

THE PREVALENCE RATES OF *THEILERIA PARVA* AND *THEILERIA MUTANS* IN CALVES, ADULT CATTLE AND BUFFALO (*SYNCERUS CAFFER*) IN TANZANIA

Mbassa G. K., Kweka L. E., Gamitwe M. G. H., ^oMlengeya T. D. K., Dulla P. N. *Pereka A. E., **Mgasa M. N., ***Matovelo J. A. and Shallua L. D., Department of Veterinary Anatomy, *Physiology, Biochemistry, Pharmacology and Toxicology, **Surgery, Reproduction and Obstetrics and ***Pathology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P. O. Box 3016 Morogoro, Tanzania and ^oSerengeti National Park, P. O. Box 3134 Arusha, Tanzania.

SUMMARY

Serum samples of 133 adult cattle, 79 calves in Eastern Tanzania, 213 adult cattle in Northern, 235 adult and 156 calves from Lake Victoria basin, 30 buffaloes (*Syncerus cafer*) from Mkomazi Game Reserve, Northern zone and 6 buffaloes from Kisaki Open Conservation Area, Eastern zone were tested for antischizont antibodies against *Theileria parva* and *T. mutans* using indirect fluorescent antibody technique at 1:640 dilution. Antibodies were detected in 63/133, 119/213 and 203/235 adult cattle for *T. parva* in Eastern, Northern and Lake Victoria basin respectively. The respective positive samples for *T. mutans* in adult cattle in Eastern, Northern and Lake Victoria basin were 71/133, 38/213 and 184/235. Antibodies were lacking in calves of the age from birth to four months old. Of the 36 buffalo samples none were positive for *T. parva* and *T. mutans*. *T. mutans* and *T. parva* occur in the same areas but the prevalence is less than for the former. The lack of antibodies in calves indicate lack of transfer of maternal antibodies, calves acquire antibodies through exposure to infections. The lack of antibodies in buffaloes indicate that *T. parva* and *T. mutans* are cattle parasites. Numerous Genera of ixodid ticks including *Rhipicephalus* and *Amblyomma* were found on buffaloes. In Eastern Zone calves haemoglobin concentration, red and white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were found to be lower in calves categorized to be of poor health compared to those apparently in good health. Prescapular lymph node length and width tended to be lower in healthy calves.

INTRODUCTION

East coast fever (ECF) is a widely spread fatal protozoan infection of cattle (Dolan, 1989), transmitted by the brown ear tick *Rhipicephalus appendiculatus*. In indigenous cattle of Ankole/Zebu cross breed under communal grazing endemic stability develops, characterized by high calf mortalities, as observed in Lake Victoria Basin (Mbassa *et al.*, 1993) with low grade syndrome in subsequent infections in recovered calves. Enormous losses result from mortality, reduced production, cost of control measures and exclusion of high productive cattle in ECF endemic areas (Young *et al.*, 1988). The treatment by parvaquone and buparvaquone (Dolan *et al.*, 1984; Dolan, 1986; McHardy *et al.*, 1985; Mbwambo *et al.*, 1987) is effective in early but not in late stages of infection. Field studies indicate that there are mixed infections that bring about a haemo-lympho-proliferative syndrome characterized by anaemia and panleukopaenia (Mbassa *et al.*, 1994), *T. parva* is lymphoproliferative while *T. mutans* is haemoproliferative (Dolan, 1989).

East Coast Fever is controlled by elimination of vectors using acaricides, or by immunization of cattle against the disease (Radley,

1981; Dolan, 1986; Mukheibi *et al.*, 1990; Nene *et al.*, 1992; Musisi and Lawrence, 1995, Mbassa and Silayo, 1995). The initial intense and later irregular use of acaricides resulted in increased incidences of tick borne diseases. Dipping has therefore become unreliable as a method of effectively controlling these diseases. A method based on a combination of therapy, vector control and immunization would be more effective.

Serological methods as DNA analytical methods (Bishop *et al.*, 1993; Mbassa and Morzaria, 1996) have facilitated understanding of the epidemiology of theileriosis and distribution of *Theileria* spp (Deem *et al.*, 1993; Perry and Young, 1993; Uilenberg *et al.*, 1993). *T. mutans* is non-pathogenic haemoproliferative parasite of cattle and buffaloes, transmitted by *Amblyomma* spp to cause African bont tick theileriosis in Africa and the Caribbean, and latent infection in sheep. *T. annulata* infects cattle and Asian (domestic) buffalo (*Bubalus bubalis*) causing tropical theileriosis or mediterranean coast fever, in South Europe, North Africa, India, Middle East, former USSR, and Central Asia transmitted by *Hyalomma* spp (Dolan, 1989). *T. orientalis* and *T. sergenti* are transmitted by *Haemaphysalis* ticks in cattle (Uilenberg, 1981. Uilenberg *et al.*,

1993) causing oriental theileriosis and benign cosmopolitan theileriosis in cattle and Asia buffalo in Asia, Australia, Europe, Africa and America. *T. verifera* is non pathogenic but is transmitted by *Amblyomma* spp in cattle and the African buffalo in Africa and the Caribbean. *T. parva* is found in Africa, causing East Coast fever, corridor disease and Zimbabwe malignant theileriosis with *Rhipicephalus* spp as vectors. *T. taurotragi* infects cattle and eland to cause benign African rhipicephaline theileriosis in Africa and is transmitted by *Rhipicephalus* spp (Uilenberg *et al.*, 1993).

In places with large number of theileria infected vectors the population of animals with antischizont antibodies is high resulting in endemic stability (Norval *et al.*, 1992). In herds with large proportion of animals having antibodies it is possible to vaccinate only calves (Mbassa *et al.*, 1996; 1998). Knowledge of the prevalence of serum antibodies in cattle and buffaloes, is a primary factor in selection of target cattle for immunization. It allows assessment of disease trends and immunization successes. Structured serum analysis for antibodies have been reported in few areas in East Africa (Deem *et al.*, 1993) although this data is essential for selecting of most

suitable methods of control of the disease (Perry and Young, 1993). The present studies were aimed at determining the prevalence of antischizont serum antibodies against *T. parva* and *T. mutans* in free grazing cattle in Eastern and Northern Tanzania, Lake Victoria basin and in Africa buffalo populations in humid subcoastal zones; Mkomazi Game Reserve and Kisaki Open Conservation Area. It was furthermore aimed at assessing calf health and the time they develop antibodies based on earlier observations of high calf mortality rates.

MATERIALS AND METHODS

Blood samples were collected from the external jugular vein in clot activated vacuum tubes (Vacutainer, Becton- Dickinson, England) from cattle in Eastern Tanzania in April and May 1996 in the herds of Kingolwira Prison dairy farm (26 calves, 53 adults), Magadu Animal Science dairy farm (16 calves, 22 adults) and Sokoine University University farm (37 calves) in Morogoro region and 58 adult cattle from small holder farms in Dar es Salaam. In northern Tanzania samples were collected in Arusha, Northern zone at Loliondo Coffee Estate (38), Mringa Estate (72), Lekenyi estate (40) and

Ndurumanga farm (40), and Moshi in Kilimanjaro region (23) similarly in April and May 1996. In Lake Victoria basin samples were collected from Nyangokorwa (22 calves, 35 adults), Mwalushu / Ng'homango (23 calves, 44 adults) in Bariadi district, Idukilo (25 calves, 38 adults), Maganzo / Kombabuki (25 calves, 42 adults) and Lubaga (33 calves, 31 adults) in Shinyanga district and Seke in Kahama district (29 calves and 45 adults) in April and May 1993 and the same months in 1996. The animals in these locations were grouped into either calves, whose exact ages were known, or adults and serum samples kept frozen at 25 °C below 0 until analysis.

Blood samples were also collected from 30 African buffaloes (*Syncerus caffer*) from Mkomazi Game Reserve in Northern Tanzania. The buffaloes were tracked and remotely injected with 9.8 mg etorphine hydrochloride (M99, imobilon, C-Vet, UK) combined with 80 mg xylazine (Chanazine®) intramuscularly using a dart gun (Pneu-dart, Williamsport, USA). Following anaesthesia blood samples were collected from the external jugular vein, then the buffalo treated with an antidote 12 mg diprenorphine (M5050) intravenously. All animals recovered fully within 2 - 16

minutes of injection of the reviver drug. Serum samples were extracted in the standard manner 24 hours later and kept frozen at -25°C until analysis. Serum samples from 6 other buffaloes were obtained during game cropping at Kisaki Open Conservation area (Gonabis) in Eastern zone and processed as above.

In Eastern zone additional blood samples from calves were collected in vacuum tubes containing potassium ethylene diamino tetraacetic acid (K₃EDTA). For the purpose of analysis the calves were grouped as 0 to 60 and 61 to 120 days old. They were examined clinically for health. Calves were decided to be in poor health if they had one or more of the following signs; emaciation, severe fungal infections, enlarged prescapular lymph nodes, pale mucous membranes, lacrimations, lameness, wounds and diarrhoea. Otherwise they were of good health. The length and width of prescapular lymph nodes were measured using a ruler by holding them tightly within skin. The calves were of *Bos taurus*, *Bos indicus* and crosses between them.

From K₃EDTA blood samples, haemoglobin level was determined by the cynomethaemoglobin method in Beckman 1201 spectrophotometer at 544 nm wavelength. Packed cell

volume (PCV) or haematocrit was determined using an M201 Sigma microhaematocrit centrifuge at 12,000 G. Red and white blood cell counts were determined using an improved Neuber haemocytometer. Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated.

Serum antibodies to schizonts of *Theileria parva* and *T. mutans* were detected by indirect fluorescent antibody (IFA) technique (Burrige and Kimber, 1972) using rabbit anti bovine fluorescent isothiocyanate conjugate (FITC). Antigens were provided by the courtesy of Dr. Frederick Musisi of Vaccine Production Centre, Food and Agricultural Organization of the United Nations Organization (FAO, Lilongwe Malawi), with negative and positive control sera and stored frozen at -70°C . The procedure involved dilutions of serum samples in microtitre plate wells using phosphate buffered saline (PBS) pH 7.0 to obtain the final dilution of 1:640. Fluorescence was assessed by photo-microscope BH2 Olympus at 10 x objective.

The ticks found on buffaloes in the inguinal region on the scrotum, udder, ears, withers, dewlap, under the tail, belly and other places were placed in 70 %

ethanol and identified by using a key of Arthur (1960) and Robinson (1926).

RESULTS

Assessment of health states of calves in Eastern zone indicated that pure *Bos taurus* calves (Friesian and Ayrshire) tended to have poor body conditions, slightly larger prescapular lymph nodes, frequent fungal skin infections and anaemia compared to the *Bos indicus* and cross breed calves. Of the 79 calves 63 were apparently in good and 16 in poor health (Table 1). Among the healthy calves, 27 were 0 - 60 and 36 of 61 - 120 days old (Table 1). Of the 16 calves in poor health 6 were 0 - 60 days and 10 at 61 - 120 days of age.

The overall length of prescapular lymph nodes of calves in good health was 1.98 ± 0.73 cm. In calves of good health at 0 - 60 days and 61 - 120 days of age the lengths were 1.65 ± 0.41 and 2.31 ± 0.84 cm respectively (Table 1). The widths also varied as indicated in table 1. The length of prescapular lymph nodes in 0 - 60 and 61 - 120 days old in calves of poor health were 1.88 ± 0.85 cm and 2.50 ± 1.27 cm respectively, with overall mean of 2.32 ± 1.17 cm. The node widths in the same ages were 1.13 ± 0.63 cm and 1.20 ± 0.55 cm respectively, with overall mean of 1.18 ± 0.54

cm. These were not significantly different from those of calves in good health.

Haemoglobin concentration in 0-60 and 61-120 days old calves in good health were 8.24 ± 2.32 mmol/l and 7.52 ± 2.29 mmol/l respectively, the overall mean being 7.92 ± 2.27 mmol/l, non significantly lower than those in poor health. In the latter, values were higher in 0-60 (10.54 ± 2.33 mmol/l) than in 61-120 days old (6.50 ± 2.27 mmol/l). The concentration was not significantly different between good and poor health calves in both age groups.

Packed cell volume of calves in good health was higher (30.28 ± 4.84 %) compared to those in poor health at both ages (29.31 ± 8.24 %). PCV values were larger in 61-120 than in 0-60 days old healthy calves. The overall means were significantly higher in calves of good than those of poor health. PCV in 0-60 days old calves in poor health were 33.00 ± 1.79 % significantly higher than 27.10 ± 9.85 % observed in 61-120 days old calves of the same health status.

Overall means of red blood cell counts (RBC) were non significantly lower in calves of good health ($7.23 \pm 2.26 \times 10^{12}/l$), compared to those in poor health within the same ages ($7.79 \pm 2.49 \times 10^{12} /l$). In 61-120 days

old calves in poor health RBC counts were higher ($7.87 \pm 2.93 \times 10^{12}/l$) than those of 0-60 days ($7.67 \pm 1.78 \times 10^{12}/l$). RBC counts of $8.45 \pm 2.95 \times 10^{12}/l$ in 61-120 days old good healthy calves were higher than those of 0-60 days old calves of the same health states ($6.26 \pm 1.86 \times 10^{12}/l$). Mean erythrocyte counts of $7.67 \pm 1.78 \times 10^{12}/l$ for 0-60 days calves in poor health were not significantly different from $7.87 \pm 2.93 \times 10^{12}/l$ found in 61-120 days old calves of the same health states.

White blood cell counts (WBC) were lower in 0-60 day old calves in poor health ($2.88 \pm 2.09 \times 10^9/l$) compared to $6.68 \pm 2.76 \times 10^9/l$ observed for the 61-120 day old calves of the same health states. WBC values were higher in calves of poor health compared to healthy calves at both ages (Table 1).

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were higher in calves of good health (49.0 ± 2.61 fl, 1.32 ± 0.98 pmol and 27.0 ± 8.00 mmol respectively) than those in poor health at both ages (39.1 ± 10.40 fl, 0.82 ± 0.46 pmol and 27.0 ± 6.00 mmol respectively). Mean corpuscular haemoglobin of 1.44 ± 0.49 pmol observed in 0-60 days old calves in poor health was

significantly higher than 0.87 ± 0.29 pmol observed in 61-120 days old calves of the same health states. MCHC in 0-60 days old calves in poor health was 32.0 ± 7.00 mmol, greater than 24.0 ± 4.00 mmol of 61-120 days old calves. MCV in 0-60 and 61-120 days old calves were 44.9 ± 0.10 fl and 35.6 ± 0.50 fl respectively and were not significantly different.

The indirect fluorescent antibody test for antischizont antibodies to *T. parva* and *T. mutans* in calves indicated no antibodies in new born calves to over 120 days (Table 2). Antibodies against *T. parva* and *T. mutans* were detected in many adult cattle (Table 3). The prevalence of *T. parva* was observed to be greater than that of *T. mutans*. The parasites appear to occur in all farms in Eastern, Northern and Lake Victoria basin of Tanzania at considerably high frequency. The highest prevalence for *T. parva* was found to be in Lake Victoria basin (86.4 %), followed by Kingorwila Prison dairy farm Eastern Tanzania (84.9 %) and Loliondo coffee estate (79.0 %) Northern zone and Mringa estate of Arusha, Northern zone (63.9 %). The highest prevalence rates for *T. mutans* were found in Kingorwila Prison dairy farm Eastern zone (83.0 %), followed by Lake Victoria basin (78.3 %) and Loliondo Coffee Estate in Northern

zone (60.5 %). The prevalence rates in other farms were low (0 - 52.6 % for *T. parva* and 0 - 46.6 for *T. mutans*).

All the 30 buffaloes from Mkomazi Game reserve and 6 from Kisi Open conservation area (Gonabis) were negative to both *T. parva* and *T. mutans* antigens (Table 3). The majority of species of ticks found on buffaloes in the ears, inguinal region, on the scrotum and udders were *Amblyomma hebraicum*, *Amblyomma personatum*, *Amblyomma cohaerens*, *Rhipicephalus appendiculatus*, *Rhipicephalus pulchellus*, *Rhipicephalus evertsi* and others that are still being identified in the laboratory. The ticks that are common in cattle for example *Amblyomma variegatum*, *A. gemma*, *A. lepidum*, *R. appendiculatus*, *Boophilus decolouratus* and others were however, very rare in buffaloes.

DISCUSSION

Assessment of health states of calves in Eastern zone indicated that pure *Bos taurus* calves (Friesian and Ayrshire) tended to have poor body conditions, slightly larger prescapular lymph nodes, frequent fungal skin infections and anaemia compared to the *Bos indicus* and cross breed calves.

Table 1: Mean \pm standard deviation of lymph node length (NL) and width (NW), haemoglobin concentration (Hb, mmol/l), packed cell volume (PCV, %), erythrocyte counts (RBC), leucocytes counts (WBC), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pmol) and mean corpuscular haemoglobin concentration (MCHC) in mixed breeds calves sampled in Eastern Tanzania in good and in poor health.

Variable	Good health calves (n=63)			Poor health calves (n=16)		
	0-60 (n=27)	61-120 (n=36)	Overall (n=63)	0-60 (n=6)	61-120 (n=10)	Overall (n=16)
Age in days						
NL (cm)	1.65 \pm 0.41	2.31 \pm 0.84	1.98 \pm 0.73	1.88 \pm 0.85	2.50 \pm 1.27	2.32 \pm 1.17
NW (cm)	0.90 \pm 0.26	1.06 \pm 0.32	0.98 \pm 0.29	1.13 \pm 0.63	1.20 \pm 0.54	1.18 \pm 0.54
Hb (mmol/l)	8.24 \pm 2.32 ^a	7.52 \pm 2.29 ^a	7.92 \pm 2.27	10.54 \pm 2.33 ^b	6.50 \pm 2.27 ^b	8.01 \pm 3.00
PCV (%)	29.20 \pm 4.29 ^d	31.63 \pm 5.42	30.28 \pm 4.84 ^c	33.00 \pm 1.79 ^{dg}	27.10 \pm 9.85 ^g	29.31 \pm 8.24 ^c
RBC ($\times 10^{12}$ /l)	6.26 \pm 1.86 ^f	8.45 \pm 2.95 ^f	7.23 \pm 2.258	7.67 \pm 1.78	7.87 \pm 2.93	7.79 \pm 2.49
WBC ($\times 10^9$ /l)	3.68 \pm 2.45	5.09 \pm 3.19	4.31 \pm 3.281	2.88 \pm 2.00 ^e	6.68 \pm 2.76 ^e	5.26 \pm 3.10
MCV (fl)	53.9 \pm 2.87	42.8 \pm 2.26	49.0 \pm 2.61	4.49 \pm 0.99	35.6 \pm 0.50	39.1 \pm 1.04
MCH (pmol)	1.59 \pm 1.21	0.99 \pm 0.48	1.32 \pm 0.98	1.44 \pm 0.49 ^h	0.87 \pm 0.29 ^h	0.80 \pm 0.46
MCHC (mmol)	29.0 \pm 9.00	24.0 \pm 8.00	27.0 \pm 8.00	32.0 \pm 6.60	24.0 \pm 4.00	27.0 \pm 6.00

Means indicated by similar superscript letters differ significantly ($p < 0.05$)

Table 2: The number of calves negative to IFA test for antischizont antibodies to *T. parva* and *T. mutans* at Kingolwira Prisons dairy farm, Sokoine University farm, Magadu Animal Science dairy farm in Eastern zone and Nyangokorwa, Idukilo, Maganzo, Lubaga and Seke herds in Lake Victoria basin. All calves were negative for both species.

Age in days	Eastern zone (n=79)			Lake Victoria Basin (n=156)					
	Kingo rwila	Soko ine	Mag adu	Nyan gokor wa	Mwa lush u	Idu kilo	Mag anzo	Lu bag a	Sek e
0-7	3	4	0	3	3	2	3	4	5
8-30	6	6	2	2	2	5	3	6	6
31-60	4	5	3	4	6	5	6	4	4
61-90	1	8	3	6	2	4	5	10	5
91-100	6	6	2	3	6	7	4	6	4
120 and above	6	8	6	4	4	2	4	3	4
Total	26	37	16	22	23	25	25	33	28

Table 3: Number of adult cattle +ve and -ve to *T. parva* and *T. mutans* in Kingorwila dairy (A), Magadu (B), Dar es Salaam (C) in Eastern zone, Loliondo Coffee Estate (D), Mringa estate (E), Lekenyi (F), Ndurumanga (G) and Moshi (H), Northern zone, Nyangokorwa (I), Mwalushu (J), Idukilo (K), Maganzo (L), Lubaga (M) and Seke (N) in Lake Victoria basin, Mkomazi Game Reserve (O) and Gonabis (P) buffaloes.

Farm	<i>Theileria parva</i>				<i>Theileria mutans</i>				Total
	+V e	%	-Ve	%	+Ve	%	-Ve	%	
A	45	84.9	8	15.1	44	83.0	9	17	53
B	0	0	22	100	0	0	22	100	22
C	18	31.0	40	69.0	27	46.6	31	53.4	58
Total	63	47.4	70	52.6	71	53.4	62	46.6	133
D	30	79.0	8	21.0	23	60.5	15	39.5	38
E	46	63.9	26	36.1	8	11.1	64	88.9	72
F	11	27.5	29	72.5	7	17.5	33	82.5	40
G	20	50.0	20	50.0	0	0	40	100	40
H	12	52.2	11	47.8	0	0	23	100	23
Total	119	55.9	94	44.1	38	17.8	175	82.2	213
I	34	97.1	1	2.9	31	88.6	4	11.4	35
J	44	100	0	0	39	88.6	5	11.4	44
K	29	76.3	9	23.7	28	73.7	10	26.3	38
L	37	88.1	5	11.9	35	83.3	7	16.7	42
M	23	74.2	8	25.8	23	74.2	8	25.8	31
N	36	80.0	9	20.0	28	62.2	17	37.8	45
Total	203	86.4	32	13.6	184	78.3	51	21.7	235
O	0	0	30	100	0	0	30	100	30
P	0	0	6	100	0	0	6	100	6

This indicates a relatively little adaptations in the tropics in these exotic cattle compared to indigenous zebu cattle. Zebu cattle are said to be adapted to a variety of tropical disease including tick bone protozoan (Deem *et al.*, 1993). The finding that out of 79 calves 63 (79.7%) were apparently in good and 16 (20.3%) in poor health (Table 1) indicate a satisfactory level of health in calves. However since these were all indoor under farm conditions, the management seems to have a large influence on calf health. Similar observations were expressed by Masanje (1989, Proceedings of Tanzania Society of Animal Production 1989 Arusha Tanzania, unpublished). The situation is different from free range cattle under pastoral system in East Africa. Calf mortality under free range grazing system is higher than in intensive systems (Mbassa, 1990; Mbassa *et al.*, 1993).

The most common diseases conditions observed in the 20.3% calves found to be in poor health were diarrhoea, wounds, fungal infections and dehydration. Lymph node sizes were relatively large and leukocyte counts high in poor health calves indicating a degree of immunological defensive adaptations.

Haemoglobin concentration in 0-60 and 61-120 days old calves in good health were 8.24 ± 2.32 mmol/l and 7.52 ± 2.29 mmol/l respectively. In poor health calves values were higher in 0-60 (10.54 ± 2.33 mmol/l) than in 61-120 days old (6.50 ± 2.27 mmol/l). The overall concentration was not significantly different between good and poor health calves in both age groups. This information indicates some degree of anaemia in calves found to be of poor health. The causes were probably infections or nutritional in origin. Simillary packed cell volume of calves of good health was higher (30.28 ± 4.84 %) compared to those in poor health at both ages (29.31 ± 8.24 %) indicating similar inferences as for Hb. PCV in 0-60 days old calves in poor health were 33.00 ± 1.79 % significantly higher than 27.10 ± 9.85 % observed in 61-120 days old calves of the same health status. It is reported that PCV values are very low in young animals (Mbassa and Poulsen, 1991). The opposite trend observed in this study is likely to have resulted from poor health rather than age because older calves have already been exposed to numerous pathogenic agents that lead to anaemia thus the lower PCV.

Overall means of red blood cell counts (RBC) were non significantly lower in calves of good health ($7.23 \pm 2.26 \times 10^{12}/l$), compared to those in poor health within the same ages ($7.79 \pm 2.49 \times 10^{12}/l$). In 61-120 days old calves in poor health RBC counts were higher ($7.87 \pm 2.93 \times 10^{12}/l$) than those of 0-60 days ($7.67 \pm 1.78 \times 10^{12}/l$). The higher values of $8.45 \pm 2.95 \times 10^{12}/l$ in 61-120 days old in good healthy calves than those of 0-60 days old calves of the same health states ($6.26 \pm 1.86 \times 10^{12}/l$) indicates the likely influence of age (Jain, 1986, Mbassa and Polsen, 1991).

White blood cell count (WBC) were lower in 0-60 day old calves in poor health ($2.88 \pm 2.09 \times 10^9/l$) compared to $6.68 \pm 2.76 \times 10^9/l$ observed for the 61-120 day old calves of the same health states. This indicates an influence by age (Jain, 1986) and by exposure to infections. The higher WBC values in calves of poor health compared to healthy calves at both ages (Table 1) probably indicates a response to infections.

The erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration

(MCHC) were higher in calves of good health (49.0 ± 2.61 fl, 1.32 ± 0.98 pmol and 27.0 ± 8.00 mmol/l respectively) than those in poor health at both ages (39.1 ± 10.40 fl, 0.82 ± 0.46 pmol and 27.0 ± 6.00 mmol respectively). Mean corpuscular haemoglobin of 1.44 ± 0.49 pmol observed in 0-60 days old calves in poor health was significantly higher than 0.87 ± 0.29 pmol observed in 61-120 days old calves of the same health states being also influenced by age. MCHC in 0-60 days old calves in poor health was 32.0 ± 7.00 mmol/l, greater than 24.0 ± 4.00 mmol/l of 61-120 days old calves. These indices are however dependent on Hb, RBC and PCV and help determination of causes of anaemia.

Antibodies against *T. parva* and *T. mutans* were detected in adult cattle (Table 3), but not in calves below 120 days (Tables 2). This indicates lack of antibodies against these parasites acquired from the dam by the calf. Burr ridge and Kimber (1973) detected low antibody levels to schizont and piroplasm antigens in colostrum. This appears to last for a short period and probably accounts for the resistance to theileriosis observed in calves (Koch *et al.*, 1990). This study did not test colostrum antibodies but calves as

young as 1 day old did not show presence of serum antibodies. This lack of serum antibody extended to up 4 months of age (day 120). Earlier observations in Lake Victoria zone have revealed antibodies in calves by 90 days of age (Mbassa *et al.*, 1993). This trend suggests that antibodies are acquired following exposure to low challenge of parasites. Lake Victoria basin is reported to be constantly infested with *Rhipicephalus appendiculatus* ticks (Moll *et al.*, 1986, Norval *et al.*, 1992), hence the observed endemic stability in this area.

Calves of the present study were kept in doors until 4 months of age so they had very little contact if any with ticks. On the other hand calves in Lake Victoria basin were of Ankole/Zebu types and were kept out doors, having frequent contact with adult cattle, thus to ticks, thereby shows early exposure to the parasites. This may be the cause of early development of antibodies earlier observed (Mbassa *et al.*, 1993). Most calves were reported to have swollen parotid and prescapular lymph nodes and the Ankole Zebu breed is reported to be resistant to *T. parva* infections (Paling *et al.*, 1991; Mbassa, 1992). This breed has therefore established itself as a sole population of animals around

Lake Victoria, preferred by farmers for its resistance to tick borne diseases.

It can be deduced that development of acquired immunity to theileriosis in calves depends on the time of exposure to ticks and parasites and also on the breed of cattle. This indicates that calves develop antibodies following exposure to parasites through tick challenge. This is corroborated with the findings that there were many positive adult cattle for both antibodies to *T. parva* and *T. mutans*. The prevalence of *T. parva* was observed to be greater than that of *T. mutans* but both occurred in all farms in Eastern, Northern and Lake Victoria basin Zones at considerably high frequency. The highest frequency (86.4%) was observed in Lake Victoria basin. This is in conformity with earlier reports on *T. parva* establishment in the basin (Norval *et al.*, 1992). Almost equal prevalence rates were observed in Eastern zone (84.9%) and these are accounted for by the wide scale movement of cattle from Lake Victoria basin to occupy these areas in search of pastures and for commercial purposes. These areas were earlier free of theileriosis (Nsengwa *et al.*, 1987). In early 1980's and 90's Eastern Tanzania area was highly

unstable epidemiologically. The increasing prevalence of antibodies in adult cattle indicate that more cattle have been exposed to the parasites and have become immune.

The results of this study further show that both *T. parva* and *T. mutans* are widely distributed (Table 3). This finding supports the report by Yeoman and Walker (1967) on the distribution of *A. variegatum* the vector for *T. mutans* being centred on the same foci as *R. appendiculatus* the vector for *T. parva*. Several host species of these ticks do not acquire a high degree of resistance to them and cattle and wild ungulates are reported to be primary hosts for all stages of their life cycles (Norval *et al.*, 1992). This is why these ticks and hence the parasites they transmit can become very abundant and so widely distributed. This is typically exemplified by the abundance of numerous species of ticks on all the 36 captured buffales in Mkomazi game Reserve and Kisasi - Gonabis conservation area. With this relationship therefore the presence of *A. variegatum* in an area strongly suggests that conditions are suitable for *R. appendiculatus*. *T. parva* and *T. mutans* pose the same threat to cattle and actually together cause severe haemolymphodepressive syndromme

characterized by anaemia and panleukopaenia (Mbassa *et al.*, 1994). Acting alone *T. mutans* is an opportunistic and benign parasite which, in conjunction with other tick borne haemoprotozoan parasites causes anaemia and lowered production (Paling *et al.*, 1981). Outbreaks of *T. mutans* causing severe disease with fatalities have been reported from East Africa (Young *et al.*, 1978a). It may at this juncture be concluded that the clinical picture seen in *T. parva* infection in cattle (Mbassa *et al.*, 1994) is a mixture with haemoproliferative *T. mutans*. *T. parva* and *T. mutans* are discriminated serologically and by enzyme linked immunosorbent assay (ELISA) (Katende *et al.*, 1990)

The distribution of ticks and their hosts is dependent on climate, subsequently serum antibody prevalence rates have been found to vary according to agroecological zone in Africa (Deem *et al.*, 1993). The present efforts in the control of theileriasis are directed mostly to the control of *Theileria parva* infection. Since this study has found that *T. parva* and *T. mutans* are found in the same environment they both pose the same threat to cattle. This means that control of *T. mutans* infection is an important activity in the control of *T. parva*. IFA results in cattle at Magadu

farm show that all the 22 sampled animals were lacking antibodies against to *T. parva* and *T. mutans* (Table 3). Acaricide use scheme in this farm is intensive and effective. These animals graze in a limited area in the morning and fed under shed in the afternoon. They do not come in contact with cattle under extensive grazing. It is apparent that ticks challenge is low in this herd as the control of ticks by acaricides is intensive and supported by limitation of movement to potentially vector infested areas. This is a different situation from the finding that all the 36 buffaloes examined were negative to both *T. parva* and *T. mutans*. This either indicates that the ticks in these areas were clean which is probable or that these parasites do not cause a serious disease in buffaloes to the level of antibody development. However, Theilerial parasites, particularly *Theileria parva lawrencei* have been observed to cause disease in the African buffalo (Young *et al.*, 1978b). *Theileria parva* parasites originating from buffaloes cause what is known as buffalo derived *Theileria parva* infections in cattle (Norval *et al.*, 1992). *Theileria taurotragi* has been observed to cause fatal disease in elands (Grototenhuis *et al.*, 1980). Attempted infections of *Theileria parva* parasites in goats have

produced no disease despite that they were fatal in cattle (Mbassa *et al.*, in preparation). There is therefore quite a wide response in different animals. The African buffalo in some places of East Africa has been found to show antigenic response *in vitro* and *in vivo* (Paling *et al.*, 1981; Maritim *et al.*, 1992).

This study has established that measured by serum antibody levels to schizonts, *T. parva* and *T. mutans* are widely distributed in Tanzania among cattle populations but not in buffalo populations, that calves maintained in doors have limited exposure to ticks and parasites thus do not develop antibodies until over four months of age, whereas calves on free grazing develop antibodies early, and that there are numerous *Amblyomma* and *Rhipicephalus* tick in buffalo populations, but there is high tick species adaptation. *R. appendiculatus*, *A. varigatum*, *A. gemma* and *A. lepidum* being very rare in buffaloes but numerous in cattle. The commonest ticks found on buffaloes were *Amblyomma hebraicum*, *Amblyomma personatum*, *Amblyomma cohaerens*, *Rhipicephalus appendiculatus* and *Rhipicephalus pulchellus*.

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REFERENCES

- Arthur, D.R. (1960). Ticks., A monograph of the Ixodidae. Cambridge University Press 1 - 247.
- Bishop, R. P., Sohanpal B.K., Allsopp B. A, Spooner P. R. and Dolan T.T. and Morzaria, S.P. (1993). Detection of polymorphism among *Theileria parva* stocks using repetitive, telomeric and ribosomal DNA probes and antischizont monoclonal antibodies. Parasitol. 107:19-31.
- Burridge, M.J. and Kimber C. D. (1972). The indirect fluorescent antibody test for experimental East Coast fever (*Theileria parva* infection of cattle). Evaluation of a culture schizont antigen. Res. Vet. Sci. 13:451-455.
- Burridge M.J. and Kimber C.D. (1973). Duration of serological response to the indirect fluorescent antibody test of cattle recovered from *Theileria parva* infection. Res. Sci. 14:270-271.
- Deen, S.L., Perry B.D., Katende J.M., McDermott J.J., Mahan S.M., Maloo S.H., Morzaria S.P., Musoke A.J., and Rowlands G.J. (1993). Variations in prevalence rates of tick borne diseases in Zebu cattle by agro-ecological zone: implications for East Coast Fever immunization. Prev. Vet. Med. 16: 171-187.
- Dolan, T. T. (1986). Chemotherapy of East Coast fever: the long term weight changes, carrier state and disease manifestations in parvaquone treated cattle. J. Comp. Pathol. 96:137-146.
- Dolan, T. T. (1989). Theileriasis; a comprehensive review. Rev. Sci. Tech. Off. Int. Epiz. 8(1):11-36.
- Dolan, T. T. Young A. S., Leitch B. L. and Stagg D. A. (1984). Chemotherapy of East cost fever: Parvaquone treatment of clinical disease induced by isolates of *Theileria parva*. Vet. Parasitol. 15:103-116.
- Grootenhuis J.G., Morrison W.I., Karstad L., Sayer P.D., Young A.S., Murray M- and Haller R.D. (1980). Fatal theileriosis in eland (*Taurotragus oryx*). Pathology of natural and experimental cases. Res. Vet. Sci. 29: 209 - 229.
- Jain, N.C. (1986). Cattle: Normal hematology with comments on

- response to disease: In: Jain N.C. 1986: *Schalm's Veterinary Hematology*, Lea and Phebiger, Philadelphia. 178-207.
- Katende, J.M., Goodeeris B.M., Morzaria S.P., Nkonge C.G. and Masoke A.J. (1990). Identification of a *Theileria mutans* specific antigen for use in an antibody and antigen detection ELISA. *Parasite Immunol.* 12: 419-433.
- Koch, H.T., Kambara I., Norval R.A.I., Ocoma J.G.R., Masaka S., Munatswa F.C., Honhold N. and Irvin A.D. (1990). Age resistance to *T. parva* infection in calves. *Vet. Parasitol.* 37:197-206.
- Maritim, A.C., Young A.S., Lesan A.C., Ndungu S.G., Stagg D.A. and Ngumi P.A.N. (1992). Transformation of *Theileria parva lawrencei* derived from African buffalo (*Syncerus caffer*) by tick passage in cattle and its use in infection and treatment immunization method *Vet. Parasitol.* 43:1-14.
- Mbassa G. K. (1990). Analytical studies on the control of Bovine East Coast Fever in traditional herds in Lake Victoria zone of Tanzania. In: *Proceedings of the 6th Conference of Association of Institutes for Tropical Veterinary Medicine, September, 1989, Wageningen, the Netherlands Edited by H. Kuil, R. W. Païng and J. E. Huhn, Utrecht.* 173-175.
- Mbassa G. K. and Poulsen J. S. D. (1991). Haematological profile in neonatal Dwarf and Landrace kids. *J. Vet. Med. A* 38:510-522.
- Mbassa, G.K. (1992). Evidence of natural resistance to East Coast fever in Ankole-zebu cross cattle in Lake Victoria zone of Tanzania. In: *Proceedings of the 7th Conference of Association of Institutes for Tropical Veterinary Medicine, September, 1992, Yamasoukrou, Ivory Coast.* 7:475-480.
- Mbassa, G. K., Balemba O. B. and Mtiba P. B. (1993). Investigations towards increasing calf survival in traditional cattle herds in Lake Victoria Basin Tanzania. *Proceedings of Workshop on cattle research network (Carnet). International Livestock Centre for Africa. Addis Ababa Ethiopia. May 1993.*
- Mbassa, G. K., Balemba, O. B., Maselle R. M. and Mwaga, N. V. (1994). Severe anaemia due to haematopoietic precursor cell destruction in field cases of East Coast fever in Tanzania. *Vet. Parasitol.* 52:243-256.
- Mbassa G. K. and Silayo R. S. (1995). The effect of massive *Theileria parva* infections on the

- trivalent sporozoite vaccine in epidemiologically unstable areas of Tanzania. *Tanzania Vet. J.* 15:60-71.
- Mbassa G. K. and Morzaria S. P. (1996). Monoclonal antibody response and *Sfi*I and *Eco*RI fragments of genome DNA of *Theileria parva* Melela of eastern Tanzania. *Tanz Vet J.* 16:39-52.
- Mbassa, G. K., Ruheta M. and Otaru M. M. (1996). Progress in the control of East Coast fever in eastern Tanzania by immunization. Proceedings of 14 th Tanzania Veterinary Association Scientific Conference, 3rd to 5th December 1996. *Tanzanian Vet. J.* Vol 17. Suppl. 2:181-184.
- Mbassa G. K., Kweka L. E. and Dulla P. N. (1998). Immunization of East Coast Fever in field cattle using low infectivity stabilate. *Vet. Parasitol.* 77:41-48.
- Mbwambo, H. A., Mkonyi P. A. and Chua R. B. (1987). Field evaluation of parvaquone against naturally occurring East Coast fever. *Vet. Parasitol.* 23:161-168.
- McHardy, N., Wekesa L. S., Hudson A. T. and Randall A. W. (1985). Antitheilerial activity of BW 720 C (buparvaquone) a comparison with parvaquone. *Res. Vet. Sci.* 39:29-33.
- Moll, G. Lohding A., Young A.S. and Leitch B.L. (1986). Epidemiology of theileriosis in calves in an endemic area of Kenya. *Vet Parasitol.* 19:255-273.
- Mukheibi, A. W., Morzaria A. P., Perry B. D., Dolan T. T. and Norval R.A.I., (1990). Cost analysis of immunization for East Coast fever by infection and treatment method *Prev. Vet. Med.* 9:207-219.
- Musisi, F.L. and Lawrence J.A. (1995). Prospects of control of tick borne diseases in cattle by immunization in Eastern, Central and Southern Africa. *Agric. Human Values* 12:95-106.
- Nene, V., Iams K. P., Gobright E. and Musoke A. J. (1992). Characterization of the gene coding a candidate vaccine antigen of *Theileria parva* sporozoite. *Molecular and Bioch. Parasitol.* 51:17-28.
- Norval, R. A., Perry B. D. and Young A. S. (1992). The epidemiology of Theileriosis in Africa. Academic Press Ltd., London pp. 63-203, 301-342.
- Nsengwa, G.R.M. and Otaru M.M.M. (1987). East Coast Fever outbreak in Southern Tanzania *Bull. Anim. Hlth Prod. Africa.* 35:79 - 80.
- Paling, R. W., Grootenhuis J. G. and Young A. S. (1981). Isolation of *Theileria mutans* from Kenyan buffalo and transmission by *Amblyomma gemma*. *Vet.*

- Parasitol. 8:31.
- Paling, R.W., Mpangala C. Luttikhuisen B., Sibomana G. (1991). Exposure of Ankole and cross bred cattle to Theileriosis in Rwanda. Trop. Anim. Hlth Prod. 23: 203 - 214.
- Perry B.D. and Young A.S. (1993). The past and future roles of epidemiology and economics in the control of tick borne diseases of livestock in Africa; the case of theileriosis in Africa; the case of theileriosis Prev. Vet. Med. 25:107-120.
- Norval, R.A.L., Peny B.D. and Young A.S. (1992). The epidemiology of Theileriosis in Africa. Academic Press, London pp 37, 63 - 98, 156-403.
- Radley, D. E. (1981). Infection and treatment method of immunization In: Advances in the control of theileriosis. A. D. Irvin, M. P. Cunningham and A. S. Young (Editors) 227-237 Martinus Nijhoff, The Hague.
- Robinson, L.E., (1926). The genus *Amblyomma*. Cambridge University Press. 1 - 302.
- Uilenberg, G. (1981). Theileria species of domestic livestock. In advances in the control of theileriosis, A. D. Irvin, M. P. Cunningham and A.S. Young (Ed) 4-37. Martinus Nijhoff publishers, The Hague.
- Uilenberg, G., Dobbelaere D.A.E., de Gee A.L.W. and Koch H.T. (1993). Progress in research on tick borne diseases: Theileriosis and heart water. Vet Quart. 15 (2) 48 - 54.
- Yeoman G.H. and Walker J.B. (1967). The ixodid ticks of Tanzania. Commonwealth Agricultural Bureaux London. 1 - 109.
- Young, A.S. Brown C.G.D., Burrige M.J., Grootenhuis J.G., Kanhai G.K., Purnell R.E., and stagg D.A. (1978a). The incidence of theilerial parasites in East African buffalo (*Syncerus caffer*). Tropenmedizin und Parasitologie 29: 281 - 288.
- Young, A.S., Purnell R.E., Payne R.C., Brown C.G.D. and Kanhai G.K. (1978b). Studies on the transmission and course of infection of a Kenyan strain of *Theileria mutans*. Parasitol. 67:99 - 115.
- Young A. S., Grocock C.M. and Kariuki (1988). Integrated control of ticks and tick borne disease of cattle in Africa. Parasitol. 1988, 96:403-432.
- Young, A.S., Leitch B. L., Dolan T. T., Mbogo S. K., Ndugu S. G., Grootenhuis J.G. and De Castro J. J., (1990). Evaluation of infection and treatment method against Theileriosis in an endemic area of Kenya. Vet. Parasitol. 35:239-257.