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**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

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

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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 والإقرمكتين 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 أيام 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 الكهربائي لبروتين الدم انخفاض في تركيز الألبومين
وزيادة في الألفا جلوبولين مع تقدم الإصابة. لم تظهر منطقة البيتا جلوبولين اختلافات معنوية
بينما حدث ارتفاع مبدئي في منطقة الجاما جلوبولين ثم انخفضت فجأة مع زيادة الطفيل في
الدم. وأظهر سلوك إنزيم AST والبيوروبين 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 حرارة ونسبة الطفيل في الدم وقيم الألبومين والألفا
جلوبولين والجاما جلوبولين أثناء الإصابة حيث هذه التغيرات أكثر 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 أعطيتا 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 drawn 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 Friesian 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 veltage, 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 5×10^{-3} (Neocidol, Hindustan Ciba Geigy Ltd.). Cows were given a single subcutaneous injection of ivermectin at a rate of $0.1 \text{ mg } 50 \text{ kg}^{-1}$ 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 $>39.5 \text{ }^\circ\text{C}$). 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* trophozoites in the stained blood smear. Blood samples were drained from each calf at the 5th 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 haematincs (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 ⁻³ ***	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 trophozoites 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.

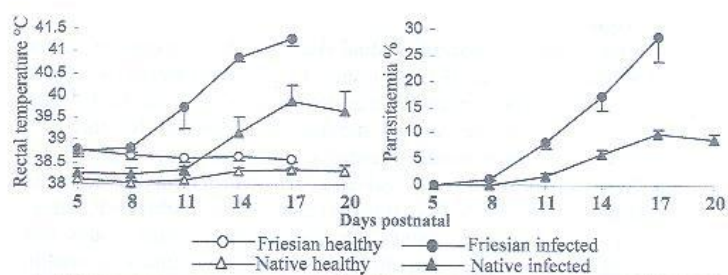


Fig. 1: Rectal temperature and percent of parasitaemia in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means \pm SEM.

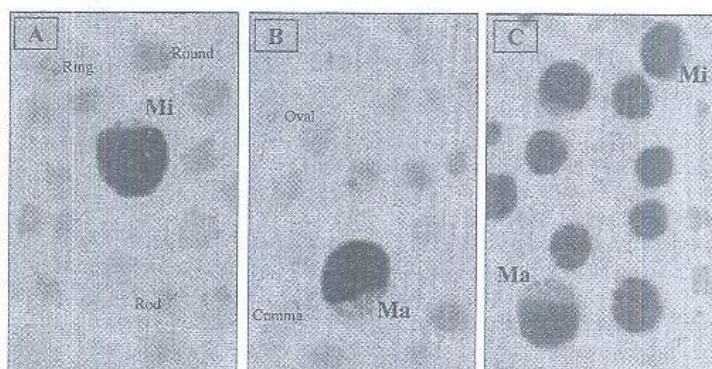


Fig. 2: Photo A & B showing different forms of intra-erythrocytic trophozoites in addition to micro (Mi) and macroschizont (Ma) stages in blood smear. Photo C showing micro (Mi) and Macroschizogony (Ma) stages in lymph node smear stained with Geimsa x1000.

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

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

1- Changes in blood serum proteins:

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

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

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

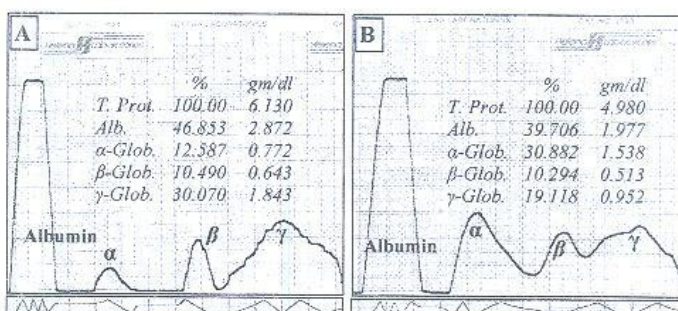


Figure (3): Scanning graph of blood serum protein electrophoresis in 17-day-old Friesian calf showing: A. Normal pattern. B. Patterns in calf infected with *T. annulata*.

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 and 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 \pm SD of serum albumin and levels of significance (P).

	Friesian		P	Native		P
	Control	Infected		Control	Infected	
Day 5	2.56 \pm 0.357	2.48 \pm 0.274	0.8635 ^{ns}	2.64 \pm 0.395	2.50 \pm 0.305	0.7864 ^{ns}
Day 8	2.80 \pm 0.259	2.36 \pm 0.267	0.2712 ^{ns}	2.72 \pm 0.329	2.46 \pm 0.271	0.5591 ^{ns}
Day 11	2.70 \pm 0.234	2.06 \pm 0.216	0.0795 ^{ns}	2.74 \pm 0.229	2.34 \pm 0.201	0.2265 ^{ns}
Day 14	2.94 \pm 0.260	1.84 \pm 0.075	0.0105*	2.82 \pm 0.146	2.20 \pm 0.145	0.0168*
Day 17	2.86 \pm 0.144	1.55 \pm 0.086	0.0001***	2.70 \pm 0.128	2.13 \pm 0.188	0.0160*
Day 20	-	-		2.76 \pm 0.196	1.94 \pm 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 -globulin. (Mean \pm SD and level of significance)

	Friesian		P	Native		P
	Control	Infected		Control	Infected	
Day 5	1.26 \pm 0.136	1.18 \pm 0.120	0.6713 ^{ns}	1.16 \pm 0.121	1.18 \pm 0.159	0.9231 ^{ns}
Day 8	1.20 \pm 0.100	1.14 \pm 0.103	0.6869 ^{ns}	1.12 \pm 0.102	1.14 \pm 0.133	0.9078 ^{ns}
Day 11	1.06 \pm 0.068	1.34 \pm 0.075	0.0242*	1.02 \pm 0.066	1.18 \pm 0.132	0.3201 ^{ns}
Day 14	0.90 \pm 0.083	1.42 \pm 0.139	0.0151*	0.98 \pm 0.080	1.18 \pm 0.107	0.1775 ^{ns}
Day 17	0.82 \pm 0.073	1.54 \pm 0.108	0.0009***	0.92 \pm 0.091	1.16 \pm 0.108	0.1281 ^{ns}
Day 20	-	-		0.86 \pm 0.093	1.22 \pm 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 -globulin. (Mean \pm SD and level of significance)

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

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

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

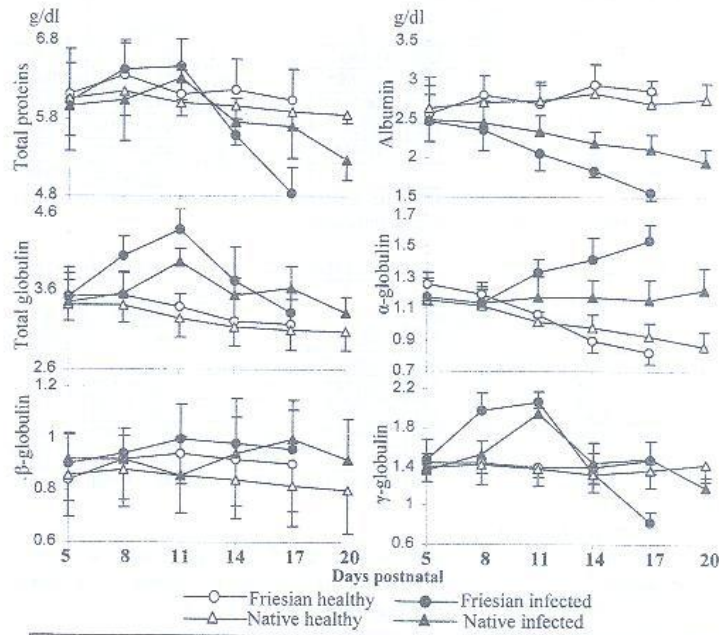


Fig. 4: Total blood serum protein and protein electrophoretogram in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means \pm SEM.

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

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

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

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

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

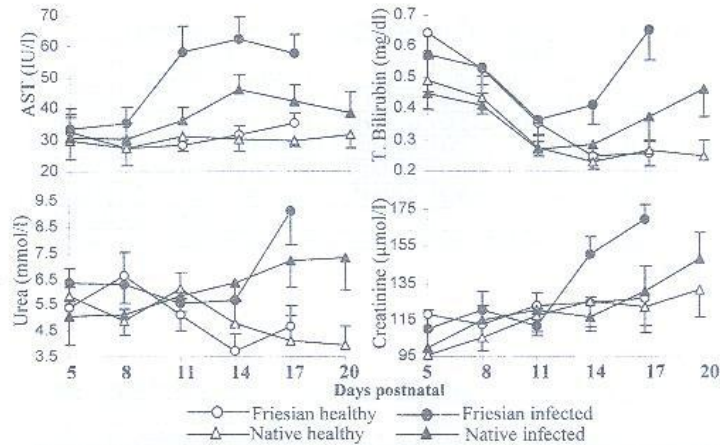


Fig. 5: The behaviour of AST, bilirubin, urea and creatinine in healthy and naturally infected neonatal calves with *Theileria annulata*. Data are expressed as means \pm SEM.

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

	Friesian		P	Native		P
	Control	Infected		Control	Infected	
Day 5	118.2 \pm 08.4	109.8 \pm 10.7	0.554 ^{ns}	095.4 \pm 11.8	099.6 \pm 10.7	0.799 ^{ns}
Day 8	112.4 \pm 09.2	120.4 \pm 10.2	0.575 ^{ns}	105.4 \pm 06.9	114.6 \pm 08.4	0.423 ^{ns}
Day 11	123.0 \pm 14.6	111.8 \pm 09.5	0.482 ^{ns}	117.2 \pm 11.1	120.6 \pm 09.6	0.822 ^{ns}
Day 14	124.6 \pm 16.0	151.0 \pm 09.2	0.203 ^{ns}	125.2 \pm 14.6	116.4 \pm 11.3	0.647 ^{ns}
Day 17	126.4 \pm 15.0	169.8 \pm 08.2	0.044*	121.8 \pm 14.5	130.8 \pm 13.5	0.662 ^{ns}
Day 20				131.6 \pm 15.2	148.4 \pm 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}
F- 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 (Kühne *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), Gründer 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 (TNF α), interleukin-1 (IL₁), interleukin-2 (IL₂), interleukin-6 (IL₆) and interferon- γ (IFN γ) 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_1 AGP) 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_1 AGP 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, 2001 and Glass, 2001a,b) about the innate resistance of tropical breeds to *T. annulata* infection.

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