Clinicopathological Studies on Theileria annulata Infection in Siwa Oasis, Egypt

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One hundred and twenty five (125) cross and native cattle breeds were examined for prevalence of *T. annulata* infection for the first time in Siwa Oasis and evaluated ts effect on some blood constituents before and after treatment with buparvaquone. The prevalence of tropical theileriosis was 40.3 and 29.4 % in cross and native breed respectively using blood smear examination. Immunofluorescent antibody technique (IFAT) could identify *T. annulata* in 80.7 % of cross breed and 70.5 % of native cattle. In addition, there was seasonal variation in prevalence. The tick species *H. anatolicum* was recovered from 65.6% of examined cattle. Cattle clinically infected with *T. annulata* had significantly low levels of total proteins, albumin, magnesium, potassium and iron concentrations ($P \leq 0.05$) but AST, L γ glutamyl transferase activities, total, direct and indirect bilirubin, creatinine levels were significantly high ($P \leq 0.05$). Buparvaquone was effective against both stages of *T. annulata* and succeed to control fever and temperature returned to normal range by 7th day post treatment. In addition, some serum elements returned to its normal values post treatment especially in native but not in cross breed cattle. In brief, our data showed that tropical theileriosis is prevalent in Siwa Oasis especially among cross breed cattle and the disease has some effects on hepatic and renal functions. There is a need for using immunization methods to reduce the losses from the disease.

Tropical theileriosis is severe, often fatal disease of cattle caused by *Theileria annulata*. The parasite is transmitted trans-staidly by the bite of Hyalomma spp. Ticks are distributed from Morocco eastwards across North Africa, the near and Middle East to India, Central Asia and China (Norval *et al.*, 1992 and Payne and Wilson, 1999). Clinical *T. annulata* infection is traditionally diagnosed by the demonstration of schizont-infected cells in the superficial lymph nodes draining the site of the tick bite, or of piroplasms in peripheral blood vessels (Anon, 1997). In recovered carrier animal, only the piroplasm stage can be demonstrated, often with great difficulty. Siwa Oasis is a virgin area and considered one of the most promising areas for agricultural expansion in Egypt. Many problems face cattle farming in Siwa including parasitic infections especially tick-borne diseases. Theileriosis is one of the most devastating blood parasites affecting cattle in Egyptian Oases (Harfoush, 2001 and Saleh and Mahran, 2003). Indirect fluorescent antibody technique (IFAT) has been effectively employed by many authors as rapid and accurate serological test for detection of bovine theileriosis (Farah, 1995; Handemir and Dik, 1998 and Lawal *et al.*, 1998). The aim of this study was directed to investigate tropical theileriosis for the first time in Siwa Oasis and clarifying its effects on some blood biochemical constituents as well as treatment of diseased cattle and evaluate the effect of buparvaquone on the health of treated animals.

Materials and Methods

Study area and examined animals. Siwa Oasis lies south to Matrouh Governorate in the western desert 306 km far from Mersa Matrouh. One hundred and Twenty five 1-3 years old cattle including 57 local and 68 crossbred cattle were subjected to careful clinical examination as well as investigation of theileriosis after (Rosenberger, 1979).

Blood and serum samples. Blood samples were collected from jugular vein. Serum samples were divided into two portions, one for IFAT and the other was used for biochemical analysis. Blood of ear veins was used for preparation of blood smears. Smears were air dried, fixed in methanol and stained with Giemsa (Levine, 1985).

Indirect fluorescent antibody technique (IFAT). All sera were tested by IFAT against *T. annulata*. Schizont antigen was prepared according to (Burridge and Kimber, 1972). The

technique adopted for IFAT was described by (Burridge, 1971) using rabbit antibovine IgG fluorescent isothiocyanate (FITC).

Biochemical analysis. Serum samples were subjected to biochemical examination for total proteins, albumin, total direct bilirubin, L-γ-glutamyl-transferase, aspartat amino trasferase, sodium, potassium, chloride, iron and magnesium according to (Weichselbaum, 1946; Doumas *et al.*, 1971; Walter and Gerade, 1970; Persijn and Vanderslik, 1976; Trinder, 1951; Sunderman and Sunderman, 1958; Schönfeld and Lowellen, 1964; and Husdan, 1968) respectively.

Tick identification. All visible ticks were removed and preserved in 70% alcohol. Ticks were identified according to (Hoogstraal, 1956).

Treatment. Naturally infected animals were treated with single dose of Buparvaquone (Butalex) (Schering Plough Animal Health) by i/m injection at a rate of 2.5 mg / kg. b.wt (1ml / 20 kg. Bwt) in neck muscles.

Results and Discussion

Tropical theileriosis or Mediterranean coast fever caused by *Theileria annulata* is one the most important diseases of cattle in Egypt, representing a major threat to the expansion and improvement of livestock production. In the present study, the clinical signs of infected animals were pyrexia, anorexia, emaciation, enlargement of lymph nodes, and lacrimation (Fig. 1, 2). These signs were more prominent in cross breed than in native cattle. These results agreed with that of (Papdopoulos *et al.*, 1999; Radostits *et al.*, 2000; Saleh and Mahran, 2003 and Omer *et al.*, 2003b) and this supports the suggestion that crossbreeds of cattle are more sensitive to theileriosis.

The seasonal prevalence of *T. annulata* infection using blood smears examination showed that infection rate of tropical theileriosis in cross-breed cattle was higher than that of native cattle 40.3% and 29.4% respectively (Table 1). The peak of infection of crossbreed cattle was recorded in summer 42.8%, while autumn season recorded the highest infection rate of native cattle 35%. Similar results were recorded by (Mahmoud, 1991; Farah, 1995 and Mahmoud, 1996). Many authors recorded different infection rates: 15 % (Bansal *et al.*, 1987), 15.7% (Handemir and Dik, 1998) 16.5% (EL-Metennawy, 2000). These results suggested that imported pure-breed or crossbreed cattle are more sensitive to theileriosis than native breeds which have the ability to limit the macroschizont index (Preston *et al.*, 1992; Papdopoulos *et al.*, 1999; Bakheit and Latif, 2002 and Saleh and Mahran, 2003).

Results of seroprevalence of *T. annulata* revealed that (80.7%) of cross-breed cattle were found harboring antibodies against *T. annulata* with maximum rate of infection in autumn season (86.6%), where as, (70.5%) of native cattle were positive by IFAT. Summer season recorded the peak of *T. annulata* antibodies (78.9%). Similar results were recorded by (EL-Bahy, 1986 and Abd EL-Kader, 1991 and 1995) who observed that maximum infection rate occurred during summer and autumn seasons. Variable infection rates were recorded by many authors; 90% (Bansal *et al.*, 1987), 71.9% (Hamed, 1993), 40% (D'Oliveira *et al.*, 1997), 27.2% (Handemir and Dik, 1998), 4% (Lawal *et al.*, 1998), 31% (Eren *et al.*, 1998), and 72.6% (Manish *et al.*, 2001). Such variations may be due to breed difference, immune status of the animals and prevalent tick species. In conclusion IFAT constitutes a valid serodiagnostic technique for use in epidemiological investigations and surveillance in order to explore latent infections that may be missed by blood film examination. (Fig. 4, 5)

With respect to tick infestation, the investigated cattle harbored *Hyalomma anatolicum*. The *overall* rate infestation of both breeds was 65.6%; crossbreed infestation rate was 72.4% while that of local breed was 58.8%. The obtained results showed that, the highest infestation rate of crossbreed (80%) and local (65%) cattle was achieved in autumn (Table 3). On the other hand, the minimum infestation rate in crossbreed cattle was recorded in winter (60%), while summer was the season of lowest infestation rate of local cattle (52.6%). Many authors reported different infestation rates of *H. a. anatolicum* 6.04% (Ayden, 2000), 1.4% (EL-Kammah *et al.*, 2001), 6.7% (Mazyad and Khalaf, 2002) and 61.1% (Razmi, 2003). Such variations were attributed to variation in the nature of investigated areas and breed of cattle. They found that crossbreed and pure-breed cattle were highly susceptible to tick infestation that agreed with our results.

(Liebisch *et al.*, 1984 and Abdel-Rahman *et al.*, 1989) reported that H. *a. anatolicum* was the prominent species of ticks occured in all domestic animals, they added that *H. a. anatolicum* is the tick species which serves as a vector of Mediterranean theileriosis (*T. annulata*) under natural condition in Egypt.

female

Male

2,3): 1 year old cross breed calf infected with theileriosis showed pronounced emaciation and enlarged prefemoral lymph node .There is ticks infestation(*H. a.anatolicum*) on the perneal region.

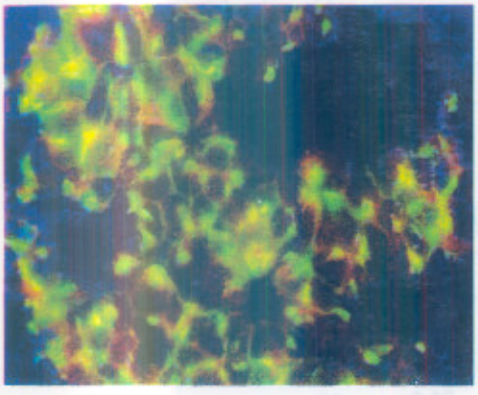

Fig. (4): Postive IFT reaction of *T. annulata* Schizont antigen with serum of infected animals

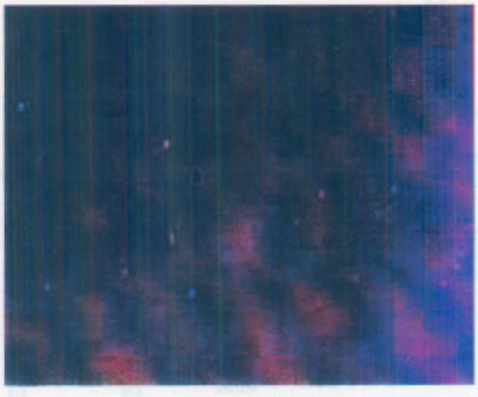

Fig. (5): Negative IFA reaction of *T.annulata Schizont* antigen with serum of control negative animals

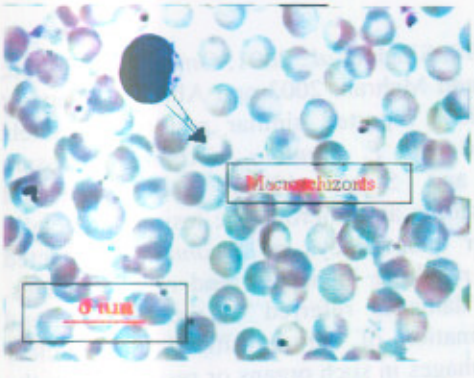

Fig. (6) Micrschizont (A) and (B) Piroplasms of *T. annulata* in blood smear of infected animals.

Table (1): Seasonal prevalence of *T. annulata* among cattle using blood smears.

Season	Total animals examined			Cross breed			Native breed		
	No.	+ve	%	No.	+ve	%	No.	+ve	%
Winter	25	9	36	15	6	40	10	3	30
Spring	32	11	34.3	13	5	38.4	19	5	26.3
Summer	33	12	36.3	14	6	42.8	19	5	26.3
Autumn	32	13	37.1	15	6	40	20	7	35
Total	125	42	36	57	23	40.3	68	20	29.4

No. = number of animals +ve. = Positive. % = Percent

Table (2): Seasonal prevalence of *T.annulata* among cattle using IFAT.

Season	Total animals examined			Cross breed			Native breed		
	No.	+ve	%	No.	+ve	%	No.	+ve	%
Winter	25	17	68	15	10	66.6	10	6	60
Spring	32	24	75	13	11	84.6	19	12	63.2
Summer	33	28	84.8	14	12	85.7	19	15	78.9
Autumn	32	29	82.2	15	13	86.6	20	15	75
Total	125	98	78.4	57	46	80.7	68	48	70.5

No. = number of animals. +ve. = Postive. % = Percent

Table (3): Seasonal prevalence of *Hyalomm . anatolicum* ticks.

Season	Total animals examined			Cross breed			Native breed		
	No.	+ve	%	No.	+ve	%	No.	+ve	%
Winter	25	15	60	15	9	60	10	6	60
Spring	32	21	65.6	13	10	76.9	19	11	57.8
Summer	33	21	63.6	14	11	78.5	19	10	52.6
Autumn	32	25	71.4	15	12	80	20	13	65
Total	125	82	65.6	57	42	72.4	68	40	58.8

No. number of animals +ve. = Positive. % = Percent.

In the current work, the mean values of blood serum total, direct and indirect bilirubin, L- gamma-glutamyl transferase, aspartate aminotransferase (AST) and creatinine revealed significant elevation in both theileria-infected native and cross breed cattle (Table 5). In contrary, total serum proteins showed significant decrease in mean values of both infected native and cross-breed cattle in comparison to control animals. These significant changes probably indicated inflammatory changes in hepatic and glomerular cells that in turn affected their functions. These results are in agreement with (Azza, 1995; Abou-ELHassan, 1997; Sandhu *et al.*, 1998; Singh *et al.*, 2001; Omer *et al.*, 2003a; Saleh and Mahran, 2003 and Abou-EL Naga *et al.*, 2004) reported similar results in camels infected with *T. annulata*. (Abou-El Hassan, 1997 and Singh, 1998) reported several macroscopic and microscopic lesions especially in liver and kidneys of *T. annulata* infected calves resulting in hepatic and renal damages. These damages referred to proliferation and dissemination of the parasitized mononuclear macrophages in such organs or resulted from the excessive production of cytokines. No significant changes were recorded in mean values of albumin or globulin in infected cattle

Table (4): Some serum biochemical parameters of cattle affected with tropical theileriosis.

Item	Native breed			Cross breed		
	Control	Infected	Treated	Control	Infected	Treated
Total bilirubin mg/dl	0.299± 0.016 ^{c}	0.398± 0.004 ^{a}	0.360±0.009 ^{b}	0.293± 0.010 ^{c}	0.392± 0.004 ^{ad}	0.364± 0.010 ^{bd}
Direct bilirubin mg/dl	0.179± 0.006 ^{b}	0.234 ± 0.013 ^{a}	0.188± 0.006 ^{b}	0.182± 0.003 ^{b}	0.235± 0.012 ^{a}	0.219± 0.013 ^{a}
Indirect. bilirubin mg/dl	0.120 ± 0.016 ^{bc}	0.164 ± 0.010 ^{a}	0.171± 0.011 ^{a}	0.110± 0.007 ^{c}	0.157± 0.013 ^{ab}	0.145± 0.014 ^{abc}
γ. glutamyl transeferse iu/l	24.40 ± 1.600 ^{b}	35.28 ± 2.330 ^{a}	31.92± 1.649 ^{b}	21.60± 1.568 ^{b}	37.14± 2.011 ^{a}	32.42± 1.228 ^{a}
AST iu/l	41.4 ±1029 ^{b}	68.2±3.152 ^{a}	45.8±1.959 ^{b}	41.6±1.208 ^{b}	67.6±3.264 ^{a}	47.6±1.860 ^{b}
Creatinin µmol/l	116.82 ± 1.912 ^{c}	131.52 ± 4.677 ^{b}	119.76± 3.671 ^{bc}	120.26± 3.057 ^{bc}	149.00± 4.351 ^{a}	128.58± 4.026 ^{bc}
Total protein gm/dl	7.672 ± 0.157 ^{abc}	6.850 ± 0.237 ^{d}	7.200± 0.213 ^{cd}	8.142± 0.080 ^{a}	7.420± 0.185 ^{bc}	7.840± 0.128 ^{ab}
Albumin gm/dl	3.808 ± 0.169 ^{a}	3.208 ± 0.095 ^{b}	3.346± 0.119 ^{b}	3.950± 0.127 ^{a}	3.126± 0.094 ^{b}	4.046± 0.741 ^{a}
Globulin gm/dl	3.864 ± 0.159^{a}	3.642 ± 0.174 ^{a}	3.854± 0.171 ^{a}	4.192± 0.120 ^{a}	4.294± 0.120 ^{a}	3.790± 0.760 ^{a}
Sodium mmol/l	161.18± 2.408^{a}	127.16±3.212 ^{d}	149.66±1.144 ^{b}	163.24±2.943 ^{a}	120.80±0.755 ^{d}	139.00±1.776 ^{c}
Potassium mmol/l	10.420 0.749 ^{bc}	5.84±.0545 ^{d}	13.14±1.47 ^{a}	10.74±0.586 ^{b}	5.24±0.503 ^{d}	8.580±0.336 ^{c}
Chloride mmol/l	110.38±1.238^{a}	109.36±1.648 ^{a}	111.48±0.717 ^{a}	110.42±1.590 ^{a}	109.14±2.059 ^{a}	110.94±0.613 ^{a}
Magnesium mg/dl	3.080±1.238^{a}	1.840±0.087^{c}	2.22±0.208 ^{cb}	3.00±0.130 ^{a}	2.00±0.063 ^{bc}	2.30±0.151 ^{b}
Iron mmol/l	134.62±1.638^{a}	120.74±2.452 ^{b}	131.42±0.810 ^{a}	136.32±1.172 ^{a}	124.00±2.844 ^{b}	131.06±2.554 ^{c}

Mean ± Standard error.
-a b c d Denotes significant differences from their respective control at $p \leq 0.05$.

Table (5): Blood films Examination of Treated Cases With Buparvaquone.

Days post-injection	Parasitemia % (Mean)		Rectal temperature (Mean)	
	Native	Cross breed	Native	Cross breed
Day 0	8	12	40.0°C	41.0 °C
Day 1	3	5	39.0°C	40.0 °C
Day 2	1	3	38.4°C	39.0 °C
Day 5	0.1	0.5	38.0°C	38.0 °C
Day7	0	0	38.0°C	38.0 °C

compared to control. Ozan *et al.* (1999) reported non-significant changes in both total protein and albumin means values. On the other hand, (Byeong *et al.*, 1992) reported significant increase in total protein with a significant decrease in albumin values in *T. annulata* infected Korean cattle. Sodium, potassium, magnesium and iron means values were significantly decreased in both native and crossbred cattle than that of control one. The same results were recorded by (Sandhu *et al.*, 1998 and Omer *et al.*, 2003). Buparvaquone (Butlex) treatment was effective against both stages of *T. annulata* (schizont and piroplasms) and succeeded to control fever as temperature returned to normal range by 7th day post-treatment. These results coincided with (Dhar *et al.*, 1990; Mahmoud, 1996; and Abou-ELNaga *et al.*, 2004). Also the drug was helpful in returning some serum elements to its normal values especially in native but not in crossbred cattle.

In conclusion, theileriosis is one of the most serious tick borne protozoan parasitic diseases of cattle in Egypt. In general, mortality, morbidity and serum biochemical changes of *T. annulata* infection are much higher in crossbreed than native animals. The conventional methods of control of bovine theileriosis include chemoprophylaxis and treatment of clinical cases in addition to rigorous dipping protocols for control of insect vector. These operations require expensive charges making them too costly and difficult to standardize. From all the above-mentioned reasons, there is a need for cattle immunization against theileriosis to reduce the losses resulted from such important disease.

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