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CLINICAL CHANGES IN THE BLOOD OF CATTLE VACCINATED AND INFECTED WITH THEILERIA-ANNULATA

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SUMMARY

Haematological and biochemical changes were studied in 10 Friesian calves after vaccination with attenuated *Theileria annulata (T. annulata)* vaccine and post challenge with the virulent strain. The parameters were compared with those of 21 animals, naturally infected with *T. annulata.*

The vaccinated gp. showed no appreciable changes either post vaccination or post challenge. On the other hand, the naturally infected animals and the non vaccinated gp. post challenge revealed a significant fall in Hb, PCV and total erythrocyte count. Levels of total serum protein, albumin, calcium, phosphorus, magnesium, sodium and potassium decreased and those of globulins, GPT, GOT and bilirubin increased with no changes in kidney functions. Several of these biochemical alterations have been explained in relation to the injury to the liver and other organs.

INTRODUCTION

Current basic knowledge of *T. annulata* incidence and the mortality of infected cattle is reviewed together with an outline of current control measures, including the use of acarcides (Hashemi-Fesharki, 1988) and treatment of clinical disease using specific chemotherapeutics (Abdel-Rahman et al., 1987).

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In endemic areas such as the case in Egypt, systematic vaccination with live attenuated vaccine is considered to be the most practical and effective control method and may be the only approach of reducing the incidence of the disease (Pipano, 1989 and Zhang, 1997).

Considerable attention has been made in the field of serology of *T. annulata* infection of bovines and on the vaccinated animals (Dhar and Gautam, 1977 and Sundar et al., 1993). However, little is known about the dynamics of metabolic process in the course of this disease.

To uncover the significance and safety of the live attenuated vaccine for preserving our rural livestock and promoting the production of milk and meat, it is deemed necessary to study the metabolism of the parasite by determining several biochemical components in bovines suffering from *T. annulata* infection.

The present study, therefore, was undertaken to determine the biochemical parameters and their levels in the blood plasma of vaccinated animals comparing with those naturally infected with *T. annulata,* inorder to ascertain the safety and the protection of applying the vaccine in the field, without causing a negative impact on the functions of the animal's internal organs.

MATERIAL AND METHODS:

(1) Animals:

A- Experimental animals:

Ten male Friesian calves, 6-7 months of age and weight from 250-300 kgs. were used in the present study. They were proven to be free from *T. annulata* infection by the conventional techniques.

The calves were divided into 2 groups as follows:

Group (1): Consisting of 6 vaccinated animals with the live attenuated vaccine (The Egyptian strain) which was isolated and attenuated by Prf. Dr. M. S. Abdel-Rahman, Parasitology Dept., Faculty of Vet. Medicine (Abdel-Rahman et al., 1989). Each animal was inoculated subcutaneously (S/C) near prescapular lymph node with 2 ml. of vaccine (containing $2x10^6$ schizont infected cells) as described by Pipano, (1977). The vaccinated animals were challenged 50 days post vaccination using the virulent strain of *T. annulata* as described by Pipano et al., (1977).

Group (2): Consisting of 4 animals and were served as non vaccinated control.

B- Field animals:

Group (3): Consisting of 21 animals of different ages from the endemic Egyptian provinces, belonging to El-Wadie el-Gedid and El-Fayoum. It was proved by Liebisch et al., (1984) and El-Bahi, (1986) that the incidence of *T. annulata* infection was high in these areas.

All the animals were observed for febrile responses and other clinical symptoms of the disease throughout the course of the study (11 weeks).

(2) Blood sampling:

Approximately 5 ml. of blood for haematology were collected from the jugular vein of each animal on EDTA every week. For other estimations, 15 ml. blood was collected in steril glass without any anticoagulant. Serum sample were aspirated after centrifugation of the blood and kept frozen at - 20°C until analysis. Blood smears were obtained weekly, stained with Giemsa stain and examined to ascertain the degree of parasitaemia.

(3) Haematological observations:

The packed cell volume (PCV), haemoglobin content (Hb) and total red blood cells (RBCs) were determined according to Schalm et al., 1975).

(4) Biochemical analysis:

Total proteins were determined using the method of Sundeman, (1964). Bilirubin was estimated by the method of Ducci and Watson, (1945). The liver functions (GPT and GOT) were estimated by the method of Bergmeyer, (1963) and the kidney functions (Urea and Creatinine) by Faulkner and King, (1970). The measurements of Magnesium, Phosphorus, Sodium, Potassium and Calcium were performed according to Oser, (1965).

(5) Statistical analysis:

Statistical analysis of the results were carried out according to Sendecor and Cochran, (1982).

RESULTS AND DISCUSSION

Creative and innovative vaccine candidates emerge from research laboratories in academic institutions, government agencies, and private pharmaceutical and biotechnology companies all over the world. The decision to begin animal testing of a candidate vaccine depends on a number of criteria, the most important of which being that the vaccine candidate must be safe and protective (Levine et al., 1997).

The haematological observations in bot the vaccinated animals with *T. annulata* schizont vaccine and the non vaccinated control gp.

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showed non significant changes in the RBCs, Hb and PCV contents (Table 1). This result could be explained by Pipano & Israel, (1971) who concluded that organisms grown in tissue culture loose the capacity to induce erythrocytic forms in cattle in parallel with the loss of virulence. Brown, (1990) and Pipano, (1995) also, added that prolonged cultivation of the *T. annulata* schizont infected cells loose their virulence for cattle and the ability to produce overt disease.

Similar results were obtained by Hashemi-Fesharki, (1988) who observed that vaccinated calves with *T. annulata* schizont vaccine showed no significant changes in Red cell count, PCV and Hb contents and no adverse reactions have been reported on the vaccinates.

The forgoing observations would imply that the live schizont attenuated vaccine is safe, with no harmful or side effects on the animals.

The obtained data in Table (2) revealed that the vaccinated animals post challenge showed negligible changes in the haematological values. This result could be explained by Subramanian et al., (1986) and Singh et al., (1993), who concluded that virulence of macroschizont infected cells with *T. annulata* was lost by several passages but immunogenicity remained and the strain was used as a vaccine which withstood challnege with no ill effects.

In contrast, the non vaccinated controls on challenge showed a marked changes in the haematological values with an associated anaemia. Our results could be explained by Chengalva-Rayulu and Hafeez, (1995a) who demonstrated that the extent of decrease in total RBCs is correlated with the intensity of infection which indicated the destruction of erythrocytes and reduction in the Hb content as evidenced by anaemia. Maxie et al., (1982) proved that anaemia resulted from a reduced production of red cells, possibly because of interference with erythropoiesis due to the toxic affect of the disease.

In our resutls, one of the four controls died with acute theileriosis 28 days post challenge while no death occurs in the vaccinated gp. post challenge. This result was supported to some extent by Hashemi-Fesharki, (1988) who observed that there were no cases of theileriosis or death among the vaccinated cattle, whereby all the non vaccinated animals became infected with a death rate of 27-40 %.

The levels of serum enzymes (GPT and GOT) and the total bilirubin showed non significant changes in the vaccinated and non vaccinated gps. (Table 1). However these values were significantly ($P < 0.001$) increased in the non vaccinated control calves post challenge, while the vaccinated gp. mainted the same values

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Table 1: Mean values of haematological and biochemical changes in animals vaccinated and non vaccinated with *Theileria annulata* attenuated live vaccine.

Measurements		N = 6 Vaccinated animals with attenuated live vaccine							N = 4 Non vaccinated animals (control)					
		0 time	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	0 time	1st week	2nd week	3rd week	4th week	5th week	6th week
RBCs (/l04 µL)	Haemato-logical values	708.3±27.87	686.33±40.7	710±29.15	695±29.05	718.3±27.86	707.5±31.26	690.16±38.52	708.3±27.87	725±26.45	707.5±51.23	685±18.92	710±26.77	701.30±16.52	696.20±24.96	692.5±35.93
PCV %		31.3±1.63	30.8±1.47	31.8±1.47	31.3±1.21	30.6±0.81	30.8±1.72	32.1±2.22	31.3±1.63	32±1.64	30.8±2.5	30.5±1.29	31.2±1.5	29.7±1.25	30.3±2.62	30.20±2.75
Hb g/dL		9.2±0.26	9.1±0.15	9.9±0.22	8.8±0.16	9.2±0.19	9.1±0.22	9.0±0.24	9.2±0.26	9.3±0.24	9.1±0.27	9.1±0.25	9.0±0.26	8.9±0.12	9.1±0.19	9.2±0.26
GPT (u/L)	Liver function	8.6±0.57	8.47±0.59	8.6±0.82	8.50±0.38	8.3±0.68	8.6±0.72	8.3±0.56	8.6±0.57	8.1±0.70	8.5±0.56	8.8±0.19	8.6±0.61	8.5±0.70	8.8±0.62	8.6±0.38
GOT (u/L)		17.4±1.23	17.36±1.01	18.75±0.81	18.76±1.46	17.62±1.12	17.05±1.26	17.60±0.99	17.04±1.23	17.65±1.24	18.75±1.92	18.35±0.85	17.02±1.46	18.14±1.91	17.57±1.3	17.33±0.92
Bilirubin (mg/100 ml)		0.78±0.07	0.74±0.06	0.92±0.14	0.94±0.14	0.70±0.30	0.84±0.05	0.82±0.06	0.78±0.07	0.83±0.04	0.90±0.10	0.89±0.09	0.87±0.05	0.84±0.04	0.86±0.06	0.85±0.05
Creatinine (mg/100ml)	Kidney function	0.87±0.03	0.84±0.06	1.07±0.29	0.80±0.08	0.94±0.25	0.73±0.31	0.74±0.31	0.87±0.03	0.84±0.03	0.92±0.09	0.92±0.05	0.83±0.06	0.91±0.10	0.81±0.05	0.83±0.05
Urea (mg/100ml)		33.70±.27	33.16±3.13	34±2.4	36.50±1.37	35.10±1.93	34.66±2.05	34.16±2.9	33.70±2.7	34±1.41	34.75±1.25	33.7±1.03	35.25±2.5	34.2±1.43	32±2.16	33±2.9
Total protein (g/100ml)	Protein value	7.22±0.73	7.06±0.31	7.08±0.37	7.37±0.47*	8.03±0.81*	8.26±0.31*	7.73±0.66*	7.22±0.73	6.95±0.38	7.02±0.43	6.9±0.21	6.85±0.39	7.0±0.45	7.2±0.14	6.88±0.46
Albumin (g/100ml)		3.41±0.34	3.43±0.31	3.53±0.2	3.25±0.23	3.71±0.19	3.82±0.31	3.56±0.38	3.41±0.34	3.55±0.12	3.52±0.24	3.35±0.38	3.23±0.26	3.4±0.21	3.7±0.18	3.55±0.45
Globulin (g/100ml)		3.75±0.41	3.60±0.26	3.53±0.22	4.08±0.38*	4.2±0.43*	4.28±0.36*	4.11±0.44*	3.75±0.41*	3.4±0.26	3.55±0.45	3.52±0.45	3.61±0.22	3.6±0.27	3.57±0.29	3.32±0.22
Calcium (mg/100ml)	Electrolytes	9.9±0.71	9.37±0.84	9.05±1.01	10.23±0.23	10.06±0.38	9.7±0.49	9.7±0.56	9.9±0.71	10.05±0.38	9.7±0.42	9.05±0.60	10.17±1.28	9.8±0.68	9.75±0.68	10±0.46
Phosphorus (mg/100ml)		5.86±0.33	5.5±0.35	5.45±0.22	5.78±0.48	5.95±0.79	5.72±0.38	5.73±0.50	5.86±0.33	5.65±0.39	5.6±0.35	5.41±0.22	5.72±0.53	5.7±0.38	6.0±0.29	6.05±0.28
Magnesium (mg/100 ml)		2.48±0.35	2.71±0.15	2.13±0.14	2.25±0.34	2.41±0.36	2.23±0.41	2.5±0.27	2.48±0.35	2.4±0.29	2.2±0.18	2.21±0.38	2.29±0.5	2.37±0.25	2.52±0.17	2.50±0.37
Sodium (meq/L)		138.61±12.30	147±17.51	141.83±20.95	147±17.53	149±12.83	152.33±17.29	155.83±19.46	138.16±12.30	152.75±10.6	140.75±18.5	145.5±18.76	150±13.60	140±17.56	143.5±17.63	147.8±10.14
Potassium (meq/L)		5.7±0.50	5.6±0.52	5.8±0.28	5.7±0.49	5.9±0.49	5.6±0.69	6.0±0.48	5.7±0.5	6.17±0.57	5.8±0.8	5.65±0.36	5.6±0.37	5.7±0.78	5.42±0.46	5.7±0.41

N. B. Data expressed as means ± standard deviation. N = Number of animals sampled.

* P ≤ 0.05

*Table 2: Mean values of haematological and biochemical changes in **Theileria annulata** vaccinated and non vaccinated challenged animals.*

Measurements		Vaccinated challenged animals N = 6						Non vaccinated challenged animals N = 4					
		0 time	1st week	2nd week	3rd week	4th week	5th week	0 time	1st week	2nd week	3rd week	4th week*	5th week
RBCs (10^4 µL)	Haemato-logical values	708.3 ± 27.87	686.33 ± 40.7	679.66 ± 46.13	681.67 ± 49.59	711.5 ± 23.98	725.33 ± 35.05	720.25 ± 26.54	658.75 ± 22.99	590.75 ± 26.55***	544.25 ± 53.59***	492.67 ± 63.89***	528.33 ± 82.81***
PCV %		31.3 ± 1.63	30.8 ± 1.47	30.0 ± 0.57	33.0 ± 1.79	32.5 ± 2.17	33.1 ± 2.23	30.80 ± 1.71	28.75 ± 1.5*	26.50 ± 1.29**	24.75 ± 1.5***	24.67 ± 1.53***	26.66 ± 1.60***
Hb g/dL		9.2 ± 0.26	9.1 ± 0.15	9.2 ± 0.16	9.4 ± 0.16	9.32 ± 0.19	9.3 ± 0.20	9.0± 0.31	8.78 ± 0.22*	8.1 ± 0.24***	7.7± 0.21***	7.5 ± 0.15***	7.9± 0.1***
GPT (u/L)	Liver function	8.6 ± 0.57	8.47 ± 0.59	8.5 ± 0.53	8.6 ± 0.57	8.8 ± 0.45	8.9 ± 0.04	8.2 ± 0.52	9.15 ± 0.36*	10.02 ± 0.46***	10.4 ± 0.39***	10.30 ± 0.46***	10.50 ± 0.53***
GOT (u/L)		17.4 ± 1.23	17.36 ± 1.01	16.87 ± 0.46	17.30 ± 0.64	17.10 ± 0.92	16.75 ± 1.02	17.18 ± 1.09	24.05 ± 4.68*	26.70 ± 1.33***	27.02 ± 1.46***	30.10 ± 1.76***	26.67 ± 0.53***
Bilirubin (mg/100 ml)		0.78 ± 0.07	0.74 ± 0.06	0.76 ± 0.08	0.72 ± 0.07	0.74 ± 0.05	0.84 ± 0.84	0.80 ± 0.03	1.12 ± 0.30*	1.31 ± 0.33***	1.83 ± 0.28***	1.57 ± 0.25***	1.06 ± 0.15**
Greatinine (mg/100ml)	Kindney function	0.87 ± 0.03	0.84 ± 0.06	0.85 ± 0.05	0.82 ± 0.04	0.97 ± 0.12	1.06± 0.36	0.86 ± 0.04	0.83± 0.05	0.89 ± 0.03	0.85 ± 0.06	0.9 ± 0.08	0.87 ± 0.04
Urea (mg/100ml)		33.70 ± 2.7	33.16 ± 3.13	32.0 ± 2.40	31.8 ± 2.5	34.5 ± 1.87	33.2 ± 3.37	33.25 ± 2.5	31.25 ± 1.89	29.75± 1.70	31.5 ± 1.73	32.0 ± 2.0	29.3 ± 2.5
Total protein (g/100ml)	Protein value	7.22 ± 0.73	7.06 ± 0.31	7.2 ± 0.34	7.61 ± 0.84	7.52 ± 0.24	7.15 ± 0.53	7.0 ± 0.53	6.45 ± 0.35*	6.18 ± 0.50***	5.90 ± 0.79***	6.23 ± 0.35***	6.1 ± 0.30***
Albumin (g/100ml)		3.41 ± 0.34	3.43 ± 0.31	3.5 ± 0.30	3.63 ± 0.30	3.70 ± 0.25	3.58 ± 0.21	3.70± 0.18	2.38 ± 0.21***	2.1 ± 0.14***	2.15 ± 0.13***	2.13 ± 0.35***	2.03 ± 0.05***
Globulin (g/100ml)		3.75 ± 0.41	3.60 ± 0.26	3.7 ± 0.24	3.9 ± 0.34	3.8 ± 0.67	3.55 ± 0.34	3.3 ± 0.25	4.07± 0.26**	4.06 ± 0.39*	3.76 ± 0.13*	4.1 ± 0.26**	4.07 ± 0.31*
Calcium (mg/100ml)	Electrolytes	9.9 ± 0.71	9.37 ± 0.84	9.55 ± 0.54	9.65 ± 1.02	9.73 ± 0.68	9.54 ± 0.74	9.85 ± 0.74	8.52 ± 0.5*	8.4 ± 0.45***	8.35 ± 0.39**	8.16 ± 0.21***	8.07 ± 0.12***
Phosphorus (mg/100ml)		5.86 ± 0.33	5.5 ± 0.35	5.62 ± 0.25	5.57 ± 0.30	5.46 ± 0.34	5.82 ± 0.26	6.0 ± 0.39	5.15 ± 0.13*	4.96 ± 0.17***	4.85 ± 0.47**	4.70 ± 0.3***	4.57 ± 0.40***
Magnesium (mg/100 ml)		2.48 ± 0.35	2.71 ± 0.15	2.78 ± 0.18	2.51 ± 0.27	2.63 ± 0.20	2.65 ± 0.31	2.6 ± 0.22	2.35 ± 0.35*	2.22 ± 0.22***	2.08 ± 0.09***	2.13 ± 0.31**	2.2 ± 0.21*
Sodium (meq/L)		138.61 ± 12.30	147 ± 17.51	149.5 ± 12.80	139 ± 12.4	140 ± 20.9	139.16 ± 13.5	136.25 ± 12.78	132.5 ± 10.85	123 ± 7.55***	121.5 ± 1.91	123.7 ± 1.53*	125.67 ± 4.04
Potassium (meq/L)		5.7 ± 0.50	5.6 ± 0.52	6.1 ± 0.49	5.9 ± 0.28	5.95 ± 0.33	5.6 ± 0.49	6.11 ± 0.31	4.72 ± 0.60**	4.45 ± 0.40***	4.34 ± 0.30***	4.77 ± 0.49***	5.17 ± 0.15*

N = Number of animals sampled.

*One of the four control animal died in the 4th week post challenge.

N.B. Data expressed as means ± standard deviation.

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

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Table 3: Mean values of haematological and biochemical changes in the vaccinated gp. pre and post challenge with *T. annulata* strain compared with the values of the natural-ly infected animals (non vaccinated).

Measurements		Vaccinated animals N = 6	Naturally infected animals (non vaccinated) N = 21	Vaccinated challenged animals N = 6
RBCs (10^4 µL) PCV % Hb g/dL	Haemato-logical values	705.05 ± 8.43*** 31.28 ± 0.59*** 9.0 ± 0.44**	517.325 ± 77.76 26.09 ± 2.60 8.1 ± 0.5	696.89 ± 20.39*** 31.88 ± 1.40*** 9.26 ± 0.12***
GPT (u/L) GOT (u/L) Bilirubin (mg/100 ml)	Liver function	**8.47 ± 0.14*** 17.75± 0.72*** 0.83 ± 0.08**	10.27 ± 0.48 24.39 ± 3.85 1.6 ± 0.25	8.65 ± 0.19*** 17.08 ± 0.26*** 0.76 ± 0.04***
Greatinine (mg/100ml) Urea (mg/100ml)	Kindney function	0.87 ± 0.13 34.83 ± 0.96	0.85 ± 0.06 34.33 ± 2.5	0.91 ± 0.01 32.93 ± 0.45
Total protein (g/100ml) Albumin (g/100ml) Globulin (g/100ml)	Protein value	7.48 ± 0.59*** 3.5 ± 0.22*** 3.9 ± 0.35*	5.73 ± 0.30 2.35 ± 0.23 4.23 ± 0.31	7.31 ± 0.24*** 3.75 ± 0.11*** 3.71 ± 0.14***
Calcium (mg/100ml) Phosphorus (mg/100ml) Magnesium (mg/100 ml) Sodium (meq/L) Potassium (meq/L)	Electrolytes	9,79 ± 0.38*** 5.74 ± 0.16*** 2.31 ± 0.15*** 146 ± 6.76*** 5.80 ± 0.15***	8.34 ± 0.26 4.36 ± 0.48 1.86 ± 0.21 124.57 ± 5.11 4.76 ± 0.37	9.57 ± 0.14*** 5.59 ± 0.15*** 2.66 ± 0.1*** 142.93 ± 4.95*** 5.83 ± 0.22***

N.B. Data expressed as means ± standard deviation. **P≤ 0.01 ***P≤ 0.001

N = Number of animals sampled.

pre-challenge (Table 2). Schlosberg et al., (1973) and Laiblin et al., (1978) ascertained that change in the activity of enzymes and the increase of bilirubin during the advanced course of the disease, are indicative of severe tissue damage in the liver. Similar occurrences were reported by Watanab et al., (1990); and Niinuma et al., (1991) who explained that the serum GOT and total bilirubin has increased rapidly in the course of sickness with theileriosis. Additionally, Dhar and Gautam, (1977) observed that Bilirubinemia resulted from enhanced destruction of erythrocytes and/or hepatic cells insufficiency, resulting in more excretion of bile.

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Neither creatinine nor urea levels were changed in the vaccinated and control gps. (Table 1). The same result applied to both gps. post challenge (Table 2). A similar view was expressed by Laiblin et al., (1978) who recorded that the level of urea was in the normal range in cattle infected with theileriosis. This seems to exclude the involvement of the kidney in the disease.

The levels of total protein and globulins (Table 1) accentuated significantly ($P < 0.05$) 3 weeks post vaccination in the vaccinated animals as compared with the control gp. Comparably, the finding of Goldman and Pipano (1978) and Subramanian et al., (1986) showed that antibodies of IgM and IgG (globulins) classes were detected 18 days after vaccination with *T. annulata* live vaccine and thus causes an elevation of total immunoglobulins. On the other hand, the total serum protein and globulin in the vaccinated gp. were non significantly changed post challenge (Table 2). The causal factor may be a higher amount of total protein and subsequently globulins (antibodies) which inhibited the parasite's harmful influence on the animal, thus the vaccine could be considered protective.

The serum protein and albumin, on the other hand, fell significantly ($P < 0.001$) in the non vaccinated gp. post challenge (Table 2). This result coincided with Sahu et al., (1996) and Nasser and Amin, (1997) who noticed that infected cow with *T. annulata* showed serum protein and albumin levels lower than those of healthy cows. Hypoproteinemia has been reported previously in *T. annulata* infection as indicated by Sharma and Yadav, (1986) and Sudhan et al., (1988). They suggested that lowering of total serum protein could be explained by lesser food intake due to anorexia or to decrease protein production due to liver disease. This result parallels with Kaneko, (1989), who deduced that diseases of severe protein-calorie malnutrition are characterized by hypoproteinemia and hypoalbuminemia.

Table (2) indicates that the globulin was significantly ($P < 0.01$) increased in the control gp. post challenge. Similar observations were outlined by Dhar & Gautam, (1979) and Ashmawy et al., (1994) who found that the experimental Theileria infection was accompained by a significant advance in the level of globulins.

Scrutiny of Table (1) also, revealed no appreciable changes in the electrolytes including calcium, phosphorus, magnesium, sodium and potassium levels in the serum for both vaccinated and control gps. That is because the vaccine proved to be safe for the animals with no debilitating effects as previously stated (Pipano, 1974).

On the other hand, the serum calcium contents gradually declined in the non vaccinated challenged animals (Table 2). The present result concurs with Yadav and Sharma, (1986) Chengalva-Rylulu and Hafeez, (1995b) and Sahu et al., (1996) who noticed a set back in the level of calcium in the *T. annulata* infected animals. Earlier studies by Bansal & Gaur, (1977) and Dhar and Gautam, (1977) revealed a direct relationship between lowered levels of calcium and the clinical manifestations of the disease. They suggested that hypocalcaemia may be attributed to hypoalbuminemia.

Infection of animals with *T. annulata* resulted in significant fall of phosphorus level ($P < 0.001$) as shown in Table (2). This observation was consistent with Chenglva-Raylulu and Hafeez, (1995b) who affirmed that a significant fall in the serum phosphorus level could be demonstrated in cattle infected with *T. annulata.* This decrease could be attributed to dispalance in parathyroid hormone as suggested by Bansal and Gaur, (1977).

A significant decline ($P < 0.001$) in the serum magnesium level was also noticed in the non vaccinated gp. post challenge. (Table 2). Similar results were reached by Yadav and Sharma, (1986) with the animals experimentally infected with *T. annulata.* Srivastava et al., (1976) and Rajkhowa, (1976) accounted for the lowering of magnesium level by its consumption in the synthesis of the alkaline phosphatase which rises naturally during the existence of *T. annulata* infection.

The sodium and potassium contents of the sera in the non vaccinated challenged calves were reduced. This fall shed light on the electrolytic imbalance in the host. Our finding is supported by Bansal and Gaur, (1977) who informed that sodium and potassium are required to maintain electrolytic balance and osmotic pressure of the tissues and blood. During theileriosis, animals suffer from severe dehydration and diarrhaea and those parameters are thus bound to be disturbed.

Regarding the vaccinated gp. post challenge, no significant changes in the electrolytes were observed as indicated in Table (2). This is may be construed to the live attenuated vaccine which induces a high degree of protection against theileriosis as emphasised by Subramanian et al., (1988).

Table (3) clarifies the results of haematological and biochemical aspects between the three gps. namely, the vaccinated, non vaccinated (naturally infected) and vaccinated challenged animals. Statistical analysis revealed a highly significant variations ($P < 0.001$) between the results of the vaccinated and the naturally infected gps. except for creatinine and urea. This indicates that *T. annulata* attenuated vaccine is safe and not harmful on the animals while the organism of *T.*

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annulata (in the naturally infected gp.) inflicts a debilitative influence on the animals.

On comparison between the naturally infected animals (non vaccinated gp) and the vaccinated challenged gp., (Table 3) the results repeated themselves.. This is an assertion for the *T. annulata* vaccine's effectiveness, a fact which was supported by Zhang (1997) who deduced that the attenuated *T. annulata* vaccine has proved to be safe and protective.

In the light of the foregoing considerations, it seems important to re-evalaute factors that may protect the animals against theileriosis. Improved vaccination programs in Egypt, could be a vital key to control tropical theileriosis, since it was proved safe, protective and free from harmful effects on the internal organs and the general health of the animals.

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