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SOME CLINICAL AND BIOCHEMICAL STUDIES OF EXPERIMENTALLY INFECTED CALVES WITH TRYPANOSOMA EVANSI

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ABSTRACT □ This study was performed on 13 calves belonging to farm of faculty of Veterinary Medicine Suez Canal University. Eight calves were inoculated with a dose of $5x10^7$ Egyptian isolates of Trypanosoma evansi, and five ones remained as non infected control group. Increase in body temperature, decrease in packed cell volume, the parasitaemia, and some clinical signs were recorded. The animals were treated with Berenil 60 days post infection and no relapsing was observed after treatment for 60 days.

Biochemical analysis of sera collected from infected animals 4 weeks after infection revealed that there were a significant increase in serum magnesium. A significant decrease in blood pyruvate, glucose, and non significant decrease in total cholesterol, calcium and inorganic phosphorus, were also recorded in serum of infected calves compared to non infected control group.

INTRODUCTION

Trypanosomiasis is one of the most serious parasitic diseases in man and animals in Africa, Asia and South America Surra, which is caused by T. evansi, is considered to be one of the most important diseases affecting large ruminants (Adiwinata and Dachlan, 1969; Denning, 1976). Bovine and buffalo calves born in an endemic area, had a greater than 50% probability of acquiring infection with T. evansi within the first 12 months of life (Payne et al. 1991). Furthermore they monitored blood samples from bovine and buffalo calves born in an endemic area for T. evansi at monthly intervals for 12 months and detected parasites by microhaematocrit centrifugation technique (MHCT) on more than one occasion in several animals in Indonesia.

Buffaloes infected with T. evansi in China usually show depressed behaviour, emaciation, and anemia, the hair becomes rough and lusterless, there is hyperemia of the conjunctiva, lachrymation and blennophthalmia in the intra-orbital with white gray exudate. Some animals show necrosis at the edges of the ears, and swollen legs, although the infection becomes chronic most animals die if not treated, (Lun et al., 1991).

Surra in cattle and buffalo is usually chronic with low mortality, progressive anemia and emaciation occur, Leading to death by exhaustion, especially in working animals. However, if infected animals are maintained

in good condition, cattle and buffalo can sometimes overcome the disease (Dieleman, 1986). Domestic animals such as buffaloes, pigs, goats, mules, and cattle usually show a chronic disease, but in equines and deer the disease is more acute and often rapidly fatal (Stephen, 1986; Luckins, 1986).

Anemia is the most outstanding clinical and laboratory feature of trypanosomalasis in man and animals. The Anemia observed in trypanosomiasis is associated with marked reticulocytosis and hepatosplenomegaly during the acute course of the disease (Amole, et al., 1982). Moreover, significant abnormalities of iron metabolism, bone marrow activity, serum and plasma proteins, together with thrombocytopenia is a consistent clinical feature of trypanosomiasis, Suliman and Feldmon (1989). The most striking abnormality of serum proteins in trypanosomal infections is the low albumin and high δ -globulin can be changed in infected animals (Rajora et al., 1986). Moreover, Boid et al., 1980 reported increased total proteins in the sera of Camels infected experimentally and naturally with T. evansi. This was associated with decreased albumin, increased δ -globulin and immunoglobulin M (IgM) but α -globulin and IgG remain unchanged.

The objective of the present investigations was intended to clarify some clinical and biochemical alterations in calves experimentall infected with an Egyptian isolates of trypanosoma evansi.

MATERIAL AND METHODS

Thirteen mixed breed calves one year old, were obtained from the facilities of the Faculty of Veterinary Medicine, Suez Canal University. These calves were proved to be free from external, internal and blood parasites by parasitological and clinical examination. These animals were housed in an open yard system with a common shaded manger and water troughs in which water was offered ad-libitum. Each animal was fed daily ration formed from concentrate mixture 4 kg, rice straw 2 kg and barseem hay 2.5 kg. The calves were divided into two groups. Group 1, of 5 male calves were used as a control. Group 2, incuided 8 male calves which inoculated subcutaneously with $5x10^7$ virulent field Egyptian isolates of T. evansi (Singh and Gaur, 1983).

Trypanosoma evansi was isolated from diseased camel at Balbies locality, and were inoculated in mice to expand the parastaemia. At the peak of parasitaemia the mice were euthanized by chloroform overdose, and the blood was collected by cardiac puncture into ice cold bicine buffered saline with 7mM glucose pH 8 (BSG) and 1% sodium citrate, and the trypanosomes were adjusted to $5x10^7$/ml with BSG.

Two blood samples were collected 4 weeks after infection, from each group from jugular vein in dry sterile centrifuge tube. The first blood samples were collected on EDTA for pyruvate and lactate determination according to Friedeman and Haugen (1943), respectively and Barker and Summerson (1941). The second blood samples were collected without anticoagulant, the sera were separated for biochemical assays. Samples were collected in heparinised haematocrit capillary tubes for PCV determination after centrifugation as well as examination of plasma-leukocyte interface in capillary tube examined for parasites according to Woo (1970) body temperature of the calves were monitored daily. The infected calves were treated 60 days following infection by intramuscular injection of aqeous solution of Diminazene aceturate (Berenil)[1] in a dose of 3.5 mg/Kg B. W. (Lun et al., 1991).

Serum glucose, total proteins, albumin, globulins, total bilirubin, creatinine, calcium, inorganic phosphorous, magensium, and total cholesterol were assayed calorimeterically according to the methods described by Trinder (1969); Weichselbaum, (1946); Bartholomeu and

1 The drug was from Hoechst Co.,

Deloney, (1966); Doumas and Biggs, (1972); Jackson and Hernaudez (1956); Schirmeister et al., (1964); Gindler and King (1972); Wootton, (1982); Gindler, (1971) and Zlatkis et al., (1953), respectively. The obtained results were statistically analyzed using student's "t" test as explained by Snedecor and Cocharn (1969).

RESULTS AND DISCUSSION

Trypanosoma evansi were detected in the infected calves 3 days after infection by MHCT, the parasitaemia levels fluctuated with frequency of 6-8 days between peaks of parasitaemia, the parasitaemia levels remain higher during the first 5 weeks as measured by number of trypanosomes / high power field. after treatment with Berenil the trypanosomes were not detected and no relapse up to 60 days post treatment.

The mean body temperature of infected animals is slightly elevated as measured for 60 days after infection, in contrast the haematocrit values were decreased during the same period of infection as shown in table (1) compared to unifected control calves. Although the infection becomes chronic and experimentally infected calves were treated after 2 months of infection. The infected calves showed slight depression, decreases appetite, rough hair, and lachrymation after infection by 8 days and different period later.

Increase in body temperature and decrease in haematocrit values appear to reflect the ability of the host to control the infection with trypanosomes and well established features of the trypanosomes (Fiennes, 1970). Elevated body temperature in T. evansi infected calves may be due to reduction of energy available for catabolic activity as the metabolic rate increased by about 15% for every 1^{0}C increase in body temperature (Stephen, 1986), and there is a positive correlation between days of elevated body temperature, and detection of T. evansi in the blood of infected calves, and decreased appetite and lachrymation these were agreement with those observed and reported by the Stephen, 1986, and Payne, et al., 1991).

The results obtained concerning the level of various blood serum parameters in normal and experimentally infected calves with T. evansi, have been statistically summarized in table (2). The mean values of blood lactate showed highly significant increase in table (2). The mean values of blood lactate showed highly significant increase in infected calves, on the other hand, the values of blood pyruvate level showed highly significant decrease in infected calves compared with normal calves. The decreased level of blood pyruvate in infected calves may be possibly due to the impairment of the appetite, partial starvation and reduced glycogen stores (Davidson and More, 1969). Such results was in parallel with that obtained by Huckabee, (1958) who proved that, glycolysis is the catabolic pathway by which glucose or glycogen is broken down to pyruvate and lactate. Pyruvate is the end product of glycolysis, could be either further metabolized in the mitochondria or reduced in the cytoplasm to lactate. The increased blood lactate levels in the infected calves may be attributed to great increased production and possible impairment of hepatic metabolism due to poor perfusion of lactic acid accumulations or it may be attributed to the impaired tissues perfusion has lead to inadequate oxygenation, despite the normal arterial Po2, with consequently increased anaerobic metabolism of glucose to lactic acid, rather than oxidative metabolism (Kurtzman and Batalle, 1983).

Regarding blood glucose level, the mean value showed a highly significant decreased in calves experimentally infected with T. evansi. These results agree with those of Goel and Singh (1969); and El-Magawry (1983) who recorded a significant decrease in the blood glucose values (44 mg%; and 56.55 $\pm$ 0.799 mg/dl. respectively). The low level of blood glucose present in infected calves may be due to the presence of high parasiteamia and abnormal rate of

Table (1): Individual reading and mean values of body temperature, and Packed cell volume (PCV), for uniinfected and Trypanosoma evansi infected calves for 60 days of infection.

Item / Animal	No. of calves	Body temperature °C	PCV Vol. %
Uninfected calves	calf No. 1	39	30.5
	calf No. 2	38.7	31
	calf No. 3	38.9	29
	calf No. 4	38	30
	calf No. 5	37.9	31.8
Total Mean		38.5	30.46
Infected calves	calf No. 1	39.5	25
	calf No. 2	39.3	24.5
	calf No. 3	39.1	23.8
	calf No. 4	39	25.6
	calf No. 5	39.6	26
	calf No. 6	39.4	22.6
	calf No. 7	38.7	23.9
	calf No. 8	39	23.1
Total Mean		39.2	24.31

Table (2): Levels of some biochemical blood parameters of clinically healthy, and 4 weeks Trypanosoma evansi infected calves.

Parameters / Groups	Normal calves Mean ± SD	Infected calves Mean ± Sd
Lactate mg/dl	11.482±0.628	46.837±1.486**
Pyruvate mg/dl	4.797±0.222	3.768±0.544**
Glucose mg/dl	56.252±3.493	44.445±3.927**
Total proteins gm/dl	7.845±1.422	9.810±2.058*
Globulins gm/dl	4.643±1.036	6.525±0.994**
Albumin gm/dl	3.202±0.412	3.285±0.439
A/G Ration	0.658±0.160	0.750±0.242
Total-cholesterol mg/dl	182.89±17.92	171.06±6.81
Total bilirubin mg/dl	0.060±0.024	0.045±0.010
Creatinine mg/dl	2.09 ±0.83	2.08 ±0.50
Calcium mg/dl	9.16 ±2.15	7.08 ±0.84
Inorganic phosphorus mg/dl	3.44 ±1.2	2.63 ±0.83
Magensium mg/dl	1.22 ±0.46	1.60 ±0.42

* (t. test) 0.05<P<= 0.1 ** (t. test) P< 0.01

consumption of blood glucose by T. evansi infection as confirmed by the results of Jatkar and singh (1974) who stated that, parasite count was inversely proportional to glucose level. Another suggestion for hypoglycemia may be attributed to partial starvation and reduced glycogen stores as supported by (Davidson and More, 1969).

Regarding serum total proteins and its friction, their was a significant increase in total serum proteins level accompanied by a highly significant increase in the values of serum globulin's in infected calves. On the other hand, the values of serum albumin showed non significant change in calves experimentally infected with T. evansi compared with values in the control calves. These results were in agreement with findings obtained by Goel and Singh (1969), and Jatkar and Singh (1974). Our results is in contrast with that reported by El-Magery (1983) who stated that, there was significant drop in serum total proteins, albumin and globulin's in naturally infected camels with trypanosomiasis. Moreover, similar results was reported by Rajora et al., (1986) who reported that, the most striking abnormality of serum proteins in trypanosome infections is high δ -globulin concentration in infected animals, furtherly, Bold et al. (1980) reported that an increased total proteins in the sera of camels infected experimentally and naturally with T. evansi this was associated with decreased albumin and increased δ - globulins level. The increased total serum proteins level in the sera of infected calves may be attributed to increased δ - globulin fraction and immunoglobulin M (IgM). Bold et al. (1980).

A non significant decrease in the total cholesterol level was recorded in experimentally infected calves with trypanosomiasis, these decrease may be attributed to the subnormal energy status in some experimental animals which may induce hypocholesterolaemia, as confirmed by Velhanker, (1973) who found that, cholesterol concentration of blood positively correlated to the energy status of the animal.

The obtained results (Table. 2) showed that the serum calcium and inorganic phosphorus levels were non significantly decreased in calves experimentally infected with trypanosoma evansi. These results were in parallel with that of Raisinghani, et al. (1981) who recorded that 27% reduction of serum calcium was noticed in artificially infected camels with trypanosoma, and with that of Goel and Singh (1969) who reported that, the mean value of serum inorganic phosphorus was decreased from 5.4 mg% reaching to 3.4 mg% in camels infected with trypanosomiasis. Moreover, El-Magawry (1983) who stated that, a highly significant decrease of serum Ca, inorganic ph. was recorded in naturally infected camels with trypanosomiasis. The decrease in serum calcium and inorganic phosphorus level, could be attributed to the blood loss as result of the parasites present in the blood and also may be due to impairment of appetite, and absorption (Chandel et al., 1992). The results also showed a non significant increase in serum magnesium, this increase may be as a result of the increased parathyroid hormone levels in response to the hypocalcemia, which reported in our results, and reported by Care, (1980) who stated that parathyroid hormone predominantly increases plasma calcium relative to magnesium, although parathyroid hormone can increase plasma magnesium by increasing intestinal absorption, increasing tubular reabsorption and increasing bone resorption.

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