Biochemistry unit, New-Valley laboratory,
Animal Health Research, ARC, Egypt.

PARASITOLOGICAL AND BIOCHEMICAL STUDIES ON ACUTE TROPICAL THEILERIOSIS IN NEONATAL INDIGENOUS AND FRIESIAN CALVES IN THE EGYPTIAN OASES

(With 13 Tables and 5 Figures)

By

MOSTAFA A. SALEH AND OSMAN M. MAHRAN*

*Parasitology unit, Shalatin lab., Animal Health Research Institute, ARC, Egypt.

(Received at 27/9/2003)

دراسات طفيلية وبيوكيميائية على الإصابة الحادة بالثيليريا الاستوائية في العجول المحلية والفريزيان حديثة الولادة في الواحات المصرية.

مصطفى أحمد صالح ، عثمان محمد مهران

لتقييم ديناميكية الأعراض الإكلينيكية ونسبة الطفيل و بعض المكونات البيوكيميائية في دم العجول حديثة الولادة الفريزيان والبلدي أثناء الإصابة بالثيليريا أنيولاتا، تم اختيار عدد ٢٠ عجل من كلا السلالتين لعمل هذه الدراسة. عشرة منهم (عدد ٥ من كل سلالة) تم معاملتهم بعد الولادة مباشرة (وكذلك الأمهات قبل وبعد الولادة) بالديازينون والإفرمكتين والبيوبارفاكون واستخدمت كمجموعة ضابطة. العشر عجول الآخرين (عدد ٥ من كل سلالة) تركت مع أمهاتها في حظائر بها نسبة عالية من القراد لإحداث إصابة طبيعية بالثيليريا أنيولاتا. تم عمل مسحات دموية يوميا كم أخذت عينات دم وريدي في اليوم الخامس بعد الولادة ثم كل ثلاثة ايام لمدة ١٧-٢٠ يوم. كانت نسبة الإصابة ١٠٠ في المائة في كل من العجول الفريزيان والبلدي التي عرضت للعدوى بينما المجموعات المعالجة كانت خالية من الطفيل. كانت درجة الحرارة (٤١,٢٨ ضد ٣٩,٨٦°م) ونسبة الطفيل في الدم (٢٨,٦ ضد ٩,٨ في المائة) أشد ضراوة في العجول الفريزيان. وقد أظهر تحليل النمط الكهربي لبروتين الدم انخفاض في تركيز الألبيومين وزيادة في الألفا جلوبيولين مع تقدم الإصابة. لم تظهر منطقة البيتا جلوبيولين اختلافات معنوية بينما حدث ارتفاع مبدئي في منطقة الجاما جلوبيولين ثم انخفضت فجأة مع زيادة الطفيل في الدم. وأظهر سلوك إنزيم AST والبيلوروبين والكرياتينين واليوريا في مصل الدم انخفاض معنوي مع زيادة نسبة الطفيل في الدم. وقد أظهر تحليل التباين الإحصائي أن هناك تفاعل واختلاف بين السلالتين في درجة لحرارة ونسبة الطفيل في الدم وقيم الألبيومين والألفا جلوبيولين والجاما جلوبيولن أثناء الإصابة حيث هذه التغيرات اكثر وضوحا في السلالة الأجنبية. وقد أوضحت هذه التغيرات البيوكيميائية مدى الخلل في التوازن الفسيولوجي للعائل أثناء الإصابة بالثيليريا أنيولاتا. على الرغم من أن كل من السلالتين أعطيا نفس البيئة وفرصة العدوى إلا أن هذا الخلل كان أقل وطأة في السلالة المحلية التي قد تكون قد أبدت مقاومة فطرية للإصابة.

SUMMARY

A total number 20 neonatal Friesian and indigenous (Balady) calves were used to evaluate the dynamics of clinical signs, parasitaemia and some blood biochemical components during Theileria annulata infection. Ten of them (5 of each breed) were medicated (just after born and weekly intervals, and their mothers before and after calving) with diazinon, ivermectin and buparvaquone, and used as control. The other ten calves and their mothers (5 of each breed) were left in pens with a highly active tick prevalence to induce natural infection with T. annulata. Blood smears were prepared daily from each calf and blood samples were drown at day 5 post natal and each 3 days for 17-20 days. The morbidity rate was 100 % in both Friesian and native calves subjected to infection, while medicated groups were T. annulata free along the study period. Pyrexia (41.28 vs 39.86 °C) and parasitaemia (28.6 vs 9.8 %) were more severe in Friesian calves. Blood serum electrophoresis revealed a steady reduction in albumin and elevation in α-globulin with advancement of the disease. The β-globulin values showed non-significant variation, while γ-globulin was elevated initially and then sharply fall when the parasitaemia was peaked. The behaviour of blood serum AST, bilirubin, creatinine and urea revealed significant elevation in Friesians when parasitaemia reached its magnitude. Analysis of variance disclosed significant breed dependence and interactions in body temperature, levels of parasitaemia, and the mean values of blood serum albumin, α-globulin and γ-globulin (P=0.015, 0.019, 0.04, 0.037 and 0.019 respectively) during infection, where these variations were more pronounced in the European breed. The biochemical changes reported herein revealed that T. annulata infection disrupt the physiological integrity of the host. In spite of these animals had the same environment and chance of infection, this disruption was less severe in indigenous tropical breeds, which may expressed an innate resistance.

Key words: Theileria annulata – new born calves – biochemical changes.

INTRODUCTION

Tropical theileriosis, caused by Theileria annulata (Dschunkowsky and Luhs, 1904) is a tick borne disease of cattle, widespread in North Africa, the Mediterranean basin of Europe (Mediterranean coast fever or Mediterranean theileriosis) and parts of

Asia (Norval *et al.*, 1992 and Payne and Wilson, 1999). The disease is an immense obstacle to livestock improvement and is considered a lethal disease for high-grade cattle and their crossbred especially neonates (Mallick *et al.* 1987; Mishra *et al.* 1994; Beniwal *et al.*, 1998; Bakheit and Latif, 2002 and Taylor *et al.* 2002). Neonatal calves are extremely susceptible to tropical theileriosis, even when their dams are immune (Khatri *et al.*, 2001). However, it was suggested that indigenous tropical breeds of cattle exhibit an innate resistance to tropical theileriosis (Preston *et al.*, 1992; Skinner, 2001 and Bakheit and Latif, 2002). In Egypt, clinical cases of the disease in imported and crossbred cattle have been reported in the Delta region of the Nile Valley (Ahmad, 1980, Hassanin, 1984 and Harfoush, 2001), and in Upper Egypt (Amer *et al.*, 1987 and El-Ballal and Abd El-Rahim, 1999).

The Egyptian oasis (New Valley Governorate) is a virgin area and represents the middle and southern parts of the western desert plateau. It considers one of the most promising areas for agricultural expansion in Egypt. Theileriosis is the main problem of cattle herdsmen in this area, which is caused by T. annulata and transmitted by the prevalent tick vector Hyalomma anatolicum anatolicum (Rezk, 1991 and Abou-El Hassan, 1997).

Up till now, the pathogenesis of the acute disease in neonatal calves either European or tropical is badly needed, and there are no reliable estimates on the basic effect or indications of how the disease may affect the general health of the host. Recently, Sandhu, *et al.* (1998) and Singh, *et al.* (2001) found some physical and biochemical alterations in calves experimentally infected with T. annulata by subcutaneous inoculation of ground Theileria annulata tick tissue stabilate (GUTS). Experimental studies of Forsyth *et al.* (1999) had established the first indication on the extent of the parasite dissemination in the body and had presented a new view on the destructive effect of the parasite on the host organs and tissues. The present work was designed to identify the effect of the progressive pathogenicity of natural Theileria annulata infection on the dynamics of clinical signs, parasitaemia and some blood biochemical components of neonatal Friesian and indigenous native calves in the Egyptian oasis.

MATERIALS AND METHODS

Twenty cows (10 Friesisan and 10 native) were expected to calve within 1-4 weeks were followed up during late summer, 2002. These cows were allocated in an endemic area of Theileria annulata infection at

El-Thawra vellage, El-Kharga oasis. Ten of them were separated into 2 groups (Friesian and native, 5 each). These cows were sprayed with diazinon at a rate of $5x10^{-3}$ (Neocidol, Hindustan Ciba Geigy Ltd.). Cows were given a single subcutaneous injection of ivermectin at a rate of 0.1 mg 50 kg^{-1} bodyweight (Campbell, 1985) and a single intramuscular dose of Butalex (Buparvaquone, BW720C, Coopers Animal Health Ltd., UK) at a rate of 5 mg kg^{-1} bodyweight (Wilkie, *et al.* 1998). These cows were transferred to calve in previously prepared tick free pens (by repairing of cracks, closing of burrows, spraying with diazinon and painting the walls with lime to close minute holes). Calves borne to these cows were immediately medicated by ivermectin and buparvaquone as a chemoprophylaxis therapy (Wilkie, *et al.* 1998) with the same doses of their mothers and served as control groups. The other 10 cows (Friesian and native; 5 each) were left naturally without medications to calve in pens with a highly active tick prevalence to induce natural infection for their borne calves with Theileria annulata.

All borne calves were daily subjected to careful clinical examination throughout 20-days experiment. The clinical condition of calves included the monitoring of body condition, activity, appetite visible mucous membranes, prescapular lymph nodes and rectal temperature (pyrexia was defined as >39.5 °C). Blood smears were prepared daily on the spot by oozing the blood from a small scission made at the rim of the ear, fixed with methanol and stained with Giemsa. Lymph node smears were prepared by dragging of injected saline in the suprascapular lymph node and stained with Giemsa. Parasitaemia was assessed by the percentage of RBCs infected with T. annulata trophosoites in the stained blood smear. Blood samples were drained from each calf at the 5^{th} day postnatal and then at 3 days interval for another 15-days in control calves, or until the peak of temperature rise in infected calves, which had to be emergency treated with buparvaquone and haematinics (Dhar *et al.*, 1988) in order to save their lives. Blood samples were drained by jugular vein puncture to obtain serum for biochemical determination of total protein, total bilirubin, urea, aspartate aminotransferase and creatinine using commercial test kits. Protein electrophoretogram was carried out by using Titan III cellulose acetate plate at pH 8.8 at ionic strength of 0.067, stained with Ponceau S dye and scanned by autodensitometer (Helena Laboratories, Cat. 1023) at absorption peak of 525 nm according to manufacture instructions.

Obtained data were subjected to a software program (SPSS) according to Borenstein *et al.* (1997) using linear model one way analysis of variance (*ANOVA*) followed by ordinary student "*t*" test.

RESULTS

Clinical signs:

Infected calves showed gradual rise in body temperature started at the 8-9th day in Friesian (F) and at the 11-12th day in native (N) calves (Tab. 1 & Fig. 1). The pyrexia reached its maximal level at the 17th day for both groups, but the highest duration was less in N. calves if compared with the corresponding control group (41.28 °C, P= $2x10^{-5}$ for F. and 39.86 °C, P=0.013 for N. calves). Prescapular lymph nodes were very enlarged (approximately 5-10 times) and may be visible in F. calves on day 11-12, while in N. calves the size of these lymph nodes was nearly doubled on palpation at the day 15. Other reactions as weight loss, anorexia, anaemia, hyperventilation, nasal discharge and bilateral lacrimation were clear in F. calves but not in N. calves. Latter on, 3 of F. calves were recumbent and one of them showed diarrhoea.

Table 1: Patterns of rectal temperature (Mean ±SD and level of significance)

	Friesian calves		P	Native calves		P
	Control	Infected		Control	Infected	
Day 5	38.80 ±0.170	38.74 ±0.051	0.749 ^{ns}	38.14 ±0.075	38.26 ±0.108	0.391 ^{ns}
Day 8	38.66 ±0.093	38.82 ±0.116	0.312 ^{ns}	38.02 ±0.058	38.24 ±0.117	0.143 ^{ns}
Day 11	38.58 ±0.097	39.74 ±0.492	0.082 ^{ns}	38.10 ±0.084	38.32 ±0.086	0.104 ^{ns}
Day 14	38.62 ±0.086	40.84 ±0.103	$2x10^{-7}$***	38.28 ±0.086	39.16 ±0.360	0.076 ^{ns}
Day 17	38.54 ±0.051	41.28 ±0.156	$2x10^{-5}$***	38.32 ±0.037	39.86 ±0.359	0.013*
Day 20	-	-	-	38.28 ±0.156	39.64 ±0.439	0.033*

* and *** are significance levels (P> 0.05 and 0.001 respectively) between control and infected within the same breed. ns: non-significant.

Parasiological findings:

Blood smears revealed different intra-erythrocytic forms of Theileria annulata piroplasm (Fig. 2) including signet ring, round and oval forms. Other forms, as comma and rod shapes were present in very low numbers. Micro and macroshizont were observed abundantly in the lymph nodes and the circulating lymphocytes. Tab. (2) and Fig. (1) showed that the prepatent period of piroplasm appearance and the magnitude of the ensuing parasitaemia were significantly breed dependent. The trophosoites began to appear at the 8th day of life in F. calves, while it appeared at the day 11 in N. calves. Parasitaemia peaked at the day 17 in both F. (before emergent treatment) and N. calves, but the magnitude was 28.6 % in F. and 9.8 % in N. calves.

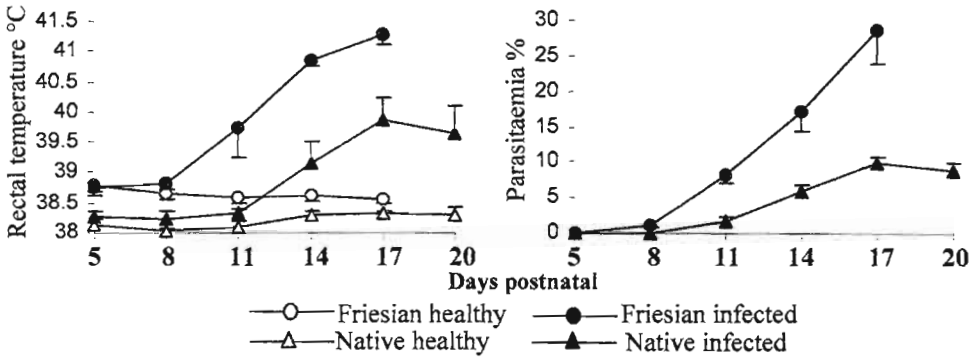

Fig. 1: Rectal temperature and percent of parasitaemia in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means ±SEM.

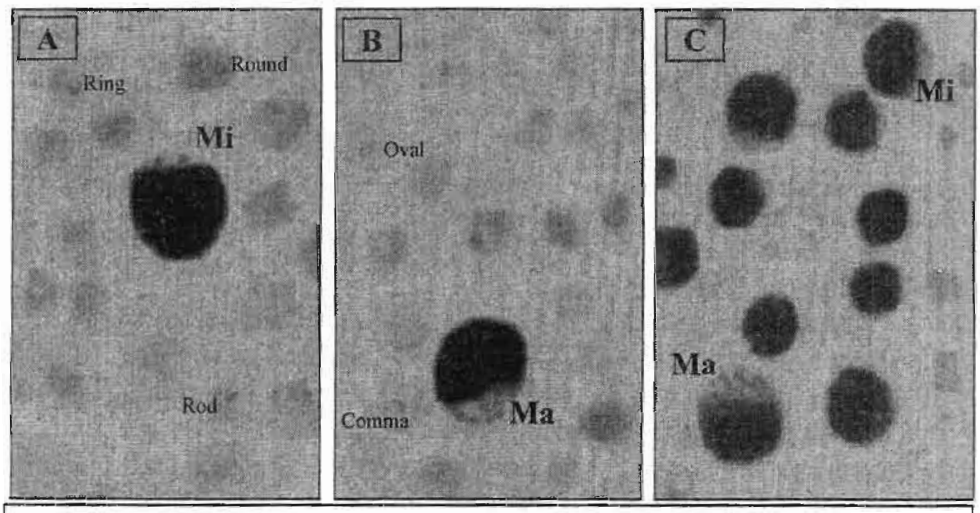

Fig. 2: Photo A & B showing different forms of intra-erythrocytic trophozoits in addition to micro (Mi) and macroschizot (Ma) stages in blood smear. Photo C showing micro (Mi) and Macroschizogony (Ma) stages in lymph node smear stained with Geimsa x1000.

Table 2: levels of parasitaemia (Mean ±SD and level of significance).

	Friesian calves	Native calves	P
Day 5	00.0	0.00	-
Day 8	01.2 ±0.490	0.00	-
Day 11	08.2 ±1.158	1.8 ±0.374	0.003**
Day 14	17.2 ±2.956	5.8 ±1.068	0.015*
Day 17	28.6 ±4.854	9.8 ±0.860	0.019*
Day 20	-	8.8 ±1.114	-

* and ** are significance levels between breeds at P> 0.05 and 0.01 respectively.

The effect of theileriosis on blood serum biochemical components in neonatal calves:

I- Changes in blood serum proteins:

Table 3: Values of serum total proteins. (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	6.10± 0.597	6.02± 0.489	0.9200 ns	6.06± 0.495	5.96± 0.575	0.8984 ns
Day 8	6.36± 0.403	6.42± 0.377	0.9162 ns	6.14± 0.307	6.04± 0.536	0.8768 ns
Day 11	6.10± 0.311	6.46± 0.364	0.4740 ns	6.00± 0.167	6.32± 0.285	0.3707 ns
Day 14	6.16± 0.397	5.58± 0.464	0.3701 ns	5.96± 0.163	5.76± 0.309	0.5880 ns
Day 17	6.04± 0.386	4.84± 0.341	0.0485*	5.88± 0.193	5.70± 0.405	0.7022 ns
Day 20	-	-		5.84± 0.098	5.26± 0.258	0.0896 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

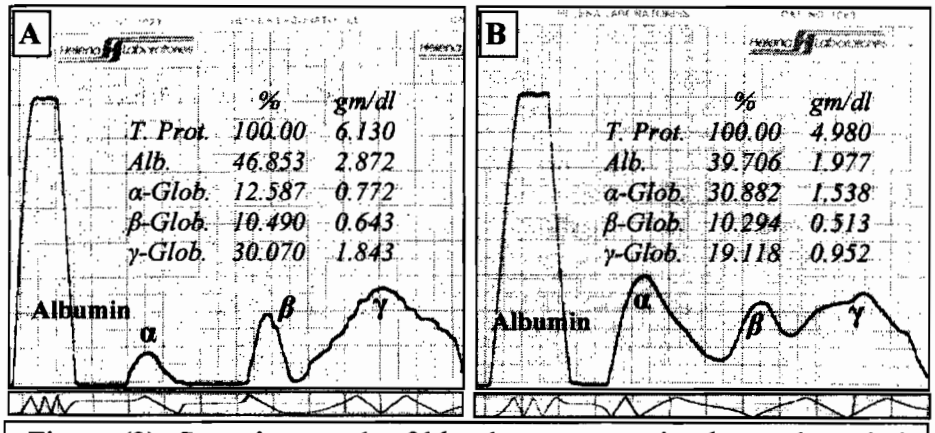

Figure (3): Scanning graph of blood serum protein electrophoresis in 17-day-old Friesian calf showing: A. Normal pattern. B. Patterns in calf infected with T. annulata.

1- Total serum proteins:

Along the course of infection, the mean values of total blood serum proteins showed insignificant fluctuation in both infected F. and

N. calves if compared by the corresponding non-infected control calves (Tab. 3 and Fig. 4). Thereafter, these values tend to decrease insignificantly (P=0.09) in N. and significantly (P=0.049) in F. calves.

2- Blood serum electrophoretogram:

Blood serum electrophoresis revealed 4 distinct bands; albumin and 3 globulin fractions (α, β αand γ) in all investigated samples (Fig. 3).

a- Serum albumin:

Infected calves in both breeds showed dramatic reduction in the mean values of blood serum albumin concentrations (Tab. 4, Fig. 4). This reduction was significant (P=0.0148) in N. calves, while it was highly significant ($P=1x10^{-3}$) in F. calves at late stages.

Table 4: Mean values ±SD of serum albumin and levels of significance (P).

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	2.56± 0.357	2.48± 0.274	0.8635 ns	2.64± 0.395	2.50± 0.305	0.7864 ns
Day 8	2.80± 0.259	2.36± 0.267	0.2712 ns	2.72± 0.329	2.46± 0.271	0.5591 ns
Day 11	2.70± 0.234	2.06± 0.216	0.0795 ns	2.74± 0.229	2.34± 0.201	0.2265 ns
Day 14	2.94± 0.260	1.84± 0.075	0.0105*	2.82± 0.146	2.20± 0.145	0.0168*
Day 17	2.86± 0.144	1.55± 0.086	0.0001***	2.70± 0.128	2.13± 0.188	0.0160*
Day 20	-	-		2.76± 0.196	1.94± 0.177	0.0148*

* and *** are significance levels (P> 0.05 and 0.001 respectively) between control and infected within the same breed. ns: non-significant

b- Serum α-globulin:

There was a steady increase in the mean values of blood serum α-globulin concentrations in T. annulata infected F. calves until reached its maximal level (1.54 g/dl) at the 17th day of life (Tab. 5, Fig. 4). This elevation was highly significant ($P=9x10^{-4}$) if compared by the non-infected control group (0.82 g/dl). On the other hand, the mean values of α-Globulin in infected N. calves showed non-significant variation than control animals.

Table 5:Values of serum α α -globulin. (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	1.26± 0.136	1.18± 0.120	0.6713 ns	1.16± 0.121	1.18± 0.159	0.9231 ns
Day 8	1.20± 0.100	1.14± 0.103	0.6869 ns	1.12± 0.102	1.14± 0.133	0.9078 ns
Day 11	1.06± 0.068	1.34± 0.075	0.0242*	1.02± 0.066	1.18± 0.132	0.3201 ns
Day 14	0.90± 0.083	1.42± 0.139	0.0151*	0.98± 0.080	1.18± 0.107	0.1775 ns
Day 17	0.82± 0.073	1.54± 0.108	0.0009***	0.92± 0.091	1.16± 0.108	0.1281 ns
Day 20	-	-		0.86± 0.093	1.22± 0.139	0.0685 ns

* and *** are significance levels (P> 0.05 and 0.001 respectively) between control and infected within the same breed. ns: non-significant

Table 6: Values of serum β β -globulin. (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	0.84± 0.143	0.90± 0.122	0.7586 ns	0.86± 0.103	0.92± 0.097	0.6826 ns
Day 8	0.92± 0.183	0.94± 0.068	0.9223 ns	0.88± 0.116	0.92± 0.120	0.8164 ns
Day 11	0.94± 0.116	1.00± 0.130	0.7404 ns	0.86± 0.147	0.86± 0.143	1.0000 ns
Day 14	0.92± 0.180	0.98± 0.174	0.8168 ns	0.84± 0.153	0.94± 0.143	0.6470 ns
Day 17	0.90± 0.181	0.96± 0.150	0.8056 ns	0.82± 0.156	1.00± 0.152	0.4323 ns
Day 20	-	-		0.80± 0.167	0.92± 0.156	0.6143 ns

ns: the values of control and infected within the same breed are non-significant

Table 7: Values of serum γ -globulin. (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	1.44± 0.196	1.46± 0.222	0.9480 ns	1.40± 0.151	1.36± 0.172	0.8659 ns
Day 8	1.44± 0.103	1.98± 0.177	0.0388*	1.42± 0.208	1.52± 0.146	0.7061 ns
Day 11	1.40± 0.114	2.06± 0.103	0.0026**	1.38± 0.188	1.94± 0.068	0.0380*
Day 14	1.40± 0.176	1.34± 0.196	0.8258 ns	1.32± 0.185	1.44± 0.209	0.6788 ns
Day 17	1.46± 0.128	0.82± 0.102	0.0046**	1.36± 0.180	1.48± 0.182	0.6529 ns
Day 20	-	-		1.42± 0.177	1.18± 0.102	0.2849 ns

* and ** are significance levels (P> 0.05 and 0.001 respectively) between control and infected within the same breed. ns: non-significant

Table 8:Values of total serum globulin. (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	3.54± 0.360	3.54± 0.287	1.0000 ns	3.42± 0.198	3.46± 0.276	0.9098 ns
Day 8	3.56± 0.265	4.06± 0.248	0.2064 ns	3.42± 0.222	3.58± 0.274	0.6626 ns
Day 11	3.40± 0.178	4.40± 0.248	0.0138*	3.26± 0.250	3.98± 0.177	0.0512 ns
Day 14	3.22± 0.360	3.74± 0.421	0.3762 ns	3.14± 0.238	3.56± 0.220	0.2315 ns
Day 17	3.18± 0.320	3.32± 0.278	0.7498 ns	3.10± 0.249	3.64± 0.282	0.1892 ns
Day 20	-	-		3.08± 0.243	3.32± 0.213	0.4797 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

c- Serum β-globulin:

Non of F. or N. calves had recorded any significant variation in the behaviour of the mean values of β-globulin during T. annulata infection if compared by control groups (Tab. 6, Fig. 4).

d- Serum γ-globulin:

Infected F. calves showed 2 phases of γ-globulin variations during the course of infection (Tab. 7, Fig. 4). Initially, the mean values were steadily increased until it peaked significantly (P=0.0026) at the day 11. As the diseased progressed, there was a sharp fall in the mean values until it reached its minimal level on the day 17. This reduction was highly significant (P= 0.0046) if compared with control animals.

The mean values of blood serum γ-globulin concentration in infected N. calves run insignificantly throughout the course of infection with an exceptional elevated (P=0.038) peak at the day 11 when compared with the corresponding control group.

e- Total serum globulin:

The results showed non-significant variation in the mean values of total blood serum globulin concentrations in both infected groups with an exception of significantly elevated peak at day 11 in F. infected calves (Tab. 8, Fig. 4).

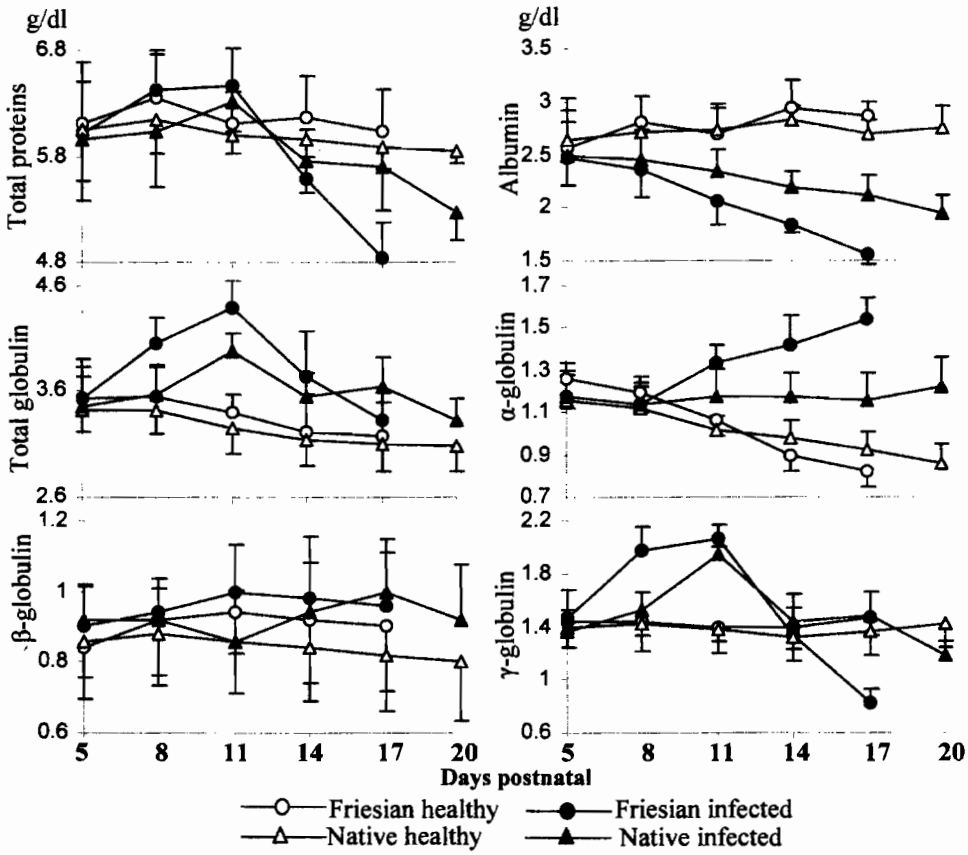

Fig. 4: Total blood serum protein and protein electrophoretogram in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means ± SEM.

Table 9: Values of serum AST (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	32.4 ±4.874	33.4 ±6.623	0.9066 ns	29.6 ±5.644	30.6 ±7.852	0.9205 ns
Day 8	27.4 ±3.140	35.2 ±5.342	0.2549 ns	27.6 ±5.706	30.2 ±5.342	0.7480 ns
Day 11	28.6 ±1.691	58.4 ±8.134	0.0230*	31.2 ±4.510	36.2 ±4.140	0.4377 ns
Day 14	31.6 ±2.960	62.6 ±7.160	0.0103*	30.2 ±3.813	46.0 ±4.827	0.0332*
Day 17	35.2 ±3.308	57.8 ±6.012	0.0165*	29.6 ±1.503	42.2 ±5.342	0.0724 ns
Day 20	-	-	-	31.8 ±4.259	38.8 ±6.414	0.3935 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

Table 10: Values of serum total Bilirubin (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	0.644 ±0.086	0.572 ±0.120	0.6409 ns	0.490 ±0.076	0.446 ±0.051	0.646 ns
Day 8	0.528 ±0.051	0.532 ±0.085	0.9690 ns	0.432 ±0.070	0.410 ±0.030	0.785 ns
Day 11	0.356 ±0.042	0.362 ±0.070	0.9438 ns	0.276 ±0.039	0.272 ±0.024	0.932 ns
Day 14	0.246 ±0.039	0.408 ±0.060	0.0578 ns	0.226 ±0.012	0.286 ±0.036	0.176 ns
Day 17	0.258 ±0.046	0.654 ±0.100	0.0113*	0.264 ±0.036	0.374 ±0.079	0.251 ns
Day 20	-	-	-	0.248 ±0.049	0.464 ±0.091	0.081 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

Table 11: Values of serum urea (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	5.42 ±0.796	6.36 ±0.717	0.4059 ns	5.86 ±1.059	5.08 ±1.153	0.6317 ns
Day 8	6.64 ±0.919	6.32 ±0.731	0.7921 ns	4.90 ±0.448	5.14 ±0.781	0.7988 ns
Day 11	5.14 ±0.627	5.56 ±1.075	0.7471 ns	6.12 ±0.655	5.88 ±0.639	0.7998 ns
Day 14	3.72 ±0.652	5.68 ±0.924	0.1267 ns	4.78 ±0.909	6.38 ±0.773	0.2166 ns
Day 17	4.70 ±0.763	9.12 ±1.321	0.0274*	4.14 ±0.919	7.20 ±0.986	0.0529 ns
Day 20	-	-	-	3.94 ±0.715	7.32 ±1.260	0.0584 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

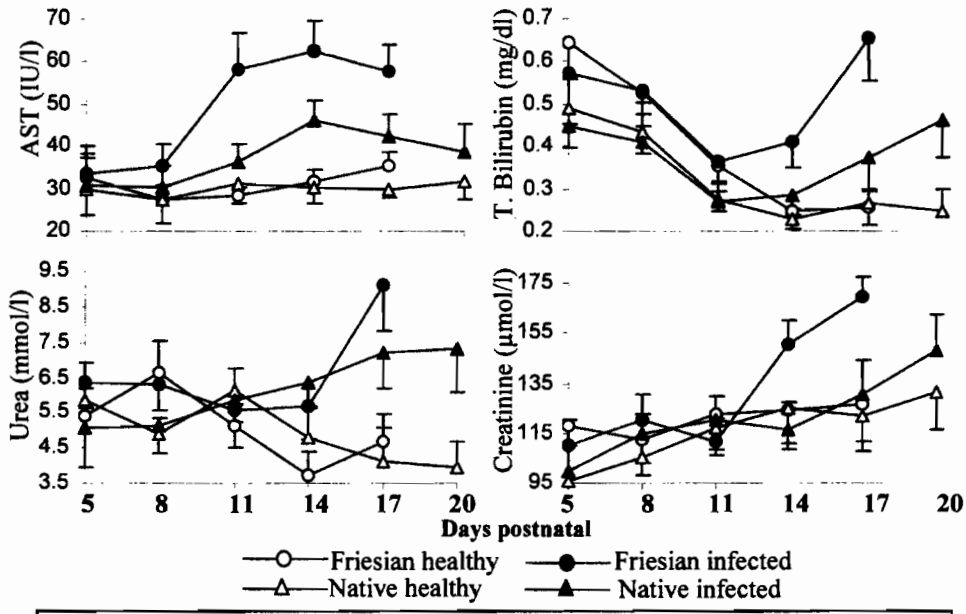

Fig. 5: The behaviour of AST, bilirubin, urea and creatinine in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means ± SEM.

Table 12: Values of serum creatinine (Mean ±SD and level of significance)

	Friesian			Native		
	Control	Infected	P	Control	Infected	P
Day 5	118.2 ±08.4	109.8 ±10.7	0.554 ns	095.4 ±11.8	099.6 ±10.7	0.799 ns
Day 8	112.4 ±09.2	120.4 ±10.2	0.575 ns	105.4 ±06.9	114.6 ±08.4	0.423 ns
Day 11	123.0 ±14.6	111.8 ±09.5	0.482 ns	117.2 ±11.1	120.6 ±09.6	0.822 ns
Day 14	124.6 ±16.0	151.0 ±09.2	0.203 ns	125.2 ±14.6	116.4 ±11.3	0.647 ns
Day 17	126.4 ±15.0	169.8 ±08.2	0.044*	121.8 ±14.5	130.8 ±13.5	0.662 ns
Day 20				131.6 ±15.2	148.4 ±14.1	0.441 ns

* is the significance level (P> 0.05) between control and infected within the same breed. ns: non-significant

II- Changes in blood serum aspartate aminotransferase (AST):

Infected F. calves showed gradual rise in the mean values of blood serum AST concentrations when compared by controls (Tab. 9, Fig. 5), which started at the day 11 and reached its magnitude at the late stage (P=0.017). The mean values in N. calves revealed significant elevation (P=0.033) in day 14 only.

III- Changes in total blood serum bilirubin:

No significant changes were observed in the mean values of total blood serum bilirubin between both infected F. and N. calves and the corresponding control groups except at day 17 (Tab. 10, Fig. 5). At this

time, the value was significantly elevated (P=0.011) in infected F. calves than their control group, in spite of absence of variation of this value than the base data (at day 5) of the same infected group.

IV- Changes in blood serum urea and creatinine:

The mean values of blood serum urea and creatinine concentrations were insignificantly fluctuated until day 14 (Tab. 11,12 and Fig. 5). There after, the values were elevated significantly (P=0.027 for urea and 0.044 for creatinine) in infected F. calves, but the elevation was non-significant (P=0.058 for urea and 0.441 for creatinine) in infected N. calves if compared with the corresponding control groups.

Breed interactions

Analysis of variance revealed significant breed dependence in the rise of body temperature, magnitude of parasitaemia, and the mean values of blood serum α-globulin (P=0.015, 0.019 and 0.037 respectively) during infection. There was also significant interaction between F. and N. calves in the decrease of the mean values of blood serum albumin and γ-globulin concentrations (P=0.04 and 0.019 respectively), where these variations were more pronounced in the European breed (Tab. 13).

Table 13: Significance levels (*P-value*) of breed interactions in control (C) and T. annulata infected (I) neonatal calves during the first 20 days of life.

Parameter		*Day 5*	*Day 8*	*Day 11*	*Day 14*	*Day 17*
Rectal temp.	C	0.0160*	$6x10^{-4}$***	0.006**	0.0230*	0.010 *
	I	0.007**	0.008**	0.046*	0.006**	0.015 *
Total proteins	C	0.960 ns	0.677 ns	0.786 ns	0.660 ns	0.724 ns
	I	0.938 ns	0.580 ns	0.769 ns	0.756 ns	0.143 ns
Albumin	C	0.884 ns	0.853 ns	0.906 ns	0.706 ns	0.688 ns
	I	0.962 ns	0.799 ns	0.371 ns	0.069 ns	0.040 *
Total globulin	C	0.780 ns	0.696 ns	0.662 ns	0.858 ns	0.848 ns
	I	0.846 ns	0.230 ns	0.211 ns	0.718 ns	0.442 ns
A- globulin	C	0.598 ns	0.590 ns	0.655 ns	0.509 ns	0.419 ns
	I	1.000 ns	1.000 ns	0.332 ns	0.213 ns	0.037*
B- globulin	C	0.913 ns	0.858 ns	0.681 ns	0.744 ns	0.747 ns
	I	0.901 ns	0.889 ns	0.490 ns	0.863 ns	0.856 ns
Γ- globulin	C	0.875 ns	0.934 ns	0.930 ns	0.762 ns	0.665 ns
	I	0.731 ns	0.080 ns	0.362 ns	0.736 ns	0.019 *
AST	C	0.717 ns	0.976 ns	0.612 ns	0.779 ns	0.174 ns
	I	0.792 ns	0.526 ns	0.051 ns	0.095 *	0.088 ns
T. bilirubin	C	0.217 ns	0.304 ns	0.201 ns	0.645 ns	0.919 ns
	I	0.378 ns	0.234 ns	0.279 ns	0.124 ns	0.059 ns
Creatinine	C	0.160 ns	0.561 ns	0.761 ns	0.979 ns	0.831 ns
	I	0.519 ns	0.672 ns	0.533 ns	0.647 ns	0.662 ns
Urea	C	0.749 ns	0.139 ns	0.311 ns	0.374 ns	0.651 ns
	I	0.377 ns	0.302 ns	0.805 ns	0.577 ns	0.282 ns

*, ** and *** are significance levels (P> 0.05, 0.01 and 0.001 respectively) between breeds in either control or infected calves. ns: non-significant

DISCUSSION

Theileriosis is an endemic problem in cattle in the Egyptian oasis. Morbidity rate may reach up to 100 % and mortality rate may exceed 50 % of untreated newly born calves (Abou-El Hassan, 1997). Buparvaquone had been described as a drug of choice for elimination of the parasite in this area (Abou-El Hassan, 1997) with high efficacy (Mourad, 1999).

In the present work, morbidity rate was 100 % in both Friesian and native calves subjected to infection, while medicated groups were shown to be Theileria free. The clinical signs appeared on infected calves and the presence of different forms of intra-erythrocytic trophozoites were completely typical to those described previously by many authors for T. annulata infection (Soulsby, 1982, Urquhart *et al.*, 1996 and Radostits *et al.*, 2000). However, the severity of clinical signs, the time of fever, the degree of enlargement of prescapular lymph nodes and the first appearance of trophozoite in the blood were shown to be breed dependent. These results support the previous experimental studies, which suggested that high graded breeds of cattle are more sensitive to theileriosis than tropical breeds (Preston *et al.*, 1992). These differences may be innate, and related to the high rate of schizont multiplication in graded cattle and ability of the tropical cattle to limit the macroschizont index (Bakheit and Latif, 2002). On the other hand, studies of Wambura *et al.* (1998) and Mattioli (2002) showed that indigenous tropical breed of cattle were more resistant to tick infestation than Friesian cattle.

In newborns, the proliferation and differentiation of the gastrointestinal tract and the variations in digestive and absorptive capacity may influence the immune status, metabolism, and endocrine systems (Hammon and Blum, 1998). So that, biochemical constituents in neonatal period have its specific characters and differ than juveniles and matures (Kűhne *et al.*, 2000). In the present study, the dynamics of the mean values of biochemical parameters in blood serum of medicated (control) calves throughout the first 20 days of life were within the ranges cited for neonatal calves by Bouda and Jagos (1984), Grűnder and Fiao (1994) and Knowles *et al.* (2000). Also, the electrophoretic pattern of blood serum proteins obtained in this study coincides with the protein electrophoretogram previously reported for neonatal calves (Kaneko, 1997 and Thomas, 2000a,b).

Blood serum proteins may provide useful markers for the pathogenesis of T. annulata infections (Skinner, 2001). In the present work, the mean values of blood serum albumin concentrations were reduced in both infected groups These results coincide with those reported in experimental infection by Theileria annulata tick tissue stabilate in calves (Sandhu, *et al.*, 1998 and Singh, *et al.*, 2001) and during natural infection in young and adult cattle (Omer *et al.*, 2003). The pyrexia accompanied by anorexia in addition to the involvement of the liver, which is highly affected (Singh, *et al.*, 2001), may be the primary factors of albumin reduction during theileriasis. The reduction of blood serum albumin in the present study was more severe in Friesian calves than indigenous native calves, which indicate the superior sensitivity of foreign breeds to the infection.

In contrast to albumin, the present study showed a steady elevation in the mean values of blood serum α-globulin concentrations during the course of T. annulata infection. This rise was noticed only in Friesian calves. These results reverse the findings of Singh, *et al.* (2001) who found severe reduction in the mean values of blood serum α-globulin concentrations during the course of experimental T. annulata infection in crossbred calves. Recent studies proved that T. annulata enters bovine macrophages, differentiates into the intracellular macroschizont stage, and induces "transformation" and functional changes resulting in a clonal expansion of infected cells (Skinner, 2001). Pivotal pro-inflammatory cytokine and monokine mRNA including tumor necrosis factor-α (TNFα), interleukin-1 (IL_1), interleukin-2 (IL_2), interleukin-6 (IL_6) and interferon-γ (IFNγ) in addition to aberrantly activated immune cells such as ^{CD4+}T and ^{CD8+}T cells were rapidly upregulated following infection (Glass, 2000 and Glass, 2001b). Acute phase proteins (APP) as haptoglobin (Hp), α_1-acid glycoprotein (α_1AGP) and serum amyloid A (SAA) were induced by these cytokines, and increased following T. annulata infection in Friesians (Glass, 2001a,b). From these results, the rise of α-globulin in the present work was expected because these APP (Hp, α_1AGP and SAA) are the major components of α-globulin band during electrophoretic fractionation of blood serum proteins (Kaneko, 1997 and Thomas, 2000a,b). The production of cytokines during T. annulata infection may be also another factor interpret the reduction of albumin in this study because the latter behave as a negative AAP reactant during acute infections (Kaneko, 1997 and Thomas, 2000a,b). In contrast, the absence of variations in α-globulin in native calves in this study coincide with the results of Glass (2001a,b) who reported that indigenous tropical breed underwent a less

severe reaction, self-limiting disease, and significantly lower levels of APP were produced.

In the current work, blood serum β-globulin fraction of infected calves in both breeds was fluctuated within narrow limits and did not differ than control group. These results contradict the results of Singh, *et al.* (2001) who found reduction in this fraction during the course of experimental T. annulata infection in crossbred calves. In the blood serum, this protein fraction consisted mainly of a positive APP (ferritin) and a negative APP reactant, transferrin (Kaneko, 1997, Eckersall, 2000 and Thomas, 2000a,b). It seems that there are no specified reports describe the behaviour of these individual proteins during T. annulata infection. However, if these reactions are true during theileriosis, the positive APP (ferritin) may antagonize the negative APP (transferrin) during the acute phase of T. annulata infection and the net result is absence of significant variation as obtained in this study.

The present study showed initial increase in the mean values of blood serum γ-globulin concentrations followed by sudden fall in these values when parasitaemia reached its peak. Similar results were obtained by Singh, *et al.* (2001). These authors found that these results were correlated with the initial leucocytosis, which latter on converted into leucopenia. In other studies, Moreau *et al.* (1999) found initial uncontrolled proliferation and neoplastic transformation of B lymphocytes (which are responsible for humoral immunity and production of immunoglobulins) followed by down-regulation of surface immunoglobulins on B lymphocytes and loss of certain surface markers on macrophages during T. annulata infection in calves. With advancement of the infection, parasite infected cells acquire dendritic cell features and over-activate CD^{4+} and CD^{8+}T cells (which are responsible for cellular immunity), while B cells are developmentally arrested (Ahmed and Mehlhorn., 1999; Glass, 2001b and Heussler *et al.*, 2001). These results were confirmed by (Khatri *et al.*, 2001) who found that protective immunity is mainly cell-mediated and antibodies may only play a small role. Furthermore, parasitised cells may induce a potent, but unspecific, proliferative response of non-infected lymphocytes followed by an unspecific lysis of infected as well as non-infected cells (Heussler *et al.*, 2001). On the other hand, Forsyth *et al.* (1999) found more cytokines production with increased population of parasitized cells, which may overwhelm its beneficial properties and suppress or dampen the active immune responses of mononuclear macrophages.

The reactions of globulin fractions toward T. annulata infection in this study were coalesced and resulted in initial hyperglobulinaemia in Friesian calves which returned to its normal levels latter on. The interaction of albumin and total globulin during the infection resulted in a consequent fall in the mean values of total serum protein concentrations. These results are similar to those reported by Singh, *et al.* (2001) and Omer, *et al.* (2003).

The behaviour of the mean values of blood serum AST, bilirubin, creatinine and urea in this study revealed significant elevation when parasitaemia reached its peak level. These results are in agreement with the reports of Sandhu, *et al.* (1998), Singh, *et al.* (2001) and Omer *et al.* (2003) during experimental and natural T. annulata infection in cattle. Pathological studies of Abou-El Hassan (1997) and Singh (1998) showed several and different macroscopic and microscopic lesions in body organs during T. annulata infection in calves, especially liver and kidney, resulting in hepatic and renal damages. These damages may be resulted from the rapid spread, metastasis, proliferation and dissemination of the parasited mononuclear macrophages in these organs or due to the excessive production of cytokines, which may overwhelm its beneficial properties, and push the host toward lethal disease than recovery (Forsyth *et al.*, 1999). The changes in the aforementioned biochemical parameters in the present work were less severe or may be absent in infected native calves.

In conclusion, the biochemical changes reported herein revealed that T. annulata infection disrupt the physiological integrity of the host. In spite of the same allow of environment and chance of infection, this disruption was more severe in high graded breeds, while it was less severe or may be absent in native tropical type. It seems that this is the first report describes the effect of natural T. annulata infection on biochemical dynamics of neonates and the interactions between European and native tropical breeds reared under tropical arid environment. This study, which dealt with simple electrophoretic scanning of serum proteins and some classic liver and kidney function tests may support the advanced immunocytochemical studies reported previously (Forsyth, *et al.*, 1999; Skinner, 2001and Glass, 2001a,b) about the innate resistance of tropical breeds to T. annulata infection.

REFERENCES

Abou-El Hassan, L. A. M. (1997): Clinicopathological study of theileriasis in New-Valley. M. Vet. Sc. Thesis, Fac. Vet. Med. Assiut Univ.

Ahmad, A. B. (1980): Some biological studies on blood parasites in Farm animals. Ph. D. Thesis, Fac. Vet. Med. Zagazig Univ.

Ahmed JS, Mehlhorn H (1999): Review: the cellular basis of the immunity to and immunopathogenesis of tropical theileriosis. Parasitol Res 85: 539-549

Amer, A. A., Mourad, M. I. and Salem, H. A. (1987): Theileriosis in Friesian cattle in Upper Egypt. Assiut Vet. Med. J. 36: 148-152.

Bakheit, M. A. and Latif, A. A. (2002): The innate resistance of Kenana cattle to tropical theileriosis (Theileria annulata infection) in the Sudan. Annals of the New York Academy of Sciences. 969: 159-163.

Beniwal, R. K; Sharma, R. D and Nichani, A. K. (1998): Susceptibility to tropical theileriosis of calves born to dams immunized with Theileria annulata (Hisar) cell culture vaccine. Trop Anim Health Prod. 30 :341-349.

Borenstein, M; Rothstein, H. and Cohen, J. (1997): Sample Power Statistics 1.0. SPSS Inc., Chicago.

Bouda, J. and Jagos, P. (1984): Biochemical and hematological reference values in calves and their significance for health control. Acta Vet. Brno. 53: 137-142.

Campbell, W. C. (1985): Ivermectin: an update. Parasitol. Today, 1: 10-16.

Dhar, S; Malhotra, D. V; Bhushan, C. and Gautam, O. P. (1988): Treatment of experimentally induced Theileria annulata infection in cross-bred calves with buparvaquone. Vet Parasitol 1988 Mar;27(3-4):267-75

Dschunkowsky, E. and Luhs, J. (1904): Die Piroplasmosen der Rinder. (Vorl. Mitt.) Zentralblatt für Bakteriologei, Parasitenkunde, Infektionskrankheit und Hygiene. Abteilung 1, 35: 486-492.

El-Ballal, S. S. and Abd El-Rahim, I. H. A. (1999): Concurrent infection with Theileria annulata and Rift Valley fever virus in a dairy cattle farm. 5th Sci. Cong., Egyptian Society for Cattle Diseases, 28-30 Nov. Assiut, Egypt. Pp 144-155.

Eckersall, P. D. (2000): Recent Advances and Future Prospects for the Use of Acute Phase Proteins as Markers of Disease in Animals. Revue Med. Vet., 151: 577-584.

Forsyth, L. M; Minns, F. C; Kirvar, E; Adamson, R. E; Hall, F. R; McOrist, S; Brown, C. G, and Preston, P. M. (1999): Tissue damage in cattle infected with Theileria annulata accompanied by metastasis of cytokine-producing, schizont-infected mononuclear phagocytes. J Comp Pathol.120: 39-57.

Glass, E. J. (2000): Acute phase protein responses to tropical theileriosis in cattle. European Colloquium report on animal acute phase proteins, April 7th 2000, University of Glasgow Veterinary School, Bearsden Road, Glasgow.

Glass, E. J. (2001a): Serum Amyloid A Responses to Tropical Theileriosis in Cattle. 2nd European Colloquium report on animal acute phase proteins May 11th-13th 2001 University of Bonn. , Germany.

Glass, E. J. (2001b): The balance between protective immunity and pathogenesis in tropical theileriosis: what we need to know to design effective vaccines for the future. Res. Vet. Sci., 70 : 71-75.

Grűnder, M. L. H. D. and Fiao, A. (1994): Hemocytological and hemobiochemical investigations on clinically healthy calves (Friesian breed) in Morocco. Dtsch. Tierärztl. Wschr. 98: 94-102.

Hammon, H; and Blum, J. W. (1998): Metabolic and endocrine changes in neonatal calves. In: J. W. Blum, T. Elsasser, and P. Guilloteau (Ed.) Symposium on Growth in Ruminants. August 1998, Berne, Switzerland. University of Berne. pp 39-48.

Harfoush, M. A. (2001): Some studies on blood parasites in both cattle and tick victor. 6th Sci. Cong. Egyptian Society for Cattle Diseases, 4-6 Nov. 2001, Assiut, Egypt.

Hassanin, H. A. (1984): Studies on bovine theileriosis in Egypt. Ph. D. Thesis, Fac. Vet. Med. Cairo Univ.

Heussler, V. T; Kueenzi, P. and Rottenberg, V. (2001): Inhibition of apoptosis by intracellular protozoan parasites. Invited review. International Journal for Parasitology. 31: 1166-1176

Kaneko, J. J. (1997): Serum Proteins and dysproteinemias, In Clinical biochemistry of domestic animals. Eds, Kaneko, J. J., Harvey, J. W., and Bruss, M. L., 5th Ed, Academic press, London. pp 117.

Khatri, N; Nichani, A. K; Sharma, R. D; Khatri, M. and Malhotra, D. V. (2001): Effect of vaccination in the field with the Theileria annulata (Hisar) cell culture vaccine on young calves born during the winter season. Vet. Res. Commun., 25: 179–188.

Knowles, T. G; Edwards, J. E; Bazeley, K. J; Brown, S. N; Butterworth, A. and Warriss, P. D. (2000): Changes in the blood biochemical and haematological profile of neonatal calves with age. Vet Rec. 147: 593-598.

Kűhne, S. Hammon, R. M. Bruckmaier, H. M. Morel, C. Zbinden, Y. and Blum, J. W. (2000): Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed

either colostrum or milk replacer at two levels. J. Anim. Sci. 78: 609–620.

Mallick, K. P; Dhar, S; Malhotra, D. V; Bhushan, C. and Gautam, O. P. (1987): Immunization of neonatal bovines against Theileria annulata by an infection and treatment method. Vet Parasitol. 24: 169-173.

Mattioli, R. C. (2002): Comparative studies on host susceptibility to ticks and tick-borne diseases in trypanotolerant N'Dama and trypanosensitive Gobra zebu cattle. 2nd International Consortium on Ticks and Tick-borne Diseases (ICTTD-2). Integrated Control of Ticks and Tick-borne Diseases. (1 July 2001-30 June 2002). The University of Utrecht, Netherlands.

Mishra, A. K; Sharma, N. N. and Srivastava, S. C. (1994): Theileria annulata infection in neonatal bovine calves. Acta Vet Hung. 42: 99-102.

Moreau, M. O; Thibaud, J; Leila Ben Miled, Marie, C; Martin, B; Davis, WC; Paola Minoprio and Gordon Langsley (1999): Theileria annulata in CD51 Macrophages and B1 B Cells. Infection and immunity. 67: 6678–6682.

Mourad, M. I. (1999): Efficacy of Butalex (Buparvaquone) against naturally infected cattle with theileriasis in El-Wadi El-Gadid Governorate (Upper Egypt). 86-88.

Norval, R. A. I; Perry, B. D. and Young, A. S. (1992): The Epidemiology of Theileriosis in Africa. San Diego: Academic Press. pp. 481.

Omer, O. H; El-Malik, K. H; Magzoub, M; Mahmoud, O. M; Haroun, E. M; Hawas, A. and Omar, H. M. (2003): Biochemical profiles in Friesian cattle naturally infected with Theileria annulata in Saudi Arabia. Vet Res Commun. 27: 15-25.

Payne, W. J. A. and Wilson, R. T. (1999): An Introduction to Animal Husbandry in the Tropics, 5th ed., Blackwell Science Ltd.

Preston, P. M; Brown, C. G; Bell-Sakyi, L; Richardson, W. and Sanderson, A. (1992): Tropical theileriosis in Bos taurus and Bos taurus cross Bos indicus calves: response to infection with graded doses of sporozoites of Theileria annulata. Res Vet Sci., 53: 230-243

Radostits, O. M; Blood, D. C. and Gay, C. C. (2000): Veterinary Medicine, 8th Ed. Baillier Tindall, London. pp 1230.

Rezk, E. F. (1991): Studies on behavioural and clinical patterns in relation to blood parasites infection of cattle and buffaloes in Northern dry region in Africa, with special reference to New-Valley. M. Vet. Sc. Thesis, Fac. Vet. Med. Cairo Univ.

Sandhu, G. S; Grewal, A. S; Singh, A; Kondal, J. K; Singh, J. and Brar, R. S. (1998): Haematological and biochemical studies on experimental Theileria annulata infection in crossbred calves. Vet. Res. Commm. 22: 347-354.

Singh, A. (1998): Clinicopathological studies on experimental Theileria annulata infection in crossbred calves. MVSc thesis, Punjab Agric. Univ. India.

Singh, A; Singh, J; Grewal, A. S. and Brar, R. S. (2001): Studies on some blood parameters of crossbred calves with experimental Theileria annulata infections. Vet. Res. Commm. 25: 289-300.

Skinner, J. G. (2001): International standardization of acute phase proteins. Vet Clin Pathol. 30: 2-7.

Soulsby, E. J. L. (1982): Helminth, arthropods and protozoa of domesticated animals. 7ed. The English language Book Society. Balliere, Tindall and Cassel. London.

Taylor, L. H; Welburn, S. C. and Woolhouse, M. E.. (2002): Theileria annulata: virulence and transmission from single and mixed clone infections in cattle. Exp Parasitol. 100 :186-195

Thomas, J. S. (2000a): Overview of Plasma Proteins. In Schalm's Veterinary Hematology. 5th Ed. Feldman, B. F., Zinkl, J. G. and Jain, N. C. Lippincott Williams & Wilkins, Philadelphia, Baltimore. pp. 891-898.

Thomas, J. S. (2000b): Protein Electrophoresis. In Schalm's Veterinary Hematology. 5th Ed. Feldman, B. F., Zinkl, J. G. and Jain, N. C. Lippincott Williams & Wilkins, Philadelphia, Baltimore. pp. 899-903.

Urquhart, G. M; Armour, J; Duncan, J. L; Dunn, A. M. and Jennings, F. W. (1996):. Veterinary parasitology, 2nd ed., Blackwell Science.

Wambura, P. N; Gwakisa, P. S; Silayo, R. S. and Rugaimukamu, E. A. (1998): Breed associated resistance to tick infestation in Bos indicus and their crosses with Bos taurus. Vet. Parasitol., 77: 63-70.

Wilkie, G. M; Brown, C. G; Kirvar, B. E; Thomas, M; Williamson, S. M; Bell-Sakyi, L. J. and Sparagano, O. (1998): Chemoprophylaxis of Theileria annulata and Theileria parva infections of calves with buparvaquone. Vet. Parasitol. 78: 1-12.