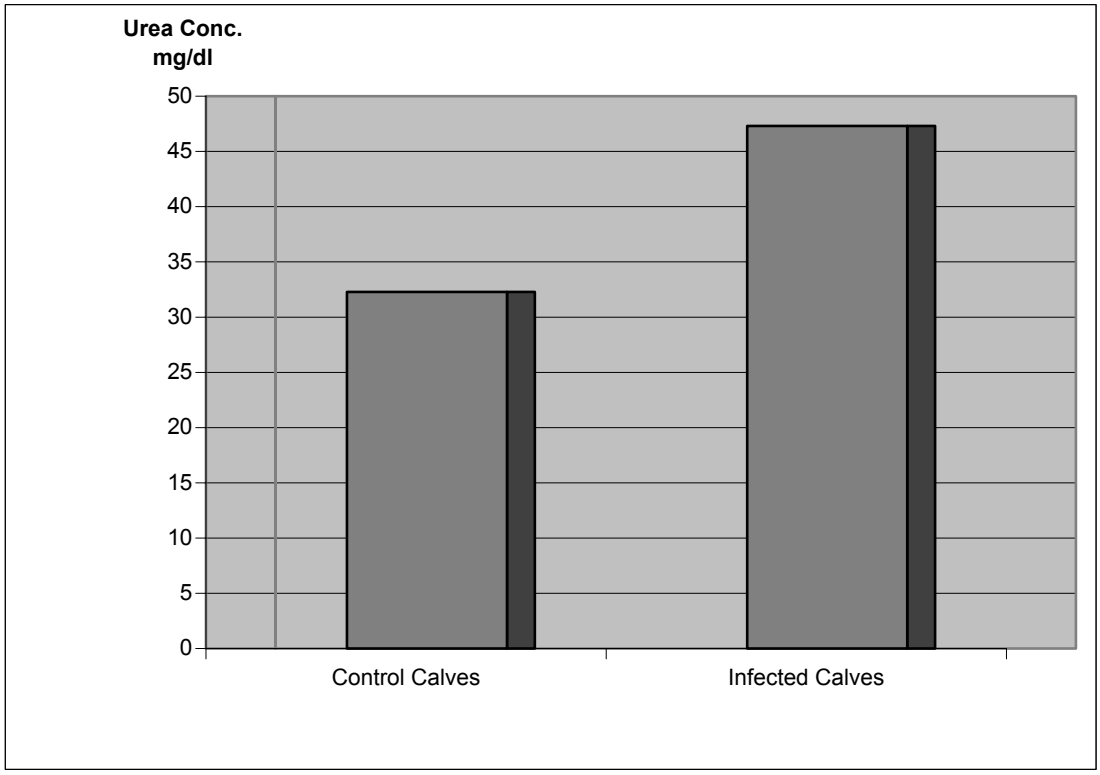
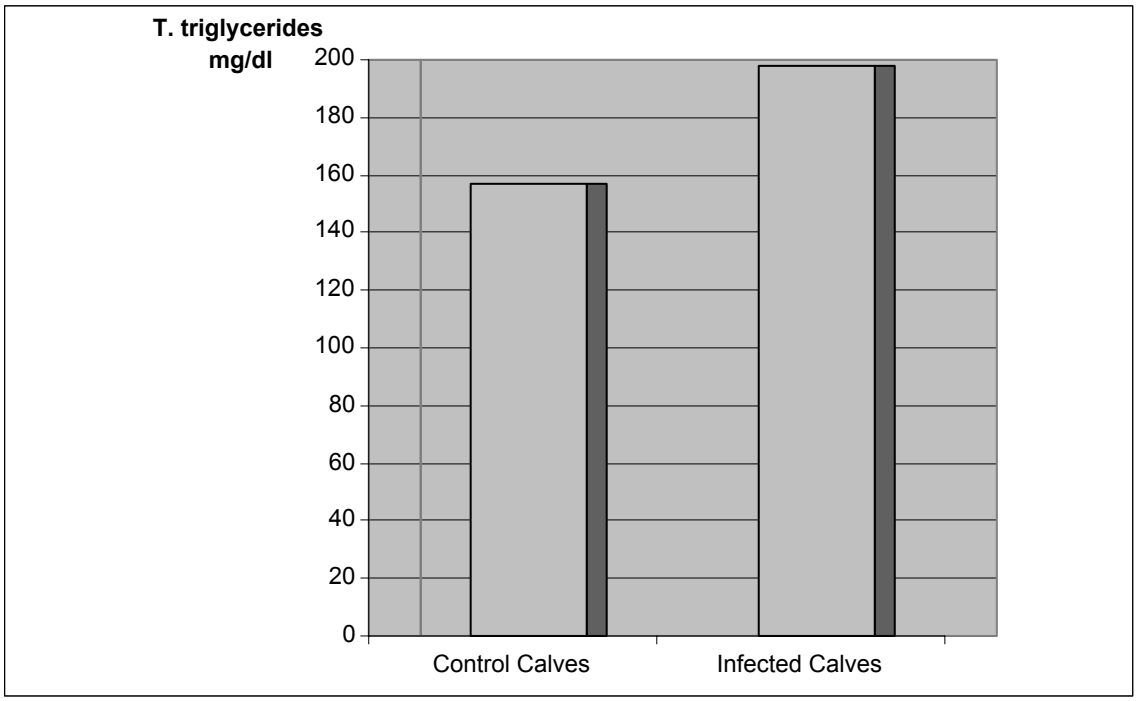
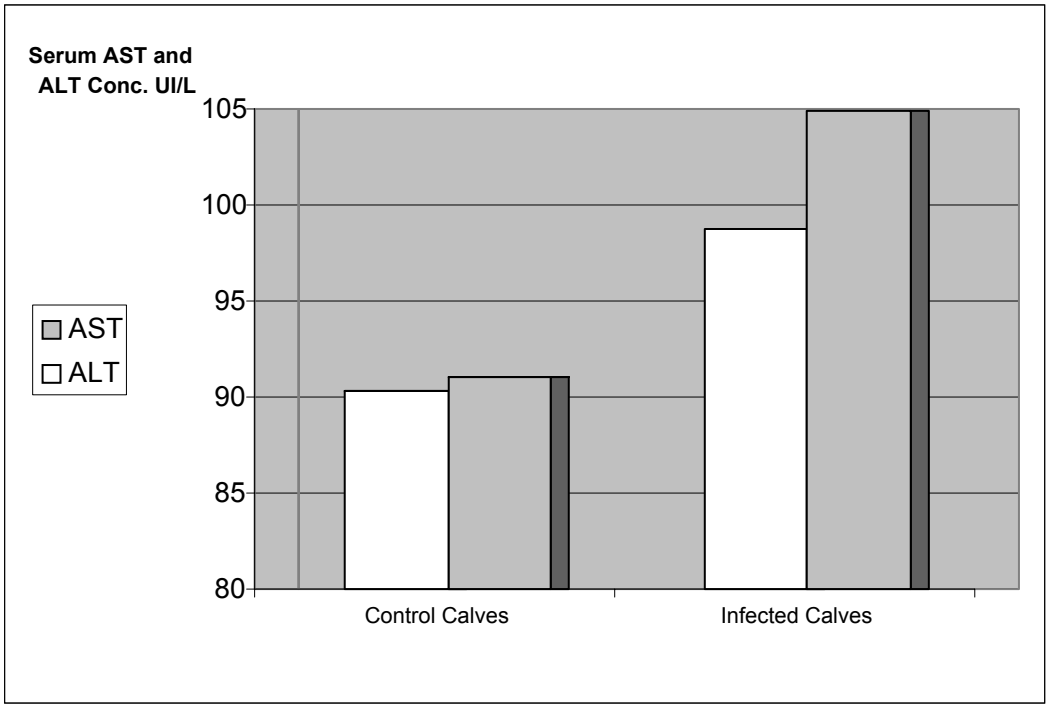


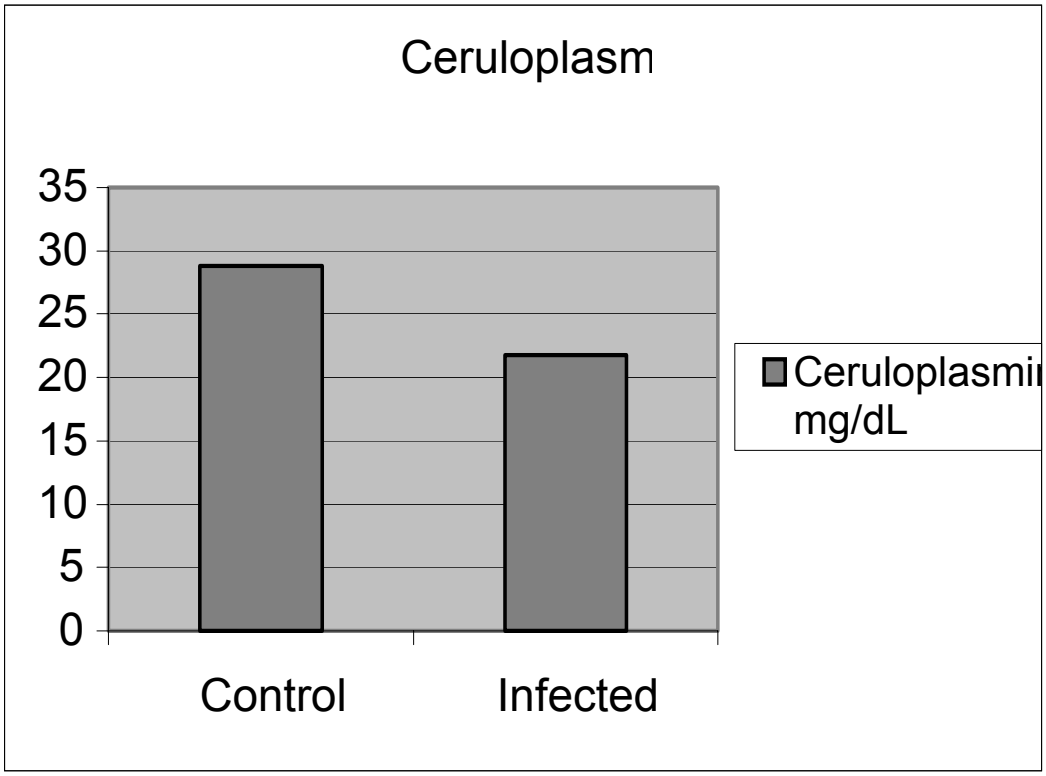
Fig (4-2-3) Serum triglycerides concentration mg/dL of theileriosis infected calves compared to control calves (Mean $\pm$ SD):



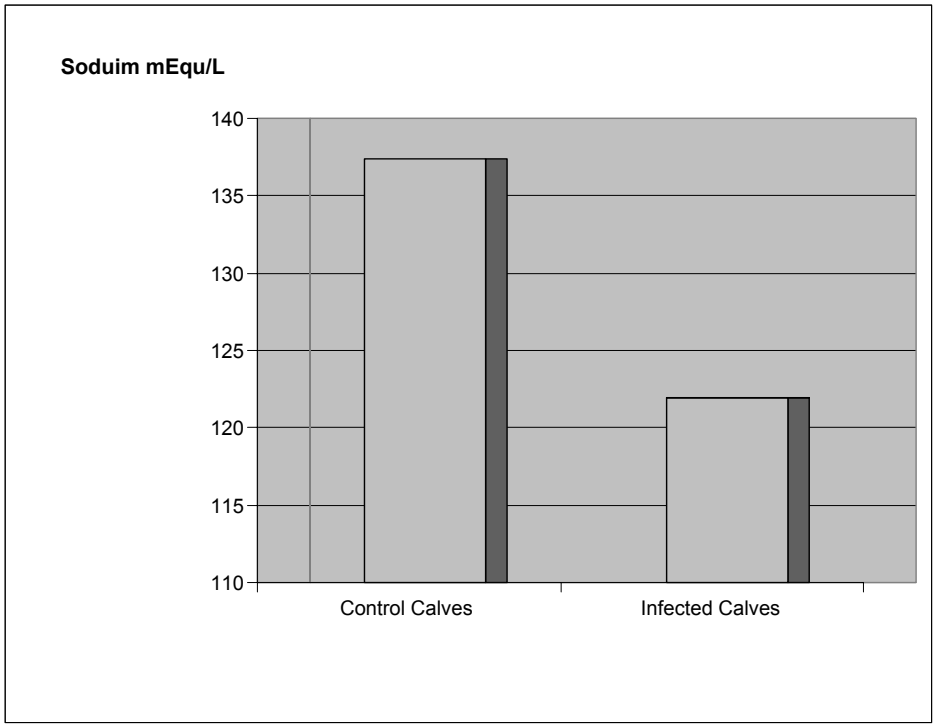
Fig(4-2-4) Serum AST and ALT concentration UI/L of theileriosis infected calves compared to control calves (Mean $\pm$ SD):



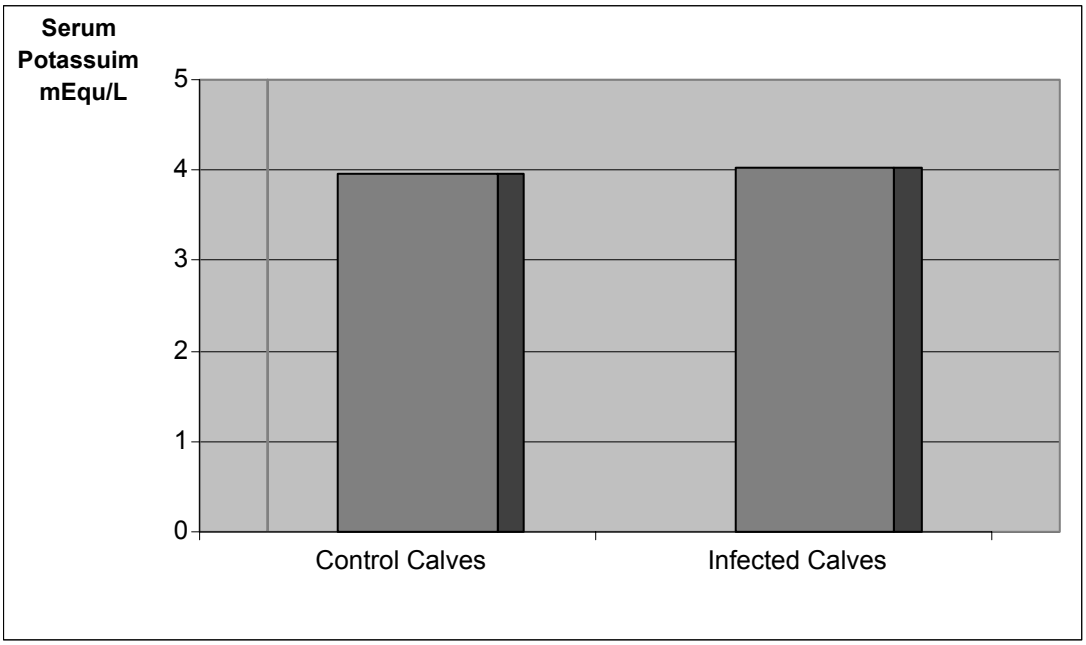
Fig(4-2-5)Serum ceruloplasmin concentration mg/dL of theileriosis infected calves compared to control calves (Mean $\pm$ SD):



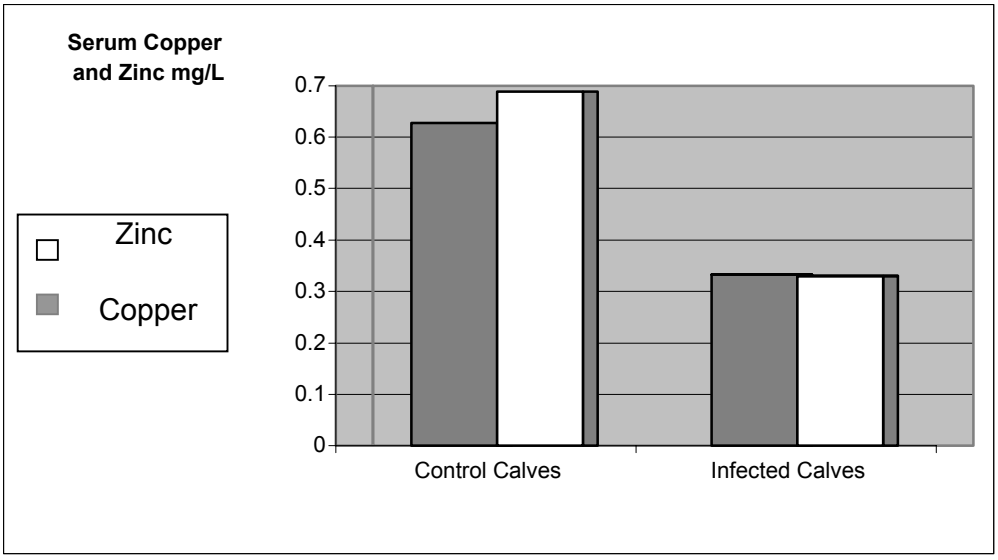
Fig(4-2-6) Serum sodium concentration mEqu/L of theileriosis infected calves compared to control calves (Mean $\overline{+}$ SD):



Fig(4-2-7) Serum potassium concentration mEqu/L of theileriosis infected calves compared to control calves (Mean $\pm$ SD):



Fig(4-2-8) Serum copper and zinc concentration mg/L of theileroisis infected calves compared to control calves (Mean $\pm$ SD):



Chapter five

Discussion

Thirty one cross-bred calves naturally infected with theileriosis were studied so as to examine the effect of the infection. The results obtained obviously emphasized the seriousness of theileriosis in this category of age.

The haematological findings of the infected calves examined in this study confirmed that severe anaemia was very distinctive.

The packed cell volume (PCV) and haemoglobin concentration decreased significantly in the infected calves compared to the control calves. Severe anaemia accompanied theileriosis infection in the young infected calves agrees with the work of Sharma, (1971) and Gautum et al , (1970). The parasites in the stage of piroplasmosis invaded the red blood cells (RBCs) (intracellular parasite). This invasion results in the destruction of the red blood cells (RBCs) and releasing of hemoglobin. Invasion of the parasites to red bllod cells and bone marrow results in low PCV values and production of immature RBCs.

Destruction of the red blood cells results in releasing of hemoglobin in plasma. Hemoglobin can not stay for long in plasma so it converted to bilirubin (haemolytic jaundice) after many biochemical changes.

Blood glucose concentration decreased in the infected calves. Decreased food utilization may due to quick food passage from the gastrointestinal tract in the form of diarrhea. In addition to that the animal became inappetite. So blood glucose concentration in theileriosis naturally infected calves have low levels compared to control calves.

Serum total protein and serum albumin values decreased in theileriosis naturally infected calves compared to the control calves. The

hypoproteinaemia detected in the infected calves is in the line with the findings of Dhar and Gautam, (1979). Hypoproteinaemia may occurs as a result of the reduction in serum albumin.

It was shown that hepatic damage and gastroenteritis due to theileriosis infection cause hypoalbuminaemia Mayer et al., (1992). This hepatic damage can cause inadequate synthesis of protein. Moreover the gastroenteritis results in inadequate absorption in the gastrointestinal tract. In addition to that the amino acids synthetized are directed to form globulins as a defense line instead of albumin.

Serum urea values of the infected calves increased significantly compared to the control calves. Increased levels of urea in the infected calves serum may seriously showed the effect of theileriosis in the infected calves kidneys. The effect may results due to antigens and antibody reaction (immuno complexes). This reaction can cause destruction of the blood capillaries in the kidney and increasing of the permeability of renal arteries. So the kidneys became oedematous, congested with red and white infection resulting in renal dysfunction.

Serum triglycerides values are increased significantly in theileriosis naturally infected calves compared to the control calves. Elevation of serum triglycerides may due to mobilization of long term energy store in the body, which considered as fat, from muscle and adipose tissues. This mobilization occurs as a result of the drop in blood glucose to compensate energy. Triglycerides are divided to free fatty acids and glycerol. Free fatty acids can be consumed in B-oxidation to produce acetyl coA which consumed in Kreb's cycle as a source of energy. Glycerol is utilized through gluconeogenesis to produce glucose.

Serum aspartate transaminase (AST) and serum alanine transaminase (ALT) were increased in theileriosis naturally infected calves compared to the control calves. The hepatic damage due to

theileriosis was indicated by changes in the activities of (AST) and (ALT) enzymes Laiblin et al., (1978).

These enzymes are of wide distribution in the body cells. Hepatic cells damage and destruction of the red blood cells many result in releasing of these enzymes in the plasma.

Ceruloplasmin which exhibits copper-dependent oxidase activity decreased in theileriosis naturally infected calves compared to control calves. The amount of ceruloplasumin in plasma is decreased in liver diseases. In particular, low levels of ceruloplasmin are found in hepatolenticular degeneration diseases due to abnormal metabolism of copper. Theileriosis directly affects liver cells resulting in retention of copper in the liver and by so doing low levels of ceruloplasmin occurs.

In general, cells maintain a low intracellular Na^+ concentration and a high intracellular K^+ concentration a long with a net negative electrical potential inside. The pump that maintain these gradient is an ATPase that is activated by Na^+ and K^+. The ATPase has catalytic centers for both ATP and Na^+ on the cytoplasmic side of the membranes, but the K^+ binding site is located on the extracellular side of the membrane.

Na^+-K^+ ATPase pump moves these Na^+ ions from inside the cell to the out side and brings two K^+ ions from the outside to the inside for every molecule of ATP hydrolyzed to ADP by the membrane associated ATPase.Inhibition of the extracellular domain due to the disease inhibits this ATPase. Inhibition of ATPase can be antagonized by extracellular K^+.

Serum copper and zinc are decreased in the infected calves. This decrease may due to that the storage of copper and zinc is in the liver, when they are required they were released and transported to the deficient areas. During theileriosis they

may be ceased and can not be released due to hepatic dysfunction and remain stored in the liver.

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Appendix (1)

Hematological values of theileriosis infected calves compared to healthy calves and the international hematological values (Meyer, 1992).

Case Int	PCV% 24-46	Hb conc. g/dl 8-15	Glucose conc. mg/dl 55-120
			Control
1	28	13.3	54.2
2	29	12.7	70.9
3	29	14	58.4
4	27	13.9	75.1
5	27	11.9	91.7
6	30	12.6	61.1
7	31	13.3	95.9
8	31	15	96.2
9	26	12.7	70.9
10	29	12.1	70.1
			Infected calves
1	19	7.73	54.2
2	22	8.13	58.4
3	20	7.93	59.6
4	21	8.40	53.3
5	21	7.21	57.8
6	21	8.73	49.3
7	17	7.5	59.1
8	18	8.5	59.7
9	16	7.2	62.4

Appendix (1) continue

10.	14	5.96	58.1
11.	22	8.0	64.5
12.	16	6.3	66.2
13.	22	7.1	69.2
14.	11	5.7	59.2
15.	15	5.9	48.2
16.	14	6.25	62.7
17.	14	8.2	51.8
18.	14	8.1	67.2
19.	19	5.5	68.7
20.	13	7.1	59.9
21.	14	8.70	51.4
22.	20	7.62	63.3
23.	15	7.34	67.8
24.	19	7.7	68.2
25.	22	7.9	59.0
26.	22	8.1	64.4
27.	14	7.2	52.3
28.	19	7.7	66.7
29.	17	7.4	62.9
30.	16	6.9	57.3
31.	21	7.2	51.9

Appendix (2)

Serum chemistry values of theileriosis infected calve compared to healthy calves and the international serum levels (Meyer, 1992).

S/No	T.P. g/L	Alb. g/L	Urea mg/dl	T.G mg/dl	Cp mg/dl	AST lU/L	ALT lU/L	Na mEqu/L	K mEqu/L	Cu mg/L	zn mg/L
Case Int.	6.6-7.8	3.0-3.6	21.2-36.5	<165	24-44	60-150	65-150	132-152	3.9-5.8	0.7-1.4	0.5-1.2
Control											
1	7.6	4.0	26.4	142.9	34.6	73	109	131.2	4.4	0.643	0.811
2	8.2	3.5	30.8	130.4	26.8	129	116	128.2	4.9	0.781	0.612
3	7.9	4.2	35.2	153.3	25.3	117	76	137.9	4.3	0.661	0.711
4	6.8	4.2	35.2	162.0	26.8	81	97	138.2	3.2	0.677	0.622
5	8.8	5.1	39.0	169.2	25.3	98	112	144.1	3.2	0.791	0.337
6	7.4	4.2	30.8	183.6	32.8	54	84	140.9	3.1	0.566	0.622
7	7.2	4.3	44.0	135.9	29.8	121	71	136.7	4.4	0.701	0.593
8	6.9	3.9	19.8	162.6	29.8	95	93	141.3	3.2	0.688	0.713
9	7.7	3.5	33.0	172.4	26.8	51	79	139.4	4.7	0.751	0.669
10	7.4	3.5	28.6	157.7	29.8	92	66	135.4	4.3	0.692	0.789
Infected calves											
1	6.3	3.3	39.6	206.0	28.3	96	99	119.1	3.9	0.380	0.421
2	6.8	3.3	30.8	196.7	28.3	139	117	112.2	3.2	0.360	0.371
3	6.2	3.4	46.2	199.1	20.8	56	83	125.9	4.3	0.269	0.345
4	5.6	2.3	35.2	192.2	19.3	128	106	118.0	4.2	0.487	0.355
5	6.1	2.9	88.0	252.7	17.8	72	81	126.6	3.2	0.511	0.413
6	6.9	3.0	68.2	192.9	17.8	121	101	129..9	3.8	0.231	0.222
7	6.2	3.1	22.0	154.8	19.3	101	98	120.5	4.9	0.199	0.379
8	3.9	3.2	44.0	208.5	17.8	1142	122	124.7	4.3	0.354	0.217
9	6.2	2.2	63.8	186.1	19.3	82	89	119.2	5.2	0.232	0.221
10	5.1	2.2	35.2	192.6	20.8	111	91	123.8	3.2	0.275	0.385
11	6.3	3.1	28.6	153.7	25.3	137	121	128.1	3.5	0.154	0.585
12	7.1	3.5	46.2	212.3	20.8	77	71	121.4	3.7	0.323	0.224
13	6.0	2.7	26.4	168.1	14.8	91	87	127.2	4.1	0.211	0.217
14	6.1	2.5	30.8	184.3	17.8	93	117	122.2	3.4	0.237	0.359
15	3.9	1.9	33.0	178.7	26.8	120	105	126.3	4.5	0.167	0.358
16	6.1	3.0	61.6	159.1	28.3	81	99	124.9	3.5	0.183	0.348
17	6.3	2.1	59.4	175.5	20.8	136	102	122.1	4.4	0.278	0.417
18	5.9	2.6	90.2	199.2	17.8	125	105	125.5	4.2	0.223	0.295
19	6.4	2.2	70.4	194.1	22.3	68	91	127.9	4.6	0.503	0.305
20	6.8	3.7	52.8	228.9	14.8	133	91	120.7	5.5	0.334	0.316

Appendix (2) continue:

21	5.5	2.7	37.4	192.2	25.3	57	86	123.4	4.2	0.219	0.279
22	5.7	1.4	33.0	181.2	14.8	65	83	126.3	4.1	0.371	0.501
23	6.6	3.5	22.0	162.4	25.3	123	112	120.2	5.3	0.322	0.391
24	6.6	3.2	11.0	157.2	23.8	136	100	122.9	4.2	0.291	0.411
25	6.1	2.9	15.4	201.2	19.3	77	93	127.1	3.9	0.311	0.267
26	5.9	2.9	101.2	144.4	19.3	122	107	123.4	4.9	0.329	0.271
27	6.8	3.1	59.4	144.4	28.3	136	93	125.2	5.2	0.224	0.313
28	6.2	3.4	33.2	188.9	26.8	142	102	122.1	4.1	0.411	0.394
29	5.7	2.7	33.9	166.7	26.8	148	128	124.3	3.8	0.352	0.511
30	5.5	2.2	43.4	115.7	20.8	144	126	127.4	3.8	0.319	0.362
31	6.7	3.4	64.9	162.4	25.3	123	93	126.2	4.1	0.329	0.283

Appendix (3)
Statistical Analysis

Varley, (1980a)

The significant differences between the values of theileria infected calves and the control calves in this study were determined using the following statistical formula.

$$t = \frac{\bar{X}_c - \bar{X}_t}{\sigma \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

t = Is the value of t

$\overline{X_c}$ = Is the mean of the control calves

X_t = Is the mean of the infected calves

σ = Is the pooled variance

N_1 = Number of the control calves

N_2 = Number of the infected calves

P = is the calculated probability of the Null hypothesis.

In practice it's often accepted that a result occurring with a probability of 0.05 is significant. This statement is usually put in the following form. (The result is significant when $P < 0.05$).

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TOTAL PORTEIN CONTROLL	7.590	10	.603	.191
	TOTAL PORTEIN_INF	6.230	31	.570	.180

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	TOTAL PORTEIN CONTROLL - TOTAL PORTEIN INF	1.360	.786	.249	5.470	39	.0

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ALBUMIN CONTROLL	4.040	10	.490	.15
	ALBUMIN INF	2.890	31	.477	.15

Paired Samples Test

		Paired Differences			t	df	Sig. (2-taile
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	ALBUMIN CONTROLL - ALBUMIN INF	1.150	.591	.187	6.152	39	.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TOTAL TRI GLYCERIDE CONTROLL	157.000	10	16.719	5
	TOTAL TRI GLYCERIDE INF	198.140	31	24.228	7

Paired Samples Test

		Paired Differences			t	df	Sig. (2-taile
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	TOTAL TRI GLYCERIDE CONTROLL - TOTAL TRI GLYCERIDE INF	-41.140	24.456	7.734	-5.320	39	.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	UREA_CONTROL	32.280	10	6.736	2.1
	UREA INF	47.300	31	20.117	6.3

Statistical analysis of serum total protein and serum albumi

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	UREA_CONTROL - UREA INF	-15.020	20.819	6.584	-2.281	39	.0

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	AST CONTROLL	91.100	10	26.831	8.485
	AST INF	104.8000	31	28.7935	9.1053

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	AST CONTROLL AST INF	-13.7000	39.1267	12.3730	-1.107	39	.297

Statistical analysis of serum tota

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	Na CONTROLL - Na INF	15.340	3.482	1.101	13.930	39	.000

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	K_CONTROLL	3.97	10	.71	.22
	K_INF	4.020	10	.702	.222

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	K_CONTROLL - K_INF	-5.00E-02	.914	.289	-.173	39	.867

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CU_CONTROLL	.688	10	6.056E-02	1.915E-02
	CU_INF	.330	31	.108	3.411E-02

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	CU_CONTROLL - CU_INF	.358	.103	3.263E-02	10.979	39	.000

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ZN_CONTROLL	.628	10	.150	4.759E-02
	ZN_INF	.333	31	8.121E-02	2.568E-02

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	ZN_CONTROLL - ZN_INF	.295	.183	5.772E-02	5.111	39	.001

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	GLUCOSE_CONTROLL	71.750	10	13.345	4.220
	GLUCOSE_INF	57.190	31	3.828	1.211

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	GLUCOSE_CONTROLL - GLUCOSE_INF	14.560	12.746	4.031	3.612	39	.006

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Hb_CONTROLL	13.150	10	.950	.300
	Hb_INF	7.729	31	.815	.258

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	Hb_CONTROLL - Hb_INF	5.421	.813	.257	21.093	39	.000

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Err Mean
Pair 1	PCV_CONTROLL	28.700	10	1.703	.
	PCV_INF	18.900	31	2.601	.

Paired Samples Test

		Paired Differences			t	df	Si (2-ta
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	PCV_CONTROLL - PCV_INF	9.800	3.225	1.020	9.610	39	

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Erro Mean
Pair 1	CERULOPLASMIN CONTROL	.202	10	1.984E-02	6.273E
	CERULOPLASMIN INF	.153	31	2.712E-02	8.576E

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	CERULOPLASMIN -CONTROLL CERULOPLASMIN INF	4.880E-02	2.891E-02	9.142E-03	5.338	39	.00

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