S. A. Ajayi [1]
I. L. Oyetunde [1]
G. A. Ogbonna [1]
O. O. Dipeolu [2]

Bovine anaplasmosis : clinical, haematological and blood biochemical changes in experimentally infected Nigerian cattle.

AJAYI (S. A.), OYETUNDE (I. L.), OGBONNA (G. A.), DIPEOLU (O. O.). Anaplasmose bovine : changements cliniques, hématologiques et biochimiques du sang chez des bovins infectés expérimentalement au Nigeria. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 41-47.

Un veau d'un an de pure race Fulani (zébu) a été choisi après sélection sur ses anticorps humoraux contre *Anaplasma marginale* en utilisant les tests de fluorescence indirecte et d'agglutination par tubes capillaires. Il a été splénectomisé et expérimentalement infecté avec un stabilat de *A. marginale*. 2,0 ml du stabilat ont été inoculés par voie intraveineuse, puis on a observé les signes de manifestations chimiques et les changements hématologiques et biochimiques du sang.

Bien que l'anémie n'ait pas été proportionnelle au degré de parasitémie, les deux facteurs étaient étroitement et négativement corrélés avec le pic de parasitémie se produisant deux jours avant l'anémie maximale. Les valeurs les plus basses de l'hématocrite et de l'hémoglobine, respectivement de 7 p. 100 et 1,8 g/100 ml, signes de l'anémie maximale, ont été enregistrées au 21e jour de l'infection. Les numérations de leucocytes totaux ont montré une augmentation marquée durant le pic de parasitémie.

Le taux de glycémie est descendu de 80,0 g/dl, valeur moyenne lors de la pré-infection, au niveau minimal de 41,6 g/dl au 19e jour. Il n'y a pas eu d'augmentation ou de diminution significatives du niveau des protéines totales du sérum durant la période patente. Cependant, les fractions de globuline et d'albumine ont légèrement augmenté et diminué respectivement au début puis au maximum de l'anémie.

Le taux de fer du sérum est passé de 46,54 mmol/l en moyenne lors de la pré-infection au maximum de 114,56 mmol/l au 19e jour, alors que, d'un autre côté, les niveaux de zinc et de cuivre ont baissé respectivement de 46,80 mmol/l et 14,36 mmol/l à 18,60 mmol/l et 7,95 mmol/l. Par ailleurs, il n'y a pas eu de changement marqué dans les taux de calcium, magnésium, sodium et potassium. *Mots clés* : Zébu Peul - Veau - Anaplasmose - *Anaplasma marginale* - Hématologie - Nigeria.

INTRODUCTION

Bovine anaplasmosis is an infectious and haemolytic disease of cattle characterized in its form by anaemia and jaundice. The disease is caused by the rickettsial *Anaplasma marginale* or *A. centrale* in cattle and is tick-borne.

The pathogenesis and pathology of bovine anaplasmosis have been investigated extensively under experimental and field conditions (1, 9, 10, 11, 13, 14, 20, 22). Although in those investigations the general pattern of the clinical disease signs were similar, the severity of the infection was variable in different breeds of cattle. The purpose of the present study was to investigate the clinical, haematological and blood biochemical changes during fever caused by *A. marginale* in Nigerian local breed.

MATERIALS AND METHODS

Experimental animal

A one-year old pure white Fulani (zebu) calf was chosen for the study after screening it for *A. marginale* humoral antibodies using the indirect fluorescent antibody and capillary tube agglutination tests (18). The presence of *A. marginale* and other parasites was also investigated in thick and thin blood smears stained with Giemsa stain. The calf was also treated with Panacur (*) for worm infestation before it was splenectonized. After splenectomy it was kept indoor in tick free environment and placed under observation for signs of clinical infection for a period of 3 weeks before inoculation with *A. marginale*. During the experimental period the animal was fed on a diet of hay, wheet offal, concentrates and water *ad libitum*.

Anaplasma stabilate

The strain of *A. marginale* used for inoculation was isolated from a splenectomized bull purchased locally. The isolate was preserved as described by DALGLIESH and MELLORS (6) and held at - 80 °C until used.

Infection

The clinically healthy calf was inoculated approximately 3 weeks after splenectomy using 2.0 ml of *A. marginale* stabilate intravenously.

1. National Veterinary Research Institute, Vom, Nigeria.
2. Faculty of Veterinary Medicine, Ibadan, Nigeria.

(*) Hoechst AG. Frankfurt-am-Main-Germany

Samples of blood were then collected in vacutainer tubes once every week until erythrocyte parasitaemia was observed and thereafter daily until parasitaemia subsided. Serum was separated from clotted blood into clean plastic containers and stored at - 20 °C until use.

Clinical observations

At each sampling period the calf was examined carefully for clinical signs of illness and visible mucous membranes were examined for signs of anaemia. Rectal temperature was taken and recorded daily from the day of inoculation until the experiment was terminated. Haematological and blood biochemical analysis :

(i)Packed cell volume (PCV) was determined by employing a microhaematocrit techniques as described by SHALM (19).

(ii)Total leucocyte count was determined using haemocytometers (Assistent) by the standard method.

(iii) Haemoglobin was estimated by cyanmethaemoglobulin method as described by COLES (5) and expressed in gm/100 ml.

(iv) Anaplasma counts : the parasites were counted in Giemsa stained thin blood smears at 1000 x magnification. At least 1,000 erythrocytes were counted on each smear and the percentage of cells containing *Anaplasma* organisms was calculated.

(v) Blood glucose values : blood used for the glucose determination was collected in bijou bottles containing sodium fluoride as anticoagulant. Glucose level was estimated by glucose oxidase method as described by TRINDER (24) and expressed in g/dl.

(vi) The total serum protein was determined by BIURET method (25) and albumin by Bromocresol Green method (8). The globulin values were later obtained by substracting albumin values from total protein values and express in g/100 ml of serum.

(vii) Serum iron, copper, zinc, calcium, magnesium, sodium and potassium levels were determined by Atomic absorption method as described in *Pye Unicam Company technical Bulletin.* The values were expressed in micromole per litre (mmol/l).

TABLE I Haematological and body temperature values in Experimental Anaplasmosis.

Day Post infection	Body Temp. °C	PPE (p. 100)	PCV (p. 100)	Haemo-globin gm/100 ml	Total leucocytes × 103
*Pre-infection	37.8	ND**	31	8.5	12.5
10	38.5	ND	30	9.4	ND
11	37.5	ND	ND	9.0	ND
12	38.1	ND	28	9.2	ND
13	38.0	1.9	26	7.3	12.3
14	38.4	2.1	26	7.5	8.5
15	38.5	5.7	23	–	8.5
16	38.8	6.9	18	5.0	7.45
17	39.0	10.8	17	5.6	10.9
18	39.8	11.6	12	4.0	11.45
19	40.3	13.0	11	2.5	15.75
20	39.9	10.0	9	2.0	14.5
21	39.6	5.0	7	1.8	19.2
22	38.9	2.2	14	2.7	7.5
23	39.2	–	13	2.8	11.5
24	39.0	2.7	12	–	11.5
25	38.7	–	12	–	12.3

* *Mean of estimations for 2 days immediately prior to infection.*
** *ND = Not Done.*

TABLE II Blood biochemical values in Experimental Bovine Anaplasmosis.

Day Post infection	Glucose	Total serum Protein gm/100 ml	Albumin gm/100 ml	Globulin gm/100 ml
*Pre-infection	80	8.2	4.4	3.8
10	ND**	ND	ND	ND
11	ND	ND	ND	ND
12	ND	ND	ND	ND
13	60	8.2	2.7	5.5
14	52	7.0	2.9	4.1
15	–	7.3	3.2	4.1
16	60	9.3	3.2	6.1
17	62.9	8.0	3.4	4.6
18	50.0	7.3	3.5	3.8
19	41.6	8.9	3.1	5.8
20	62.5	6.6	3.1	3.5
21	85	8.0	3.2	4.8
22	80	7.3	3.0	4.3
23	–	6.8	2.9	3.9
24	–	8.7	2.8	5.9

* *Mean of estimations for 2 days immediately prior to infection.*
** *ND = Not Done.*

RESULTS

Clinical observation

(i) Preinfection period : the animal was in good physical condition before it was inoculated with *A. marginale* stabilate. The preinfection mean rectal temperature, packed cell volumes (PCV) and haemoglobin values were 37.8 °C, 31 p. 100 and 8.5 g/100 ml respectively. Other haemat-

TABLE III Serum mineral values in Experimental Bovine Anaplasmosis.

Day Post infection	Iron mmol/l	Copper mmol/l	Zinc mmol/l	Calcium mmol/l	Magnesium mmol/l	Sodium mmol/l	Potassium mmol/l
*Pre-infection	46.55	14.36	46.80	3.50	1.39	137.02	5.63
6	50.12	–	–	3.65	1.84	144.20	3.61
8	50.12	13.36	33.60	3.75	1.44	151.38	4.42
13	42.96	12.72	–	3.00	1.39	144.42	4.42
14	53.70	13.67	66.66	2.50	1.52	147.45	2.88
15	–	–	–	2.50	0.98	147.46	4.74
16	78.76	12.00	27.90	3.50	1.43	144.21	3.65
17	96.66	9.86	30.60	3.75	1.52	–	6.27
18	89.30	13.99	54.90	3.00	1.48	137.05	5.25
19	144.56	10.40	18.60	3.50	1.35	143.98	5.25
20	85.92	10.81	20.40	2.75	1.15	117.45	3.84
21	110.98	9.38	18.60	3.75	1.64	139.55	4.03
22	96.66	8.27	–	3.25	1.48	–	3.97
23	93.08	7.95	29.10	3.50	1.68	133.54	5.31
24	71.60	8.50	24.60	3.15	1.07	–	4.67

* *Mean of estimations for 2 days immediately prior to infection.*

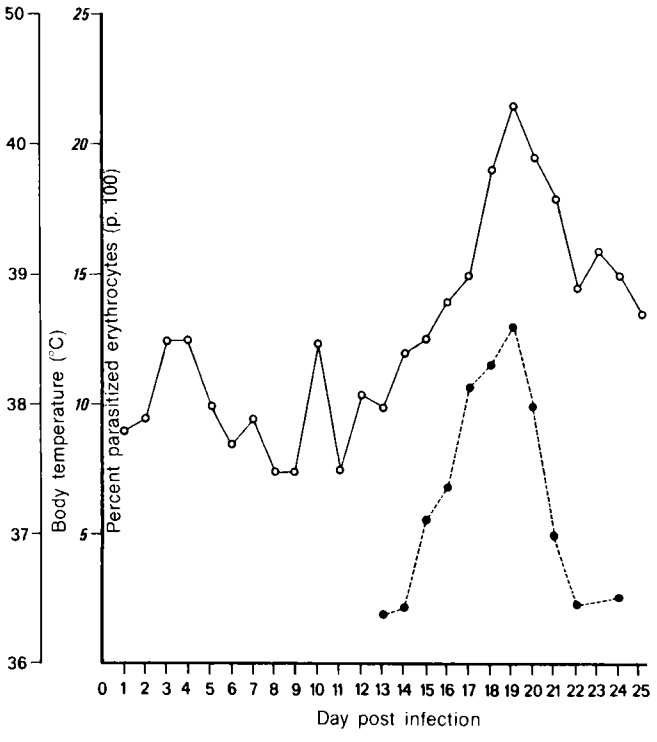

Fig. 1 : Body temperature and percent parasitized erythrocytes (PPE) in experimental bovine anaplasmosis.

ological and biochemical parameters (Tables I-III) were also within normal during this period.

(ii) Prepatent or incubation period : this period extended from the day of inoculation until the first organisms were detected in the circulating erythrocytes. No evidence of pyrexia or anaemia was observed during this stage of the infection (Table I).

(iii) Patent disease : this includes phases of early and maximal anaemia. Visible clinical signs of disease appeared only after the parasites had increased in number and caused severe anaemia and high fever.

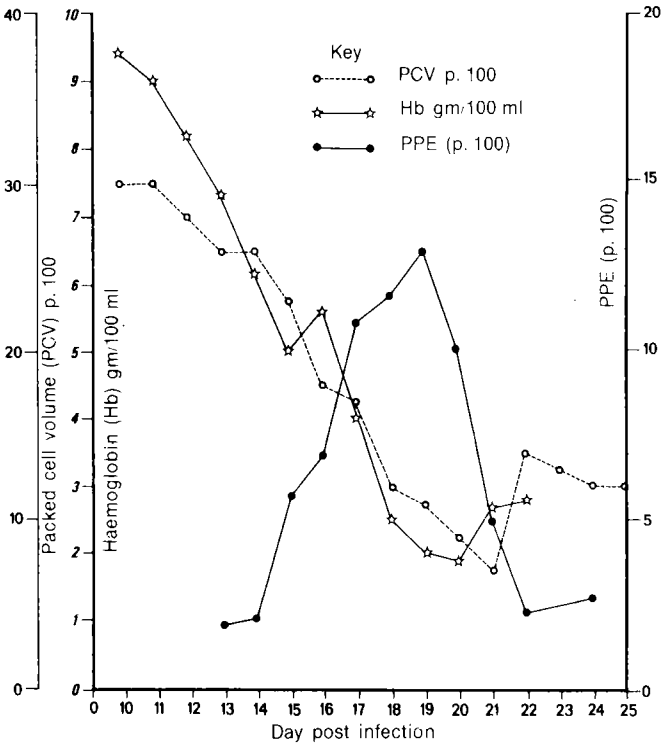

Fig. 2 : PCV, Hb and PPE values in experimental bovine anaplasmosis.

Maximal pyrexia and peak parasitaemia were observed on the same day (Fig. 1).

Other clinical signs observed during this period included pallor of the nasal, oral and conjunctival mucous membranes, general weakness, loss of appetite and signs of depression. There were also an accelerated respiratory rate, muscle tremors and the peripheral blood was less concentrated in appearance.

Haematology

The pattern of parasitaemia and its relations to fever and anaemia are shown in Fig. 1 and 2. Maximum level of parasitaemia occurred two days before maximal anaemia (Fig. 2), while the lowest haematocrit and haemoglobin values of 7 p. 100 and 1.8 g/100 ml respectively, indicative of maximal anaemia, were recorded on day 21 of infection.

Following a transient leucopenia between days 14 and 16, total leucocytic counts showed marked increase during peak parasitaemia and maximal anaemia period (Fig. 3), but the highest total leucocytic count of 19.2 x 10^3 ml^3 was recorded on day 21 of infection.

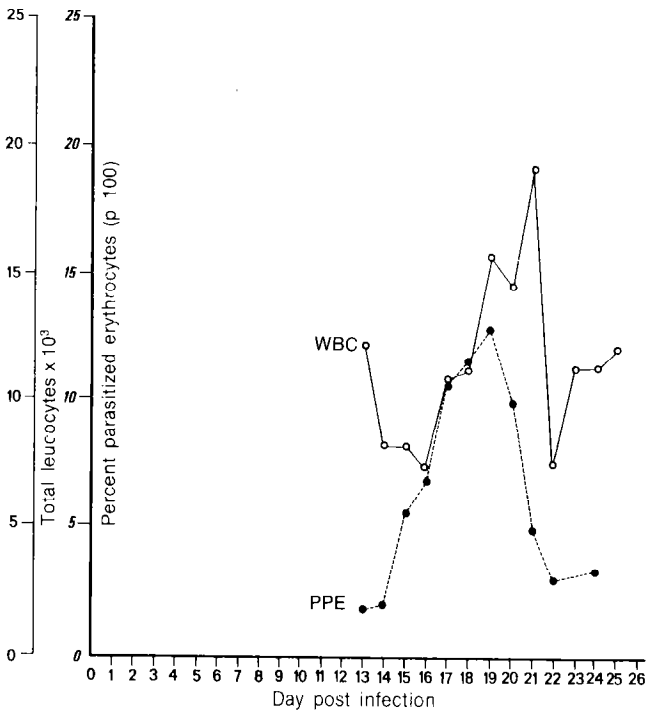

Fig. 3 : Total leucocytes and percent parasitized erythrocytes in experimental bovine anaplasmosis.

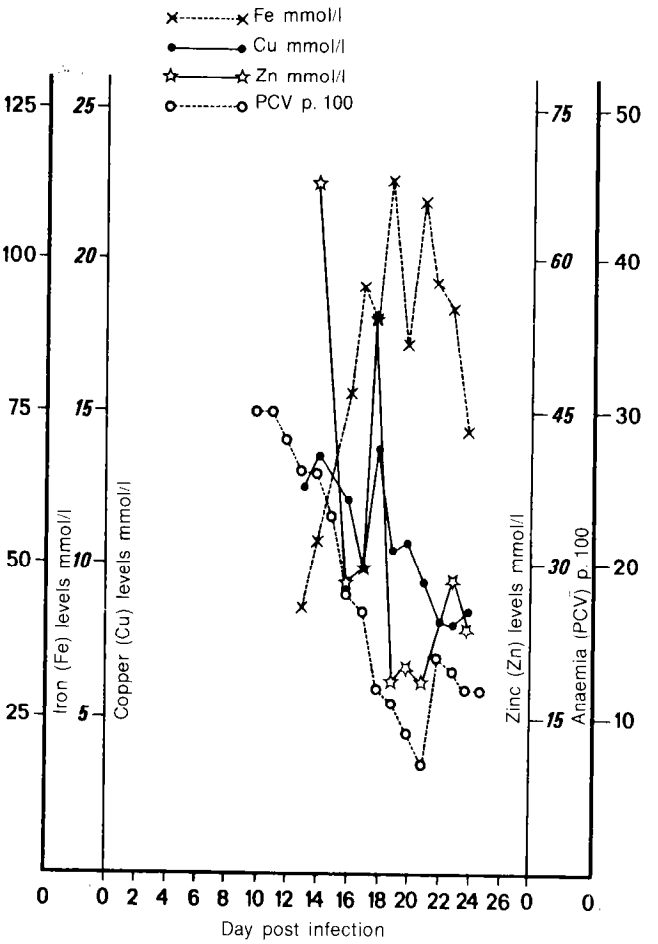

Fig. 4 : Changes in serum iron, copper and zinc levels during anaemia (PCV) in experimental bovine anaplasmosis.

Biochemistry

(i) Blood glucose level dropped from pre-infection mean value of 80.0 g/dl to minimum level of 41.6 g/dl on day 19, coinciding with the period of maximal pyrexia and peak parasitaemia phase.

(ii) The pattern of total serum protein (TSP) in relation to anaemia is shown in Fig. 5. There was no significant increase or decrease in the total serum protein level throughout the patent period. However, the globulin and albumin fractions slightly increased and decreased respectively during the early and maximal anaemia phases (Table II).

(iii) Table III summarises the level of seven serum minerals before and after infection. The pattern of iron, zinc and copper levels in relations to anaemia is shown in Fig. 4. Iron level elevated significantly from day 16 rising to peak (114.56 mmol/l) on day 19, while zinc and copper levels, on the other hand, decreased

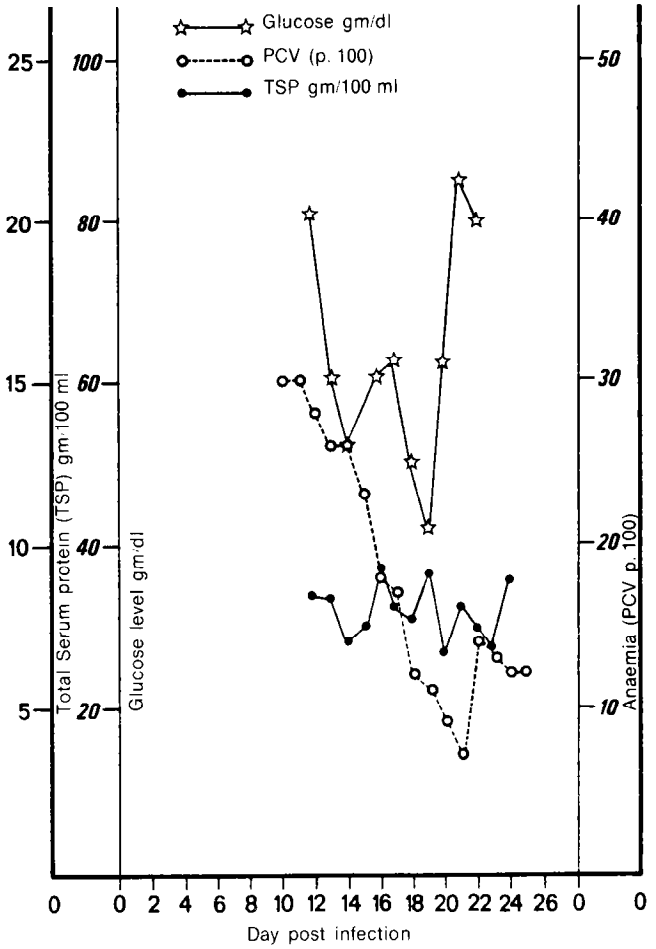

Fig. 5 : Glucose and total serum protein levels during anaemia in experimental bovine anaplasmosis.

during the same period (Fig. 4). However, there was no marked change in the levels of calcium, magnesium, sodium and potassium (Table III).

DISCUSSION

All the clinical disease signs observed in the present study have been reported previously by other investigators (1, 9, 11, 14, 20). Fever as an important clinical sign of anaplasmosis (3) has been attributed to the rapid release of products of haemoglobin breakdown (19). In this study, fever occurred prior to severe loss of erythrocytes, thereby confirming the findings of other workers and indicating that there may be other factors that cause rise in body temperature.

An outstanding feature of erythrocytic infections is the occurrence of anaemia that is often not in proportion to the prevailing parasitaemia (21). Anaemia in *Anaplasma* infected animals has been shown to be caused by extensive erythrophagocytosis initiated by parasitic damage to erythrocytes and antierythrocyte antibody (2, 4, 10, 22). The severe anaemia in spite of relatively low parasitaemia observed in this study may be as a result of autoimmunization as demonstrated in rats infected with *Babesia rodhaini* or *Plasmodium berohei* in which anaemias were not in proportion to the degree of parasitaemia (21). RISTIC (17) and MANN and RISTIC (15) have earlier demonstrated autoimmune response in calves infected with anaplasmosis.

The blood glucose concentration depends upon a wide variety of factors and the concentration at any time is the net result of the rates of entry and of removal of glucose in the circulation (12). The decrease in the blood glucose level observed in this study (Fig. 5) may be a result of two main factors ; (i) reduced dietary glucose intake by the animal caused by loss of appetite, and (ii) increased body tissue utilization of the available blood glucose removed from circulation for energy and conversion into other essential products.

Many disease conditions as lymphocytoma, acute bacterial and protozoan infection have been known to cause an elevation in the γ-globulin level (12). Increase in globulin fractions levels during *Anaplasma* infection have also been reported (3, 7, 10, 22). The findings in this study showed decrease in albumin fraction level as reported by ALLEN *et al.* (3) and MURPHY *et al.* (16).

In haemolytic anaemia serum iron concentration increases (12) thus the observed increase in the serum iron level in this study may be due to the haemolytic nature of anaemia in anaplasmosis.

ACKNOWLEDGEMENTS

Our appreciation is extended to Mr. Sunday ATAWODI, Miss Bunmi ABE and Mr. Samson EDOKPOLO for technical assistance. The authors thank Dr. A. A. MAKINDE, for his useful criticism of the manuscript.

AJAYI (S. A.), OYETUNDE (I. L.), OGBONNA (G. A.), DIPEOLU (O. O.). Bovine anaplasmosis : clinical, haematological and blood biochemical changes in experimentally infected Nigerian cattle. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 41-47.

AJAYI (S. A.), OYETUNDE (I. L.), OGBONNA (G. A.), DIPEOLU (O. O.). Anaplasmosis bovina : modificaciones clinicas, hematologicas y bioquímicas de la sangre en bovinos infectados experimentalmente en Niger. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1) : 41-47.

S. A. Ajayi, I. L. Oyetunde, G. A. Ogbonna, O. O. Dipeolu

A one-year old pure Fulani (zebu) calf was chosen for the study after screening it for *A. marginale* humoral antibodies using the indirect fluorescent antibody and capillary tube agglutination tests. The calf was splenectomized and infected experimentally with a stabilate of *A. marginale*. 2.0 ml of the stabilate was inoculated intravenously and observed for signs of clinical manifestations, haematological and blood biochemical changes.

Although anaemia was not proportional to the degree of parasitaemia, both factors are closely and negatively correlated with peak parasitaemia occurring two days before maximal anaemia. The lowest haematocrit and haemoglobin values of 7 p. 100 and 1.8 g/100 ml. respectively, indicative of maximal anaemia, were recorded on day 21 of infection. Total leucocytic counts showed marked increase during peak parasitaemia. Blood glucose level dropped from pre-infection mean value of 80.0 g/dl to minimum level of 41.6 g/dl on day 19.

There was no significant increases or decreases in the total serum protein level throughout the patent period. However, the globulin and albumin fractions slightly increased and decreased respectively during the early and maximal anaemia phases.

Serum iron level elevated from pre-infection mean value of 46.54 mmol/l to maximum level of 114.56 mmol/l on day 19, while zinc and copper levels, on the other hand, decreased from 46.80 mmol/l and 14.36 mmol/l to 18.60 mmol/l and 7.95 mmol/l respectively. However, there was no marked change in the levels of calcium, magnesium, sodium and potassium. *Key words* : Fulani zebu cattle - Calf - Anaplasmosis - *Anaplasma marginale* - Hematology - Nigeria.

Se escogió un ternero de un año de edad, de raza pura Fulani (cebú) después de selección a partir de los anticuerpos humorales contra *A. marginale* al utilizar las pruebas de fluorescencia indirecta y de aglutinación por tubos capilares. Fué esplenectomizado y experimentalmente infectado con un « *stabilate* » de *A. marginale*. Se inocularon 2 ml del « *stabilate* » por vía intravenosa, y se observaron los signos de manifestaciones hematologicas y bioquímicas de la sangre.

Aunque la anemia no sea proporcional al nivel de parasitemia, los dos factores eran estrechamente y negativamente en correlación con el pico de parasitemia produciendose dos días antes de la anemia. El 21o día de la infección se notaron los valores más bajas del hematocrito, 7 p. 100 y de la hemoglobina, 1,8 g/100 ml indicando la anemia máxima. Las numeraciones de leucocitos totales mostraron una aumentación marcada durante el pico de parasitemia. La tasa de glicemia bajó de 80 g/dl, valor medio durante la pre-infección, al nivel mínimo de 41,6 g/dl al 19o día.

No se observaron aumentación o disminución significativas del nivel de las proteinas totales del suero durante el periodo patente. Sin embargo, las fracciones de globulina y de albúmina aumentaron ligeramente y disminuyeron respectivamente al principio y al máximo de la anemia. La tasa de hierro del suero llegó de 46,54 mmol/l por termino medio durante la pre-infección hasta el máximo de 114,56 mmol/l al 19o día, mientras que los niveles de cinc y de cobre bajaron respectivamente de 46,80 mmol/l y 14,36 mmol/l a 18,60 mmol/l y 7,95 mmol/l. No se observó modificación marcada en las tasas de calcio, magnesio, sodio y potasio. *Palabras claves* : Cebú Fulani - Ternero - Anaplasmosis - *Anaplasma marginale* - Hematologia - Nigeria.

REFERENCES

1. AJAYI (S. A.), WILSON (A. J.), CAMPBELL (R. S. F.). Experimental bovine anaplasmosis clinico-pathological and nutritional studies. *Res. vet. Sci.*, 1978, **25** : 76-81.
2. ALLBRITON (A. R.), SEGER (C. L.). The transport and excretion of bile pigments in anaplasmosis. *Am. J. vet. Res.*, 1962, **23** (96) : 1011-1018.
3. ALLEN (P. C.), RUTTLER (K. L.), AMERAULT (T. E.). Clinical chemistry of anaplasmosis : comparative serum protein changes elicited by attenuated and virulent *Anaplasma marginale* isolates. *Am. J. vet. Res.*, 1981, **42** (2) : 326-328.
4. BAKER (N. F.), OSEBOLD (J. W.), CHRISTENSEN (J. F.). Erythrocyte survival in experimental anaplasmosis. *Am. J. vet. Res.*, 1961, **22** (88) : 590-596.
5. COLES (E. H.). Veterinary clinical pathology. Philadelphia, W. B. Saunders, 1967.
6. DALGLIESH (R. J.), MELLORS (L. T.). Survival of parasitic protozoan *Babesia bigemina*, in blood cooled at widely different rates to 196 °C. *Int. J. Parasit*, 1974, **4** : 169-172.
7. DIMPOULLOS (G. T.), FOOTE (L. E.), SCHRADER (G. T.). Electrophoretic studies of bovine serum. I. Changes in the serum proteins after splenectomy. *Am. J. vet. Res.*, 1959, **20** : 270-272.
8. DOUMAS (B. T.), WATSON (W. A.), BIGGS (H. G.). Determination of serum albumin with bromocresol green. *Clinica chim. Acta*, 1971, **31** : 87.
9. GAUTAM (O. P.), SHARMA (R. D.), SINGH (B.). Anaplasmosis. II. Clinical cases of anaplasmosis in cattle, buffaloes and sheep. *Indian vet. J.*, 1970, **47** : 1012-1019.
10. JATKAR (P. R.), KREIER (J. P.). Pathogenesis of anaemia in *Anaplasma* infection. *Indian vet. J.*, 1967, **44** : 393-399.
11. JONES (E. W.), NORMAN (B. B.). Bovine anaplasmosis. The disease, its clinical diagnosis and prognosis. Nat. Anaplasmosis Conf., 4th Proceeding.
12. KANEKO (J. J.). Iron metabolism. *In* : CORNELIUS (C. E.), KANEKO (J. J.). Clinical biochemistry of domestic animals. New York, Academic Press, 1970.
13. KREIER (J. P.), RISTIC (M.), SCHROEDER (W.). Anaplasmosis. xvi. The pathogenesis of anaemia produced by infection with *Anaplasma*. *Am. J. vet. Res.*, 1964, **25** (105) : 343-352.

14. LOTZE (J. C.). Variables and constants in experimental bovine anaplasmosis and their relationship to chemotherapy. *Am. J. vet. Res.*, 1947, **8** : 267-274.

15. MANN (D. K.), RISTIC (M.). Anaplasmosis, xiii. Studies concerning the nature of autoimmunity. *Am. J. vet. Res.*, 1963, **24** (7) : 703-708.

16. MURPHY (F. A.), OSEBOLD (J. N.), AALUND (A.). Hyper-γM-globulinaemia in experimental anaplasmosis. *Am. J. vet. Res.*, 1966, **27** : 971-974.

17. RISTIC (M.). Studies in anaplasmosis. III. An autoantibody and symptomatic macrocytic anaemia. *Am. J. vet. Res.*, 1961, **22** : 871-876.

18. RISTIC (M.). A capillary tube-agglutination test for anaplasmosis. Preliminary report. *J. Am. vet. med. Ass.*, 1966, **141** (5) : 588-594.

19. SCHALM (O. S.). Veterinary haematology. Philadelphia, Lea and Febiger, 1967.

20. SCHIMDT (H.). Manifestations and diagnosis of anaplasmosis. *Ann. N.Y. Acad. Sci.*, 1956, **64** (1-5) : 27-30.

21. SCHROEDER (W. F.). Blood serum factors associated with erythrophagocytosis in anaplasmosis. Ph.D. Thesis University of Illinois, Urbana, Illinois, 1966.

22. SHARMA (S. K.), BANERJEE (D. F.), GAUTAM (O. P.). Serum protein changes in bovine anaplasmosis. *Haryana Vet.*, 1981, **20** : 54-56.

23. SINGH (B.), GAUTAM (O. P.). Anaplasmosis. III. Experimental anaplasmosis induced by splenectomisation in indigenous and crossbred calves. *Indian vet. J.*, 1971, **48** : 1215-1222.

24. TRINDER (P.). Blood glucose method by glucose oxidase using 4 - amino phenozone as oxygen acceptor - modified. *J. clin. Path.*, 1969, **22** : 246-248.

25. VARLEY (H.). Practical clinical biochemistry. 4th ed. Great Britain, Willian Leieman Medical Books Ltd, 1967.

26. WEICHSELBAUM (T. E.). Estimation of total proteins in serum by Biuret method. *Am. J. clinical Path.*, Techno. Section, 1946, **10** : 40-43.