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**INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES  
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## OCCURRENCE OF THE MULTIVESICULAR FORM OF HYDATIDOSIS IN CATTLE IN MOZAMBIQUE.

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### SUMMARY

Classical hydatid disease infection with *Echinococcus granulosus* was found in 336 out of 13 390 slaughtered cattle at Maputo slaughterhouse. The cysts were mainly found in the lungs (2,3%) and liver (0,2%).

The occurrence of the multivesicular form of hydatidosis was rarely observed in bovine livers. The evolution of multivesicular hydatidosis is described and illustrated with photomicrographs. A comparative study of the two types of larval echinococcosis was carried out. The gross anatomical and histological aspects in cattle are very similar to those described in humans. The main feature of multivesicular hydatidosis was its invading and necrosing tendency. This is the first time that multivesicular forms of hydatid disease have been described in the southern hemisphere.

Five dogs were experimentally infected by means of ingestion of cattle hydatid cysts (2) and multivesicular cysts (3). Among the former embryonated eggs were found after a 67-day period. Among the latter one had unembryonated eggs and the other two had matured eggs after a 29 and 45 day period respectively.

### INTRODUCTION

Hydatid disease caused by the cystic larval stages of *Echinococcus granulosus* is mentioned by many authors as having a world-wide distribution. In contrast, the cystic larval stages of *E. multilocularis* seem to be limited to

the northern hemisphere. (Rausch 1954; Rausch & Schiller 1954; Rausch & Yamashita 1957; Rausch & Gentoft 1957; Gemmel, 1979; Shantz *et al* 1969; Dada, 1978).

In this report we record, for the first time, the occurrence of the multivesicular form of hydatid disease in cattle in southern Africa, suggesting that the epizootology of this disease should be considered in a new perspective. Our results are based on an analysis of a survey carried out in the municipal slaughterhouse of Maputo, from November 1971 to March 1972.

Some particular aspects of normal hydatid cysts are discussed. The step-by-step evolution of multivesicular hydatid lesions is also briefly described and illustrated with photomicrographs.

### MATERIALS AND METHODS

The hydatid lesions found in livers and lungs were collected from 13 390 slaughtered cattle and the pathological description of the lesions was based on gross examination of 336 lesions of which 30 lesions were examined histologically. Five dogs were experimentally infected by means of ingestion of cattle hydatid cysts (2) and multivesicular cysts (3) and were sacrificed three months later.

Necropsies were carried out in these dogs and lung, liver, spleen, kidney and intestine were removed for histological study. For histopathological examination tissues of both cattle and dogs were fixed in 10% formal saline solution.

Paraffin sections were stained with Haematoxylin-Eosin and some were stained with periodic Acid-Schiff (PAS), Van Gieson, Gomori's elastin Weigert's, La matta and Cleyden's methods.

Taeniae were collected from the small intestine, cut into pieces of about 4 cm each and dipped in 9% NaCl solution at 38° C. They were fixed in 3% Acetic-Alcohol for 4 hours and stained with Mayer's Hemalumen and Mayer's Paracarmin according to Drury (1967).

Data on hydatid cysts in man (recorded during the past 23 years) were provided by the Central Hospital of Maputo.

## RESULTS

### Bovines

#### General Aspects

Classical hydatid disease infection with *E. granulosus* was found in 336 animals; the incidence thus being 2.5%. The cysts were mainly found in the lungs (2.3%) and liver (0.2%).

The cysts were usually fertile although a few regressive changes identified as

suppurative and serocavitary Echinococcosis were found.

The fertility of the cysts was not related to their size. Fertile cysts 8 mm in diameter with protoscolices were found, while others of much larger size were infertile.

#### Histo-Pathology

Histological observation of hydatid cysts did not demonstrate eosinophilia.

In the hydatid cysts observed, the lesions were completely, localized and the surrounding liver tissue was normal.

The multivesicular form of hydatidosis, anatomically and clinically different from the normal hydatid cysts, was rarely observed in the livers.

The main feature of multivesicular hydatidosis was its invading and necrosing tendency resulting in circulatory disturbances and regressive alterations which were seen in the surrounding liver tissue (Fig. 1).



Fig. 1: The alveolar structure is well developed in this specimen.

Following the first stages of the inflammatory cellular reaction, larval migration, and development of the granuloma, (Fig. 2) there was a sudden transition to another stage with an increase in larval size and a predominance of degenerative cells.

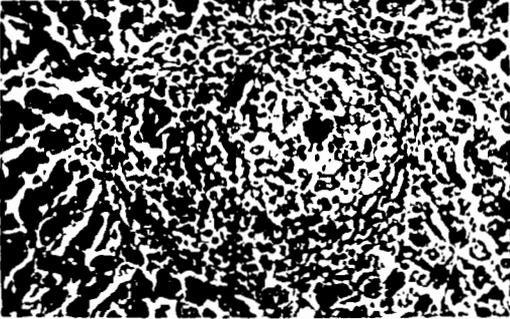


Fig. 2: Granuloma showing polymorphism with few connective fibres. The parasite can be seen in the centre surrounded by eosinophiles. H. E. x 250.

As growth of the larva proceeded, the germinal membrane, became thicker as did its supporting connective tissue. More vesicle developed (either endogenous or oxogenous) by means of proliferation and multiplication of the germinative layer and necrosis of hepatic cells (Fig. 3). The alveolar structure became more evident (Fig. 4).

Rapid larval growth was indicated by many factors: 1. the presence of numerous isolated masses of germinal tissue at the edge of the stroma which were the forerunners of new vesicles; 2. by the increase of necrotic and degenerative lesions of hepatic cells, and 3. the development of new vesicles and formation of new scolices.

In this stage of development, germinal membranes were found in the interlobular veins; these membranes had the same capacity for multiplication as those from which they were derived. New vesicles developed by this process.

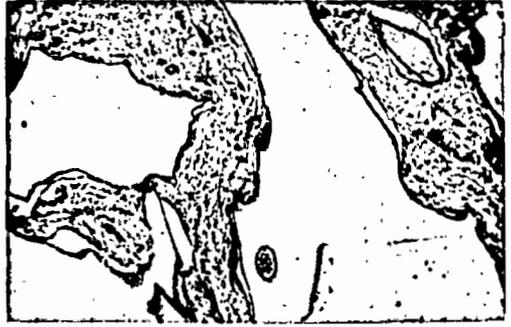


Fig. 3: Endogenous vesicle with germinal layer-lower left corner. A protoscolex can be seen in the upper middle centre. H.E. x 100



Fig. 4: The alveolar structure is well developed. H.E. x 70.

Degenerative changes of the surrounding hepatic tissues included disruption of nuclear elements (pionosis and cariorrhesis) and faint staining of the cytoplasm.

The circulatory disturbances were evidenced by dilation of portal vein branches and congestion of the sinusoids of the remaining liver tissue.

A considerable eosinophilic reaction was always evident in these stages.

## Dogs

### General Aspects

The experimental infection of dogs by means of ingestion of bovine hydatid cysts demonstrated the viability of the

cysts. Although Thomas (1880) Smyth (1964), Moreno (1965), and others referred to the difficulty of infecting dogs older than two years, we were able to infect a dog of three and another of eight years.

Among the three dogs experimentally infected by ingestion of multivesicular cysts, we found unembryonated eggs after a 29 day period. The other two dogs contained mature eggs after a 45 day period.

Embryonated eggs were found after 67 days in the two dogs which were infected with hydatid cysts.

The adult taeniae were mainly located in the jejunum and the ileum of the dogs.

#### *Histo-Pathology*

The intestinal lesions caused by the mechanical and irritative action of the parasites, were of a chronic catarrhal enteritis of the mucosal type in transition to the polypous phase with secondary complications.

Adult taeniae collected from experimentally infected dogs with classical hydatid cysts and multivesicular cysts were not of uniform morphology (fig. 5, 6).



Fig. 5: *Taenia* collected from dog experimentally infected with multivesicular form of Hydatidosis. Mayer Haemalumen x 80.



Fig. 6: *Taenia* collected from dog experimentally infected with unilocular form of Hydatidosis. Mayer Haemalumen x 60.

Hepatic kidney and lung lesions identical to those described in the initial stages of multivesicular bovine hydatidosis were found in two of the experimentally infected dogs with multivesicular cysts. These two animals showed eosinophilia values of 36% and 22%.

The remaining had normal eosinophilic values. All animals were free from other parasites.

#### *DISCUSSION*

The incidence of multivesicular and classical hydatid cysts in cattle is relatively low in Mozambique (approximately 25%). Although only 10 cases of human hydatidosis were recorded in Maputo Central Hospital during the past 23 years, unreported cases may exist.

The incidence may be expected to rise with an increase in cattle breeding which is planned for the following years and the establishment of communal villages.

Major public health and economic problems will arise if adequate control measures are not introduced.

The absence of eosinophilia in the normal hydatid cysts may be considered as an adaptation of the parasite to the

host which does not occur in the multivesicular cyst.

A comparative study of the two types of larval echinococcosis found in cattle livers led us to the conclusion that their histogenesis is very different.

While in the normal hydatid cyst, the germinative membrane forms a thick cuticle that does not permit the diffusion of the hydatid fluid, this cuticle acts as a foreign body giving rise to an adventicia. This adventicia circumscribes the parasitic lesion, preventing its diffusion. In the multivesicular form of hydatidosis, formation of the cuticle is delayed and interrupted allowing the diffusion of the fluid that causes necrosis of the tissue. Even the germinative membrane is interrupted and emits extensions that advance easily through the necrosed tissues, invading the vessels and giving rise to new vesicles. Due to these factors, the rapid development of the process quickly resembles the growth of a tumour.

The gross anatomical and histological aspects of hydatidosis in cattle are very similar to those described in humans. The maturing period for taenia from normal hydatid cyst infection was approximately 67 days while the maturing period for taenia obtained from dogs infected with multivesicular cysts was 45 days. This data is in agreement with Smyth (1964).

These observations and the morphological aspects lead us to believe that two different types of taenia were present. Although this aspect of the problem exceeds the scope of this work we think it worth mentioning.

Systematic investigations for the presence of Echinococcus were carried out in 643 normal dogs with negative results. On the other hand hydatid cysts are very common in buffalo (*Syncerus caffer*) impala (*Aepycerus melampus*) and warthog (*Phacocercus aethiopicus*).

Contacts of cattle with these wild animals resulting from grazing habits are very frequent in many regions of Mozambique. The incidence of the disease in man is very low. Considering all the data, we suggest that a *sylvatic* cycle involving wild animals could be the major source of infection.

Hydatidosis / Echinococcosis in Mozambique has not yet been thoroughly studied. More knowledge of the epizootiology of the disease is necessary for effective control.

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## STUDIES ON TICKS OF VETERINARY IMPORTANCE IN NIGERIA

### II. Oviposition and eclosion patterns of *Amblyomma variegatum* Fabricius, 1974 in relationship to state of engorgement.

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#### SUMMARY

The patterns of oviposition and eclosion were observed in female *Amblyomma variegatum* detached in various states of engorgement from cattle. Oviposition did not take place until females had attained a state of engorgement of 0.4 gm. A state of engorgement of 1 gm was found to be critical for the female ticks below which only a proportion of the eggs will eclode. Although there was a wide gap between the maximum and minimum number of eggs oviposited within ticks of a weight group, the construction of a frequency table allowed accurate prediction to be made on the number of eggs that will be produced by the majority of females in a given weight group. Daily egg output by females was variable; however, each weight group of engorged ticks was characterized by a definite but constant number of peaks in egg output. When these peaks were numbered, it was further observed that the highest peak of each of the members of the same weight group occurred at a constant numerical number. The degree of daily weight loss is directly proportional to the intensity of egg output and hence, the highest peak of daily egg output correlates with the highest peak of daily weight loss.

Oviposition was totally suppressed when female *A. variegatum* were maintained at temperatures of 10°C and 37°C. At 18°C, not only was the pre-oviposition period significantly prolonged,

but also the numbers of eggs laid by all the weight groups were significantly less. In the course of oviposition at this temperature, a period of decreased to insignificant oviposition activity was observed in every weight group. For this period, we propose "mobilisation phase". The oviposition capacity of female *A. variegatum* was found not to be totally injured when they were kept at temperatures 10°C, 18°C and 37°C provided they are transferred within specific days to more favourable temperatures. The field implications of these results are discussed.

#### INTRODUCTION

The importance in Nigeria of *A. variegatum* in terms of abundance (Unsworth, 1952; Strickland, 1961; Mohammed, 1974; 1977; Dipeolu, 1975) and disease transmissions (Dipeolu, 1976) had been well elucidated. However, only few field and laboratory studies had been conducted on various facets of the biology of this tick species. In a previous paper, we have reported our comprehensive studies on the life cycle of *A. variegatum* under quasi natural conditions (Dipeolu and Ogunji, 1977). Our present studies are devoted to the factors that influence the various stages of the life cycle of *A. variegatum*. In this paper we report our observations on the oviposition and eclosion patterns of this tick species. The influence of temperature on these patterns is also highlighted.

## MATERIALS AND METHODS

Collection and maintenance of ticks  
 Female *A. variegatum* in various states of engorgement were collected individually by forcible detachment with pairs of forceps from bodies of indigenous cattle breeds stationed at the veterinary control post in Ibadan. These were immediately conveyed to the laboratory in dual purpose kilner jars and the individual weights were determined and recorded using a sensitive balance. Each weighed tick was placed in a universal bottle which was then plugged with cotton wool. The maintenance of each individual tick varied according to the factor being tested. Those which were being observed under standard conditions were kept in the insectary where no attempt was made to control the temperature and relative humidity. Occasional measurements of these factors showed that they varied during the day from a minimum of 22°C and a maximum of 27°C with an average of 24°C. The relative humidity varied from 75% to 80%. When oviposition was studied under different temperatures, these were put in incubators regulated to the corresponding temperatures with saturated solutions of Sodium chloride as a source of relative humidity.

*Egg collection, egg counting and egg estimation:* After the preoviposition period the eggs laid by each tick were collected at 0800 hours everyday during oviposition and counted. Counting was done under dissecting microscope using a tally counter. Counting was facilitated by addition of oxylene which dissolved the wax which had hitherto made the eggs stick together. Our experience was that eggs produced by certain categories of ticks were usually so many that it

was too time consuming to count all of them individually. We therefore resorted to arithmetical estimation for all ticks which weighed up to 0.5 g and above. This was done by recording the weight of the whole batch of eggs to be counted (e.g. X g). A small quantity of this batch was then weighed very carefully (e.g. Y g) and the number of eggs contained in it very carefully counted (e.g. Z eggs). Estimation of the egg is done by dividing the weight of egg of the whole batch by that of the small quantity taken from the same batch and multiplying by the number of eggs in the small batch (e.g.  $\frac{X \times Z}{Y}$ ). Before this estimation was resorted to, comparison was made between figures obtained from actual and estimated counts in 5 fully engorged female *A. variegatum*. In each of the five cases there was no significant difference between both counts ( $P < 0.005$ ).

*Statistics.* The observations made in the insectary under quasi natural conditions were considered as standard results. When observations were made at temperatures other than the standard, the significance of deviation of results was tested by the Chi-square method (Snedecor and Cochran, 1973).

## RESULTS

### Standard Results

*Relationship between state of engorgement, oviposition and eclosion:* As shown in Table 1, no female *A. variegatum* below the weight of 0.2 oviposited. There was no hatching among the eggs produced by the ticks which weighed 0.2-0.3 g. All female ticks which weighed 0.4 g and above laid eggs but eclosion in all the eggs so produced did not take place until the tick had weighed 1 g. The preeclosion period was not

**Table 1: The proportion of different weight groups of engorged *Amblyomma variegatum* which oviposited and eclosed and the preecllosion times under quasi natural conditions.**

*Engorged weight in g.	Percentage oviposition	Percentage eclosion	Preecllosion period in days	
			Range	Mean
0.1	0	—	—	—
0.1 - 0.2	0	—	—	—
0.2 - 0.3	38	0	—	—
0.3 - 0.4	62	8	56 - 66	59
0.4 - 0.5	110	30	55 - 61	58
0.5 - 0.75	100	40	57 - 61	59
0.75 - 1	100	63	57 - 60	58
1 - 1.5	100	100	55 - 63	58
1.5 - 2	100	100	57 - 61	59
2 - 2.5	100	100	57 - 64	59
2.5 - 3	100	100	54 - 61	58
3 - 3.5	100	100	54 - 62	58
3.5 - 4	100	100	55 - 63	59
74	100	100	59 - 64	60

\* Number of ticks examined for each weight group is 200.

affected by the state of engorgement of the female ticks. The preoviposition periods, length of oviposition and number of eggs produced by different weight groups of engorged females are shown in Table 2. The mean preoviposition intervals which had been variable for engorgement weights from 0.2-1 g became relatively stable as from 1 g and above. The mean number of egg output was relatively low in ticks weighing 0.2 - 1 g. There was however a sharp rise in the number of eggs produced by ticks weighing 1 g and above and with the exception of the highest weight group (4g) the number of eggs produced was proportional to the weight group.

#### *Frequency table of egg production*

Since the variation in egg production within each weight group of engorged tick is very wide (Table 2), the frequency chart (Fig. 1) was constructed so as to ascertain not only the minimum and maximum number of eggs produced by the members of any weight group but also, and more importantly; the number of eggs produced by a great majority of ticks in any weight group. The frequency chart of Fig. 1 was constructed for weight groups of 1 g and above. It will be seen from the chart that most of the ticks in weight groups of 1 - 1.5 g will lay 10 000-15 000 eggs, those of 1.5 - 2 g and 2 - 2.5 g 15 000 - 20 000, those of weight groups 2.5 -

3 g and 3 - 3.5 g 20 000 - 25 000 while most of those in weight groups of 3.5 - 4 g will lay eggs up to between 20 000 and 30 000.

*Daily egg output and peaks of oviposition:* Fig. 2 shows the daily output of eggs in three weight groups of engorged females. It was observed that the daily egg output was variable and that peaks could be recognized within the oviposition period. In Fig. 2 for example, there are up to 7 peaks for the weight group of 3 - 3.5 g with the fourth peak being the highest and occurring 22 days after engorgement. The total num-

ber of peaks and the number of peak which was the highest were further analysed for each weight group of engorged ticks. Each peak of each weight group was further analyzed so as to ascertain the number of days it took to obtain the peak, the number of eggs laid and the approximate number of eggs laid per hour during the peak. All these are shown in Table 3. It was observed that each weight group of engorgement was characterized by a definite but constant number of peaks. When these peaks were numbered, it was further observed that the highest peak of each of the members of the

Table 2: The preoviposition period, length of oviposition and number of eggs laid by *Amblyomma variegatum* at 24°C.

*Engorged weight in g.	Preoviposition period in days		Length of oviposition in days		No. of eggs	
	Range	Mean	Range	Mean	Range	Mean
0.2 - 0.3	20 - 24	22.8	4 - 8	6	211 - 437	295
0.3 - 0.4	12 - 15	13.5	12 - 20	15.8	960 - 1252	998
0.4 - 0.5	12 - 18	14.2	14 - 21	16.8	2061 - 3118	2684
0.5 - 0.75	11 - 16	12.3	15 - 25	17.6	3289 - 4870	4102
0.75 - 1	11 - 20	13.2	18 - 25	22.7	3000 - 3793	3481
1 - 1.5	10 - 20	12.2	22 - 30	25.8	5958 - 24024	12946
1.5 - 2	9 - 13	11	22 - 30	27.2	7143 - 38732	16458
2 - 2.5	10 - 13	11	30 - 35	32.7	7214 - 31900	18262
2.5 - 3	9 - 14	11	38 - 55	45	8030 - 33674	20042
3 - 3.5	4 - 13	10	36 - 50	42	7934 - 35814	22801
3.5 - 4	9 - 13	11	30 - 40	35	9206 - 36206	25231
74	10 - 13	11	22 - 31	28	8724 - 32224	20896

\* Number of ticks examined for each weight group is 200.

Table 3: The peaks of oviposition in different weight groups of *Amblyomma variegatum* kept at 24°C.

	Weight in grams												
	0.2 - 0.3	0.3 - 0.4	0.4 - 0.5	0.5 - 0.75	0.75 - 1	1 - 1.5	1.5 - 2	2 - 2.5	3 - 3.5	3.5 - 4	4		
Number of peaks	1	3	3	5	5	7	7	7	7	7	7	7	
Highest Peak	-	2nd	1st	1st	1st	2nd	2nd	5th	3rd	4th	3rd	3rd	
1st Peak *Range of days obtained	25-26	26	10-12	10-12	11-13	11-13	11-13	12-14	12-14	13-15	12-14	11-13	11-13
Range of eggs laid	120-125	110-115	196-200	510-520	265-280	400-420	480-490	550-560	540-550	400-410	690-700	750-760	
App. No. of eggs laid per hour	5	5	8	21	11	17	20	23	23	17	29	31	
2nd Peak Range of days obtained	-	17-21	15-16	17-19	17-19	16-19	17-19	15-17	15-17	16-18	19-21	16-18	
Range of eggs laid	-	120-139	136-140	210-220	260-270	450-460	540-550	540-550	670-680	460-470	590-600	660-670	
App. No. of eggs laid per hour	-	5	6	9	11	19	23	23	28	19	25	28	
3rd Peak Range of days obtained	-	24-27	19-22	20-22	20-22	22-24	23-25	19-21	18-20	19-21	21-23	18-20	
Range of eggs laid	-	52-54	160-164	370-380	190-200	355-365	420-430	590-600	870-880	530-540	570-800	830-840	
App. No. of eggs laid per hour	-	2	7	15	8	15	18	25	36	22	33	35	
4th Peak Range of days obtained	-	-	-	23-26	28-30	26-28	25-27	21-23	22-24	21-23	26-28	21-23	
Range of eggs laid	-	-	-	420-430	180-190	310-320	390-400	580-590	830-840	1100-1110	510-520	650-660	
App. No. of eggs laid per hour	-	-	-	18	8	13	16	24	35	46	21	27	
5th Peak Range of days obtained	-	-	-	29-30	30-32	29-30	29-31	26-28	24-26	25-27	31-33	25-27	
Range of eggs laid	-	-	-	85-90	100-105	180-185	200-210	780-790	630-640	710-720	610-620	610-620	
App. No. of eggs laid per hour	-	-	-	4	4	8	8	33	26	30	25	25	

Table 3: The peaks of oviposition in different weight groups of *Amblyomma variegatum* kept at 24°C. (Cont.)

	Weight in grams											
	0.2 - 0.3	0.3 - 0.4	0.4 - 0.5	0.5 - 0.75	0.75 - 1	1 - 1.5	1.5 - 2	2 - 2.5	3 - 3.5	3.5 - 4	4.	
Number of peaks	1	3	3	5	5	7	7	7	7	7	7	7
6th Peak												
Range of days obtained	-	-	-	-	-	30-33	31-33	31-32	27-29	32-34	37-39	27-29
Range of eggs laid	-	-	-	-	-	188-196	130-140	250-260	510-520	680-690	370-380	470-480
App. No. of eggs laid per hour	-	-	-	-	-	8	5	10	21	28	15	20
7th Peak												
Range of days obtained	-	-	-	-	-	35-37	36-38	36-38	32-34	35-37	40-42	36-38
Range of eggs laid	-	-	-	-	-	80-85	75-85	100-110	210-220	540-550	230-240	280-290
App. No. of eggs laid per hour	-	-	-	-	-	3	3	5	9	23	10	12

+Number of days after detachment from host.  
Number of ticks examined for each weight group is 50.

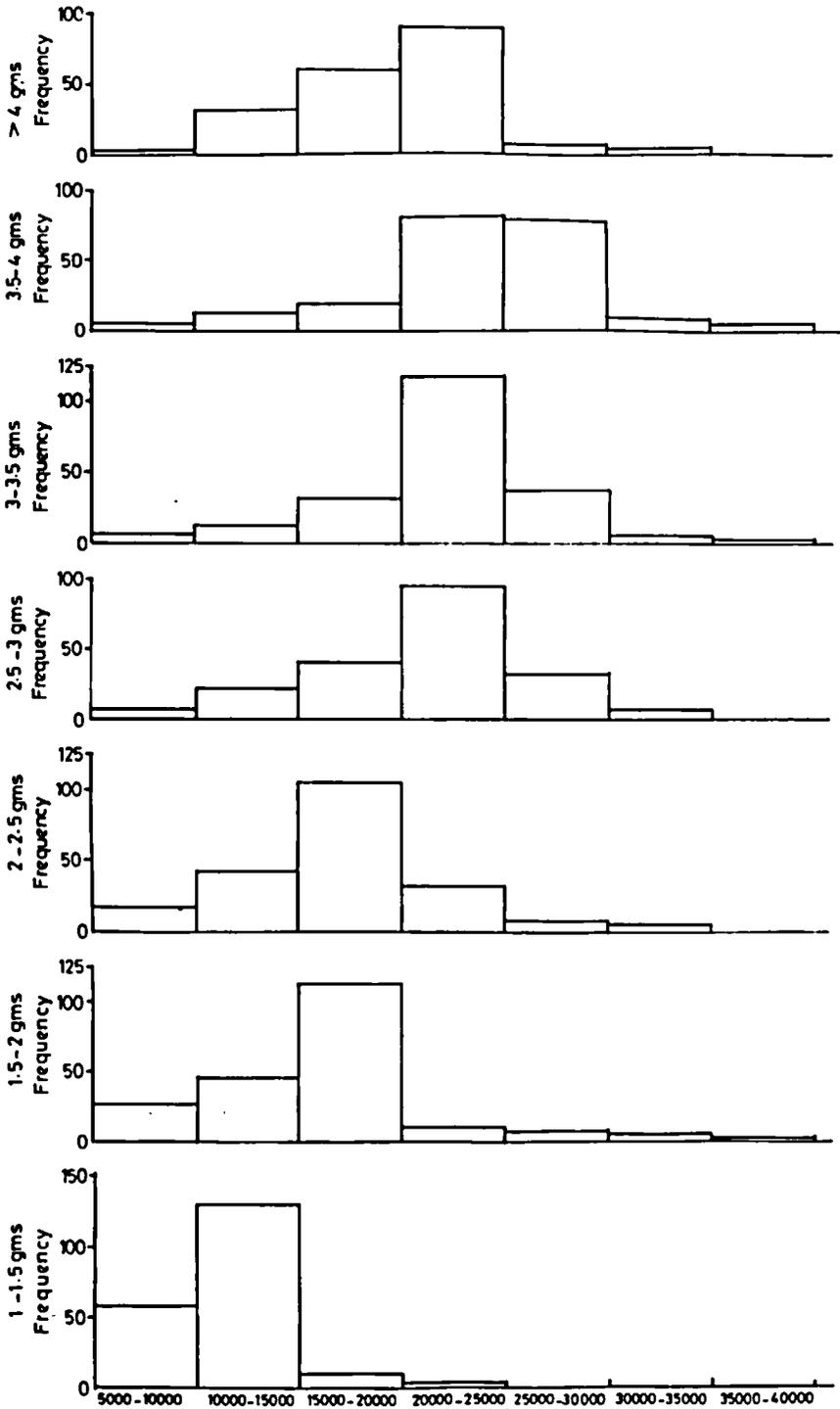


Fig. 1: Frequency chart for the range of eggs produced by different weight groups of engorged *A. variegatum*.

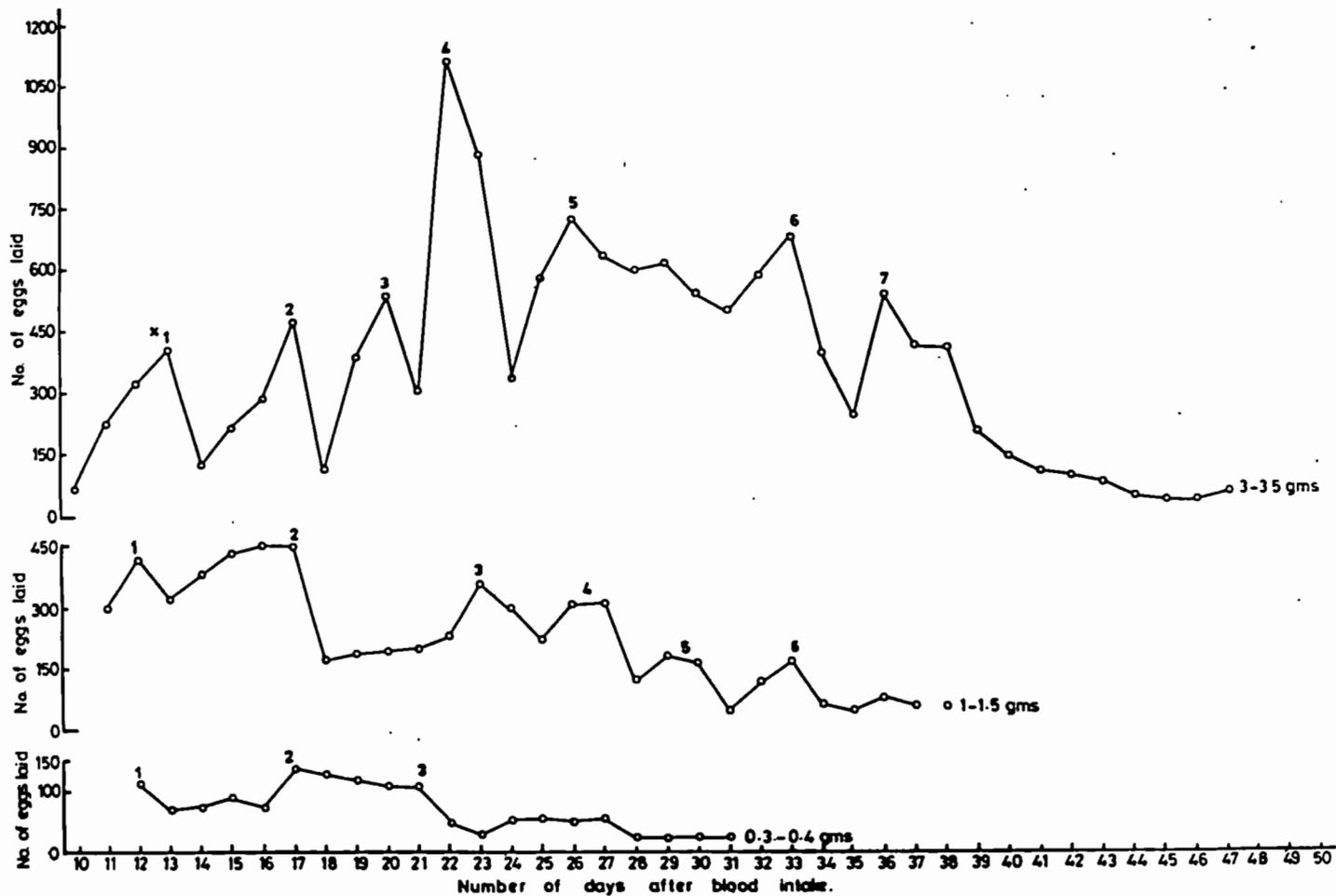


Fig. 2: Daily egg output of engorged *A. variegatum* maintained under standard procedures.

same weight group occurred at a constant numerical number. Even within each peak of any given weight group of engorged ticks, the variation of the days at which the peak is obtained and number of eggs laid at the peak do not vary widely.

*Relationship between weight loss and oviposition:* In this experiment, daily weights of different weight groups of ticks were recorded from the time of detachment to the end of oviposition. Fifty ticks were used for each weight group. It was found that in each weight group, there is a significant correlation between the daily weight loss of the engorged ticks and the number of eggs per day. An example is shown in Fig. 3 (weight group 2.5 - 3 g. There is a significant correlation ( $r = -.916$ ) between the daily weight loss and the intensity of daily egg output and here, as in all cases, the highest peak of daily egg output occurs simultaneously as the highest peak of daily weight loss.

#### Results of Modification of Standard Procedure

*Maintenance of engorged ticks at various temperatures.* In this investigation engorged ticks of various weight groups (0.1 - 4 g were kept in incubators regulated to 10°C, 18°C and 37°C. These are the ranges of temperatures which are known to prevail on the field during the major climatic seasons i.e. harmattan, dry and rainy seasons, in Nigeria. Two hundred ticks were used for each of the temperature experiments. The preoviposition periods, length of oviposition and number of eggs laid by the weight groups at these temperatures were recorded and compared with the standard results.

Results showed that oviposition was totally suppressed at temperatures of 10°C and 37°C. The ticks kept at these temperatures were discarded after observation for 6 weeks. Table 4 shows the results of the ticks kept at 18°C. In all the weight groups, the preoviposition period was significantly ( $P < 0.005$ ) prolonged. Although there was no significant difference in the length of oviposition, the numbers of eggs laid by all the weight groups of ticks were significantly less than those kept under standard procedures. Daily outputs of eggs and peaks of oviposition at 18°C were further studied for groups of 20 ticks of each weight group of engorged ticks kept at that temperature. The pattern of results was similar for all the groups and those of weight groups 1.5-2 g, 2-2.5 g and 2.5-3 g are shown in Fig. 4. Compared with ticks kept at standard procedures, oviposition at 18°C were further studied for groups of 20 ticks of each weight group of engorged ticks kept at that temperature. The pattern of results was similar for all the weight groups and those of weight groups 1.5 - 2 g 2 - 2.5 g and 2.5 - 3 g are shown in Fig. 4. Compared with ticks kept at standard procedures, oviposition did not commence until 21-22 days after detachment from the animal; daily egg output was variable and peaks can be recognized within the oviposition period. Like the standard results, each weight group of engorgement was characterized by a definite but constant number of peaks; unlike the standard results, however, the first peak was always the highest at this temperature. It was also observed that for every weight group, there was always a period of decreased to insignificant oviposition activity. The length of this period was constant for each weight group and occurs after a constant number of peaks.

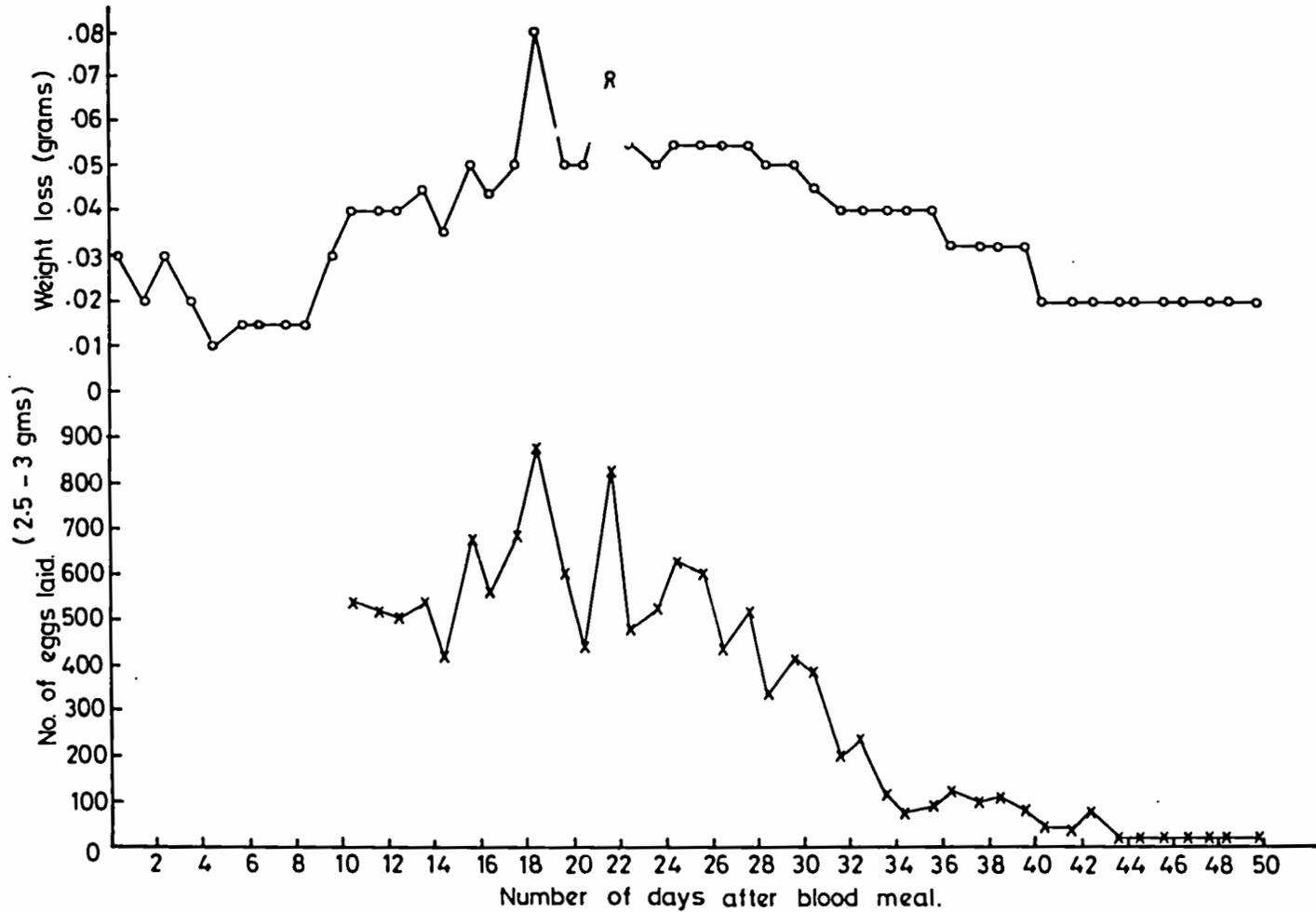


Fig. 3: Relationship between daily weight loss and daily egg output of engorged *A. variegatum* maintained under standard procedures.

Table 4: The Preoviposition period, length of oviposition and number of eggs laid by *Amblyomma variegatum* at 18°C

*Engorged weight in g.	Preoviposition Period in days		Length of oviposition in days		Percentage oviposition	No. of eggs	
	Mean	+Significance	Mean	+Significance		Mean	+Significance
0.1	—	—	—	—	0	—	—
0.1 – 0.2	—	—	—	—	0	—	—
0.2 – 0.3	.9	—	—	—	0	—	—
0.3 – 0.4	36	P .005	20	P .5	50	51	P .005
0.4 – 0.5	34	P .005	22	P .25	50	211	P .005
0.5 – 0.75	31	P .005	21	P .5	50	872	P .005
0.75 – 1	28	P .005	25	P .75	50	1024	P .005
1 – 1.5	26.5	P .005	31	P .5	100	4253	P .005
1.5 – 2	27.2	P .005	29	P .75	100	7123	P .005
2 – 2.5	29	P .005	36	P .75	100	8204	P .005
2.5 – 3	20	P 0.1	38	P .5	100	6875	P .005
3 – 3.5	25.5	P .005	45	P .75	100	5694	P .005
3.5 – 4	26.2	P .005	50	P .05	30	2112	P .005
++4							

\*Number of ticks examined for each weight group is 200

+Statistical significance tested against standard results in Table 2

++Data disturbed by very high mortality.

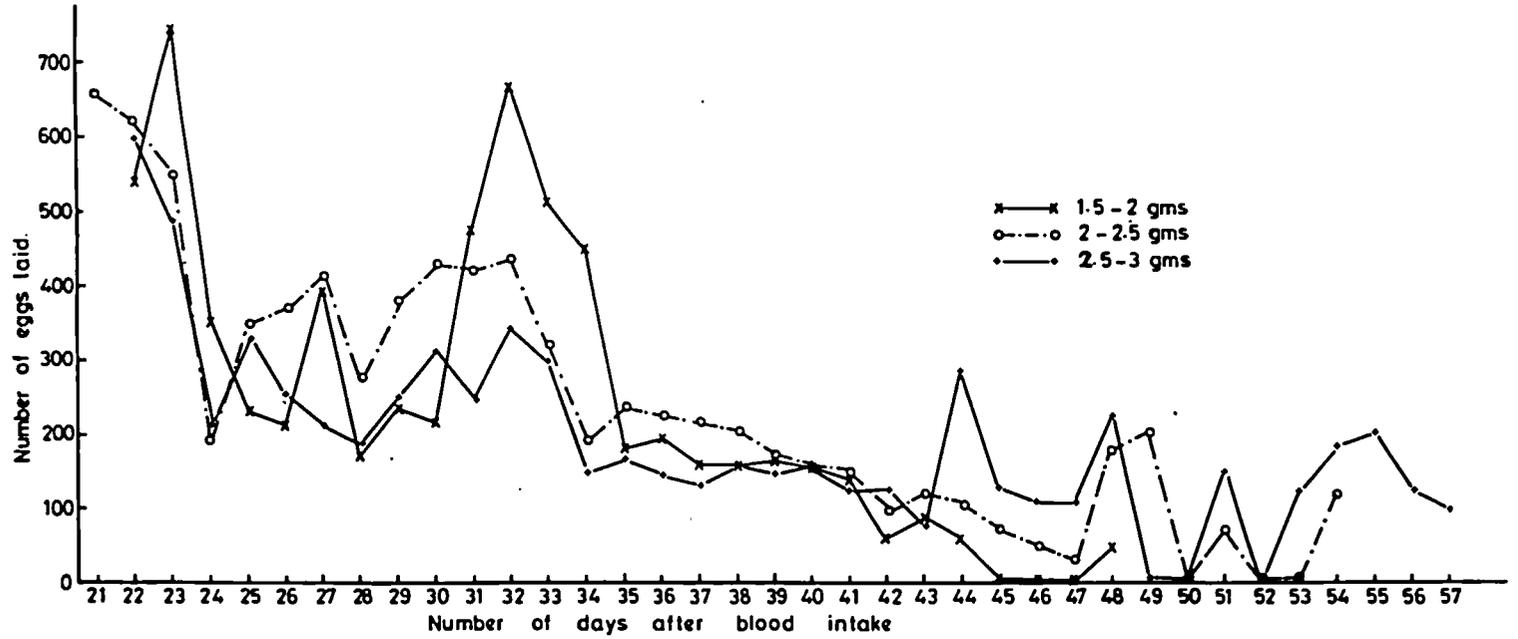


Fig. 4: Daily egg output of engorged *A. variegatum* maintained at 18°C.

As shown in Fig. 4, this period lasts for 10 days (Days 35 - 45), 13 days (Days 34 - 47) and 9 days (Days 34 - 43) for weight groups 1.5-2 g, 2-2.5 g and 2.5-3 g respectively. The period of decreased egg output also occurred after the third peak in the weight groups of 2.5-3 g.

The relationship between the oviposition at 18°C and corresponding loss in weight of ticks kept at this temperature was also studied. As exemplified by the results of 2.5-3 g weight group in Fig. 5, there was a correlation ( $r = .98$ ) between the intensity of egg output and loss in weight only at the highest first peak. The other peaks in egg output did not register significant loss in weight of the ticks.

*Preovipositional weight losses:* In this experiment engorged ticks of weight group 2.5-3 g weight were kept at temperatures of 10°C, 37°C, 18°C and at the standard temperature of 24°C and their weight loss per day was recorded up to the day when oviposition started. Fifty engorged ticks were used for each temperature and Fig. 6 shows the rate of weight loss per day. The data for each day were obtained by finding the average of the total weight loss per day for all of the 50 ticks for each temperature. The ticks kept at standard temperature showed initial peaks of loss in weight on the 1st and 3rd days after detachment from the host. Very little fluctuation was observed from the 5th day up to the beginning of oviposition on the 11th day. The ticks kept at 18°C showed a peak, 3 days after detachment and exhibited fluctuating weight losses thereafter until the 10th day when there was cessation of weight loss. This phenomenon persisted until the 17th day *post* detachment (p.d.) when the weight loss in-

creased consistently until oviposition started on the 22nd day p.d. Although two peaks in weight loss on days 2 and 4 p.d. were observed among ticks kept at 37°C, the weight loss decreased to insignificant as from day 6 onwards and ceased totally as from day 9 p.d. The ticks were discarded on day 26. Weight loss among ticks kept at 10°C was irregular until day 11 p.d. when it ceased totally until the ticks were discarded on day 27.

*Effect of Rotational temperatures:* In this experiment, fed ticks of different weight categories were rotated between temperatures of 37°C and 24°C. Enyenihi (1972) had shown that temperatures up to 40°C was recorded in Maiduguri. It was therefore necessary to know how such high temperatures could affect the biology of *A. variegatum*. Since such high temperatures are not usually continuous during the 24 hours of the day, the ticks were kept at 37°C for 12 hours (0800 - 2000 hours) and at 24°C for 12 hours (2000 - 0800 hours). As shown in Table 5 not only was the interval of preoviposition significantly prolonged ( $P < 0.005$ ) for almost all the weight groups but also the length of oviposition was significantly reduced. Furthermore, the numbers of eggs produced by the weight groups were significantly lower than ( $P < 0.005$ ) those of similar weight groups kept at standard temperature.

*Effect of daily transference of ticks from various temperatures to standard temperature:* As already highlighted in this paper, the effect of keeping engorged *A. variegatum* at temperatures of 10°C, 18°C and 37°C varies from a significant decrease to complete cessation of egg production. This experiment was therefore designed to ascertain the

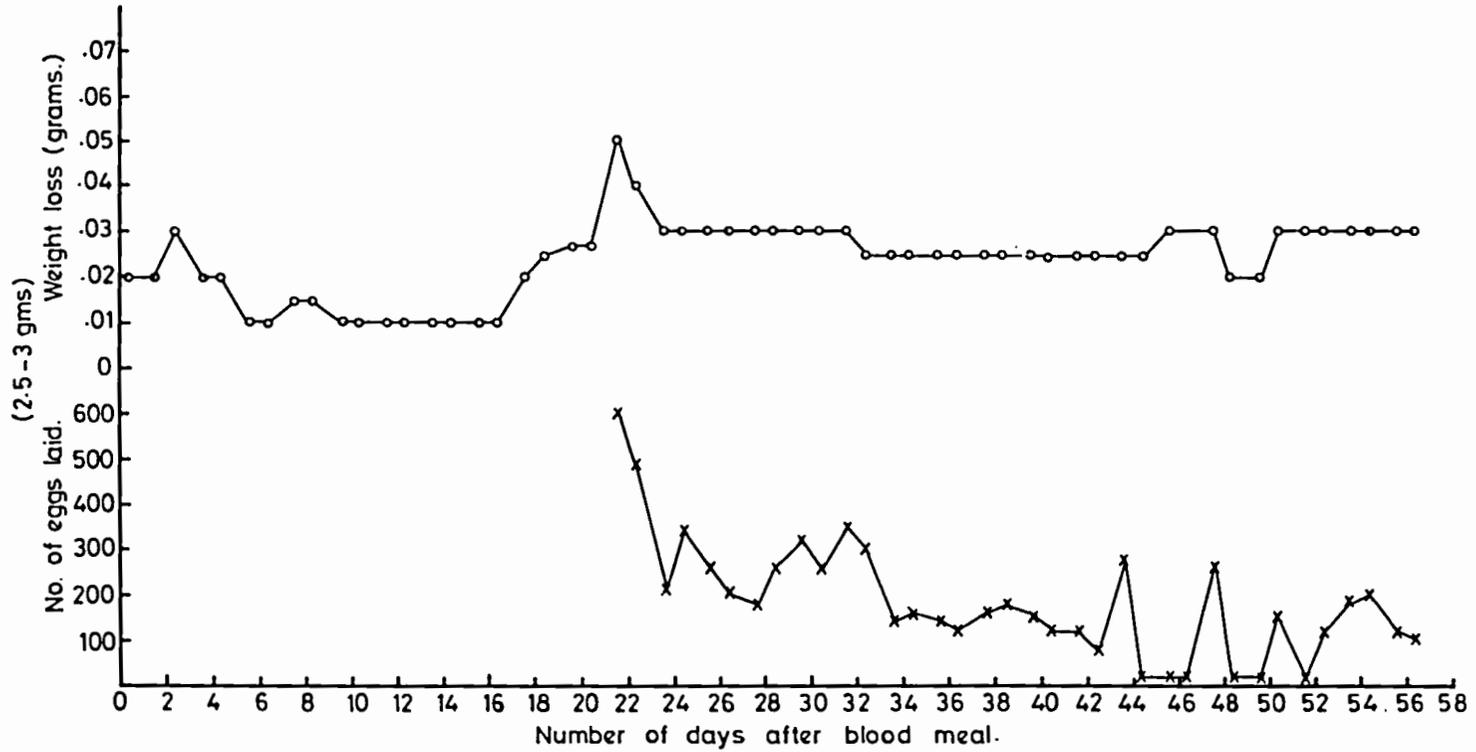


Fig. 5: Relationship between daily weight loss and daily egg output of engorged *A. variegatum* maintained at 18°C.

Table 5: The preoviposition period, length of oviposition and number of eggs laid by *Amblyomma variegatum* kept in rotational temperatures of 37°C and 24°C.

*Engorged weight in g	Preoviposition in days (means)	+Significance	Length of oviposition in days (mean)	+Significance	Percentage Oviposition	Percentage Eclosion	Mean Preeclosion period in days	No. of eggs laid	+Significance
0.2 – 0.3	—	—	—		—		—	0	
0.3 – 0.4	29	P .005	2	P .005	10	10	60	39	P .005
0.4 – 0.5	23	P .025	4	P .005	10	10	58	56	P .005
0.5 – 0.75	25	P .005	6	P .005	12.5	12.5	59	469	P .005
0.75 – 1	23	P .005	10	P .01	15	15	59.5	769	P .005
1 – 1.5	22.6	P .005	15	P .05	25	25	61	1493	P .005
1.5 – 2	21.3	P .005	15	P .025	30.5	30.5	59	2830	P .005
2 – 2.5	22	P .005	15	P .005	61	61	60	2438	P .005
2.5 – 3	22	P .005	15	P .005	70	70	58	2764	P .005
3 – 3.5	22	P .005	12	P .005	15	15	58	1241	P .005
3.5 – 4	23	P .005	3	P .005	2	2	59	270	P .005
4	24	P .005	3	P .005	0	0	—	169	P .005

\*Number of ticks examined for each weight group is 50

+Statistical data tested against control data of 24°C.

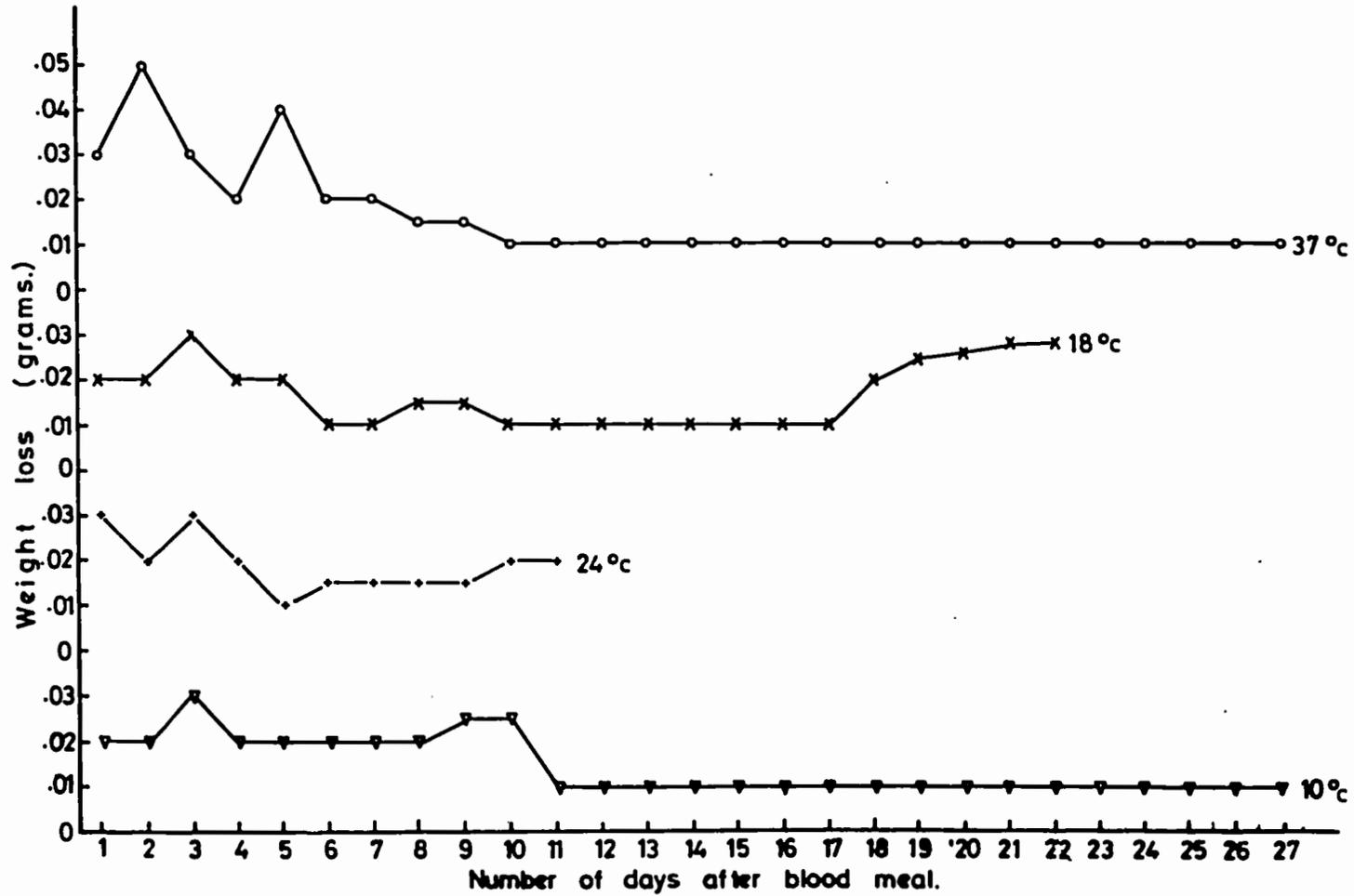


Fig. 6: Daily preovipositional weight losses of engorged *A. variegatum* maintained at 10°C, 24°C, 18°C and 37°C.

number of days the ticks can exist at these temperatures before these effects would be manifested. Ticks of 1.5-2 g weight category were kept at temperatures of 10°C, 18°C and 37°C and 10 of them were transferred daily to standard temperature where observations on their preoviposition period, length of oviposition, preeclosure period and number of eggs laid were made. The day of their detachment and preservation at the different temperature was classified as day 0. The first transference 24 hours later was classified as day 1 transference; the second transference 48 hours after day 0 is day 2 transference etc.

The results are shown in Table 6. Ticks transferred from 10°C to 24°C from day 1 to day 16 transfereces were not affected in terms of preoviposition period, length of oviposition and number of eggs produced when compared with the standard results. From the data on the column of preoviposition period after transference, it is apparent that the process for oviposition started only after transference, there was a significant decrease in the number of eggs produced and this was further reflected in the reduced preoviposition period and length of oviposition. However, the proportion of transferred ticks which produced ecloded eggs decreased consistently with increasing days of transference. Only 2% of the 10 ticks transferred on day 16 transference produced hatchable eggs while none of the eggs of ticks transferred as from day 17 and above hatched into larvae. It was also observed that oviposition ceased totally among ticks transferred from the 20th day upwards.

As for ticks transferred from 18°C to the standard temperature, the number of eggs produced, the length of preovi-

position and preeclosure periods were not affected up to 19th day transference. As from the 20th day transference, however, there was a decrease in the number of eggs produced and length of oviposition. There was also a consistent decrease in the proportion of ticks which produced hatchable eggs with increasing days of transference. Ticks transferred from 37°C began to produce decreased number of eggs as from day 11 transference. Although oviposition was recorded among ticks transferred from 37°C up to 20 day transference, the proportion of these which produced hatchable eggs decreased consistently until day 12 transference when no single tick was observed to produce hatchable eggs.

#### DISCUSSION

There is no doubt that the category of engorgement is an important factor not only in determining whether a female *A. variegatum* oviposits but also whether the eggs hatch. In this investigation, only the female ticks which engorged up to 0.4 g weight oviposited but the proportion of those in which larvae were formed varied until the state of engorgement of 1 g was reached. From this weight upwards all the ticks kept under standard procedures not only oviposited but the eggs of all of them ecloded into larvae. Since it is the eclusion of the eggs and not necessarily the oviposition that influences the abundance of any tick species on the field, a state of engorgement of 1 g must be considered as being important and critical for *A. variegatum*. When a female *A. variegatum* attains this state of engorgement, it can safely be predicted that the eggs produced

Table 6: The preoviposition period, length of oviposition and number of egg laid by *Amblyomma variegatum* transferred from 37°C, 18°C and 10°C to 24°C on various days

Days of transference	Preoviposition period in days (mean)		Mean length of oviposition (days)	mean Preecllosion period	Percentage Eclosion	mean No. of eggs laid	
	After transference	Total					
1	a	11.75	12.75	25	62	100	16001
	b	11.5	12.5	26	61	100	17291
	c	10.8	11.8	27	63	100	17368
2	a	13.25	15.25	26	62	100	17400
	b	12	14	28	59	100	17111
	c	13.25	15.25	26	58	100	16585
3	a	12.5	15.5	25	59	80	16013
	b	11.2	14.2	24	59	100	15663
	c	11.2	14.2	26	59	100	17640
4	a	11.4	15.4	25	59	80	14678
	b	11.3	15.3	25	61	100	15238
	c	11.5	15.5	25	61	100	15888
5	a	12.6	17.6	25	63	60	16011
	b	11	16	26	59	100	15688
	c	10.6	15.6	25	60	100	16222
6	a	13.8	19.8	25	60	40	15371
	b	10.4	16.4	26	60	100	16021
	c	8.5	14.5	25	60	100	16026
7	a	12.5	19.5	25	61	66	16522
	b	7.25	14.25	26	62	100	16367
	c	8.2	15.2	26	63	100	16374
8	a	10.5	18.5	25	60	70	16380
	b	7.6	15.6	26	58	100	14216
	c	8.2	16.2	25	58	100	16614
9	a	14.2	23.2	27	58	70	17360
	b	7.8	16.8	26	59	100	16228
	c	7.8	16.8	25	57	100	15677
10	a	13.6	23.6	25	58	60	17818
	b	8.4	18.4	26	59	80	15248
	c	13	23	27	62	100	14224
11	a	12.6	23.6	27	62	70	17322
	b	7.2	18.2	25	48	80	15248
	c	18	29	5	61	50	1710
12	a	15.2	27	25	63	50	15388
	b	7.7	19.7	26	57	80	154424
	c	18.8	30.7	2		0	196

Table 6: The preoviposition period, length of oviposition and number of egg laid by *Amblyomma variegatum* transferred from 37°C, 18°C and 10°C to 24°C on various days (cont.)

Days of transference	Preoviposition period in days (mean)		Mean length of oviposition (days)	mean Preecllosion period	Percentage Eclosion	mean No. of eggs laid	
	After transference	Total					
13	a	14.1	27.1	25	58	40	16556
	b	8.3	21.3	20	62	80	13807
	c	19.3	32.3	1		0	53
14	a	11.3	25.3	25	58	20	16155
	b	4.6	18.6	26	62	80	15601
	c	22	36	1		0	21
15	a	9	24	27	63	20	17211
	b	4	19	25	61	80	15703
	c	20	35	1		0	28
16	a	18	34	10	61	10	9585
	b	5.6	21.6	18	58	80	11132
	c	21	37	1		0	22
17	a	17	34	2		0	117
	b	3.4	20.4	15	59	70	8645
	c	18	35	1		0	12
18	a	20.5	38.5	1		0	25
	b	3	21	10	62	50	7721
	c	18	36	1		0	18
19	a	22.1	41.1	1		0	25
	b	4.2	23.2	5	63	20	6304
	c	11	30	1		0	25
20	a	oviposition ceases					
	b	2.2	22.2	5	56	10	5453
	c	16	36	1		0	113
21	a						
	b	1.4	22.4	3		0	2491
	c	oviposition ceases					
22	b	1	23	3		0	2196
23	b	1	24	3		0	2077
24	b	1	25	3		0	2183

\*a = 10°C.

b = 18°C

C = 37°C.

would hatch into larvae. The critical nature of this state of engorgement is further highlighted by the finding that it is from this weight upwards that the preoviposition period becomes stable. Similarly, at this weight of 1 g there was a very sharp rise in the number of eggs produced compared with the number of eggs produced by ticks below 1 g weight. Arthur (1961) observed that the male ticks were attracted to females for mating and fertilisation of the eggs until after a certain state of engorgement had been reached.

Although, as shown in this investigation, the fecundity of the individual *A. variegatum* varies widely, the frequency chart given in Fig. 1 shows that once its state of engorgement is ascertained, it is possible to predict within reasonable limits of error the number of eggs that a female can produce. Similarly, the results obtained on the rates of egg output and peaks of oviposition show that predictions could be made in the oviposition patterns of female *A. variegatum* once its state of engorgement is known.

The temperature at which the engorged *A. variegatum* is maintained plays a great role in the oviposition and eclosion. While permanent maintenance at 10°C and 37°C completely suppressed oviposition, at temperature of 18°C caused significant decrease in the number of eggs produced. The results obtained from experiments on rotational temperatures (Table 5) give an insight into what may be happening on the field. Enyenihi (1972) recorded the highest temperature of 40°C in Nigeria in Maiduguri. Since temperatures fall below 40°C, particularly during the night, the ticks are bound to be exposed, in such situations, to fluctuating temperatures which are bound to be more

variable than those used in this experiment. It is evident that exposure of female *A. variegatum* to extreme temperatures for some hours per day causes decline in egg productivity and renders a varying proportion of them incapable of oviposition. It is interesting however that the eggs of all ticks which oviposited also eclosed. This shows that there are variations within the groups of ticks of this species in their resistance to the devastating effects of extreme temperatures.

It was observed in this investigation that engorged ticks maintained at 37°C, 18°C and 10°C and later transferred to 24°C were not affected in terms of fecundity and preeclosion period when the transference took place before a definite number of days. For ticks kept at 10°C, this transference must take place within 1-15 days, for those kept at 37°C and 18°C, transference must take place within 1-10 days and 1-19 days respectively. However, it is not in all instances that eggs of ticks so transferred hatched into larvae. For the ticks transferred from 10°C, a hundred percent eclosion of the eggs took place only when transference took place within 1 and 2 days; for those kept at 18°C and 37°C only ticks transferred within 9 and 10 days respectively had all the oviposited eggs hatching into larvae. A translation of these observations to field situations indicates that each climatic condition is bound to have its own distinct effect on the biology of *A. variegatum*. Depending on the geographical zone of an area, dry season temperatures in Nigeria fluctuate between 30°C and 40°C and lasts 4-9 months. During this dry spell, only a proportion of engorged *A. variegatum* are able to oviposit. In a similar way, the cold spell of harmattan which lasts 2-4

months depending on the geographical zone of an area and during which temperatures as low as 10° C can be recorded, is bound to affect the oviposition capabilities of a proportion of the engorged ticks. From these results therefore, the wet season is the most favourable to the ticks because apart from moisture, the temperature (22° C–28° C) is optimal.

The involvement of metabolic processes in tick egg production was highlighted by the results in which weight losses were found to be directly proportional to the intensity of egg output. At 10° C, the metabolic processes were so retarded that the food digestion essential for egg laying ceased. At 37° C tick metabolism was so high within the first four days that unless the tick was transferred to more favourable temperatures, it was injured permanently. The period of decreased egg output observed during oviposition of engorged *A. variegatum* maintained at 18° C is difficult to explain. It is possible that at this temperature, the tick metabolism could not cope with the rate of egg output and the inactive phase is a resting time during

which the tick mobilises energy for the next and final phase of egg output. This explanation is strengthened by the fact that the peaks attained after this phase are never as high as any of those before. Hence, we propose the name "mobilisation phase" for this period.

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## FREQUENCY OF GROUP-SPECIFIC CHLAMYDIA ANTIBODIES IN CATTLE IN SOME AREAS IN TANZANIA

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### SUMMARY

The occurrence of group-specific complement fixing antibodies was studied in 691 cattle sera from three areas of Tanzania that differed in prevalence frequency of ticks. 352 of 691 sera were positive (50.9%). In the coastal area, where the tick population was relatively low, the antibody frequency (66.5%) was significantly higher than it was in the densely tick-populated Arusha area (32.9%) and sparsely populated Tabora and Sumbawanga (38.8%). The antibody frequency of the local cattle in the Arusha area was significantly higher than the frequency in heifers imported four months earlier from New Zealand (15.3%). The results did not support the role of ticks as a vector of chlamydial infection in Tanzanian bovine herds.

### INTRODUCTION

*Chlamydia psittaci* is known to be widespread in nature (Meyer, 1967), both in domestic animals and man. The infections are mostly subclinical, but clinical cases are also common. Carrier states seem to be frequent. Serological surveys in Europe using the complement fixation test have yielded positive findings in 10-40% of domestic animals (Schjerning-Thiesen, 1964, Ronsholt, 1977, Neuvonen and Estola, 1974, Neuvonen 1976). In Africa, *Chlamydia psittaci* has been shown to cause clinical disease in cattle

(Ehret *et al.* 1975; Schutte and Pienaar, 1977) and it also has been isolated from a wild buffalo (Rowe *et al.* 1978). In an earlier serological survey in Kenya complement fixing antibodies were found to be common; 70% of the sheep examined had titres of 1:40 or higher (Krauss *et al.* 1971). Chlamydia infections are known to spread orally and by respiration for the most part, but other routes, such as arthropods, cannot be excluded. The agent has been isolated from ticks, fleas and lice (Eddie *et al.* 1962, Eddie *et al.* 1969). A rickettsia-like agent assumed to be *Chlamydia psittaci* has been isolated from arthropods in Africa (Giroud and Jadin, 1954). In spite of isolations of *Chlamydia psittaci* from ticks, there is little information about the possible role of ticks in chlamydial epidemiology (Page *et al.* 1975).

The purpose of this study was to determine the frequency of chlamydial antibodies in bovine sera, originated from some Tanzania areas, which differ from each other with regard to their tick populations.

### MATERIALS AND METHODS

#### Bovine Sera

750 bovine sera were collected in 1976 and 1977 and stored at -20°C. The number of animals in the herds varied from a few dozen to 200. On average, a half of the adult cows in the herds investigated were randomly tested.

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The sera were originally collected for brucellosis and foot-and-mouth disease surveillance. Before testing for Chlamydial antibodies, the sera were tested for brucellosis by the routine agglutination test and only negative sera were accepted for the chlamydia investigation. The age distribution is not known.

### Herds

The herds were non-migrating and the tested animals had always belonged to the herd in question. The herds were in three various locations in Tanzania which differed in their tick population (Yeoman and Walker, 1967).

(1) In the Arusha area 240 sera from six herds were collected. In general the area is densely populated by several species of ticks. Climate is hot and drier than in the coastal area. (2) 353 sera from six herds were collected in the coastal area. Compared with the Arusha area the tick population is less dense. The climate is hot and very humid. (3) In Tabora and Sumbawanga 98 sera were collected from two herds. The

density of their population is about the same as in the coastal area. Climate is warm and humidity on the same level as in Arusha.

In addition to these Tanzanian stocks, 59 heifers imported from New Zealand and located in Arusha were bled four months after their arrival. The local herds were grazed on cultivated fields and natural pastures and were in close contact with their natural surroundings. The imported heifers were grazed carefully in a fenced, cultivated field and contact with both local cattle and game were very limited. The Tanzanian cattle were Zebu or Zebu-cross breeds and the imported heifers were Jerseys.

### Ticks

The rough frequencies of the six most common species of ticks are given in Table 1. These species are estimated to comprise about 4/5 of the total amount of ticks. The data derive from the publication by Yeoman and Walker (1967).

### Assay of antibodies

The direct complement fixation test

Table 1: Rough frequencies of the most common species of ticks

Species of tick	Arusha area	Coastal area	Tabora	Sumbawanga
<i>Boophilus decoloratus</i>	++	+	+	+
<i>Rhipicephalus appendiculatus</i>	++	+	+	++
<i>Amblyomma variegatum</i>	++	+	++	+
<i>Rhipicephalus evertsi</i>	+	+	+	+
<i>Amblyomma lepidum</i>	+	0	+	0
<i>Boophilus microplus</i>	0	+	0	+

frequency of ticks: according to Yeoman G.H. and Walker J.B. 1967.

(++) high

(+) moderate

(0) nil

was performed using a microtitre technique (Neuvonen and Estola, 1974). The sera were diluted with two fold steps, and two units of antigen and guinea pig complement were used. A titre of 1:16 or higher was considered positive. The antigen was Behringwerke ornithose antigen prepared from strain p-4.

The  $\chi^2$ - test was used for the statistical calculations.

## RESULTS

750 bovine sera were tested, of which 361 were positive (48.1%). 691 of the specimens were from native

Tanzanian cattle; 352 were positive (50.9%). Antibodies were found in 79 of the 240 samples from the Arusha area (32.9%) (Table 2), in 235 of the 353 samples from the coastal area (66.5%) (Table 3) and in 38 of the 98 samples (38.8%) (Table 4) from Tabora and Sumbawanga. Nine of the 59 heifers (15.3%) (Table 5) from New Zealand were positive. Statistically the antibody frequency in the Arusha herds was homogenic, ( $\chi^2 = 4.67$ ) and the same applies to the frequency in the herds of the coastal area ( $\chi^2 = 4.61$ ). The frequencies in Tabora and Sumbawanga were also homogenic ( $\chi^2 = 0.0089$ ).

Table 2: Prevalence of chlamydial CF antibodies in bovine sera in the Arusha area.

Herd	No. of sera	No. of posit. sera $\geq 1:16$	Titres				Percentage of posit. sera
			1:32	1:16	1:8	<1:8	
I	19	6	4	2	7	6	31.6
II	26	10	2	8	6	10	38.4
III	19	6		6	4	9	31.6
IV	56	15	3	12	16	25	26.7
V	54	13		13	14	27	24.1
VI	66	29	10	19	19	18	43.9
total	240	79	19	60	66	95	32.9
percentage of total positive			24.1	75.9			

Table 3: Percentage of chlamydial antibodies sera in the coastal area.

Herd	No. of sera	No. of posti. sera $\geq 1:16$	Titres				Percentage of posit. sera	
			1:64	:32	1:16	1:8		
I	66	34		14	20	10	22	51.6
II	69	53	4	23	26	8	8	76.8
III	49	38	4	17	17	5	6	77.6
IV	41	24		7	17	9	8	58.5
V	63	42		10	32	11	10	66.7
VI	65	44	4	16	24	54	64	67.7
total	353	235	12	87	136	54	64	66.5
percentage of total positive			5.1	37.0	57.9			

The antibody frequency of the coastal area was significantly higher than the frequency in the Arusha ( $\chi^2 = 30.56^{***}$ ), Tabora and Sumbawanga ( $\chi^2 = 9.77^{**}$ ) areas. The antibody frequencies of Arusha on the one hand, and Tabora and Sumbawanga, on the other hand, did not differ significantly ( $\chi^2 = 0.69$ ). The frequencies for local Tanzanian cattle in Arusha were significantly higher than the frequency in the heifers imported from New Zealand  $\chi^2 = 7.31^*$ . The highest antibody titres, 1:64, were detected only in the coastal area. In the other areas the highest titre was 1:32. The highest titre of the imported cattle was also 1:32.

### DISCUSSION

Regional differences in the frequency of complement fixing antibodies have been found in the human population (Wyman *et al.* 1960) and in cattle in Finland (Neuvonen and Estola, 1974). The present results show real differences also in Tanzania but they do not support the hypothesis that ticks play any significant role in chlamydial epidemiology. In the coastal area of Tanzania where the antibody frequency is highest the total number of ticks is relatively low and none of the species is as frequent as in the Arusha area. In Arusha and in Tabora and Sumbawanga, respectively the antibody frequencies are

Table 4: Percentage of chlamydial CF antibodies in bovine sera in Tabora and Sumbawanga

Herd	No. of sera	No. of post. sera $\geq 1:16$	Titres				Percentage of post. sera
			1:32	1:16	1:8	1:8	
Tabora	42	16	5	11	11	15	38.1
Sumbawanga	56	22	3	19	24	10	38.8
	98	38	8	30	35	25	38.8
percentage of total positive			21.1	78.9			

Table 5: Percentage of chlamydial CF antibodies in the sera of cattle of New Zealand origin.

Herd	No. of sera	No. of posit. sera $\geq 1:16$	Titres				Percentage of posit. sera
			1:32	1:16	1:8	<1:8	
I	59	9	1	8	6	44	15.3
Percentage of total positive			11	89			

about the same, but the total number of ticks in the latter areas is much lower than in Arusha and also the frequency of each individual species is lower or about the same as in Arusha. The significant difference between frequencies of local and exported cattle shows chlamydia in Arusha and in Tanzania in general to be widespread. The antibody frequency in the coastal area is very high. The average frequency is 66.6% and the highest, 77.6%, shows that almost every animal has been infected in the recent past. In addition to the higher frequency, the higher antibody titres support the view that infection is more frequent in the coastal area than in the other areas.

The percentages in Arusha (32.9%) and Tabora and Sumbawanga (38.8%) are also quite high and above the average frequencies in European countries. The results generally agree well with earlier studies in Kenya (Krauss *et al.* 1971) where the average percentage of CF antibodies in sheep was about 70 and show that chlamydial infections in this tropical area are very frequent.

The group specific CF test reacts with all the chlamydial agents and it is impossible to draw conclusions about the serological types and different pathogenic groups. Since there is no knowledge of any clinical cases in the country either the clinical importance of the disease or effect or different manifestations to serological reactions cannot be evaluated. Most of the serological positive reactions are caused by recent infections because CF antibodies have a short life span. This shows that the chlamydial organism is really common. The complement fixation test is widely used for chlamydiosis. The known serological cross

reactions are only few. Brucellosis and O-fever antibodies are however known to be able to react lowly with group specific chlamydial CF antigen (Meyer and Eddie, 1951, Surrey Dane, 1955). Unfortunately the sera for Q-fever and a part of the positive reactions may be caused by Q-fever infections. Their role may be of practical significance at least in the Arusha area where frequency of antibodies to Q-fever is high (Hummel, 1976). The Q-fever antibody frequency in the other areas tested is not known.

The pastoral areas of local herds are large and cattle come into close contact with the surrounding nature. The imported heifers were grazed very carefully in a small cultivated field and contact with the local cattle and game was very limited. This difference in cattle farming, together with the short time they have been in the country may be the reasons for the difference in antibody titres.

The epidemiology of Chlamydiosis in the world can vary considerably with the specific ecological situation and it is difficult to apply information from one area to another. The question of arthropods as a possible reservoir and vector in the epidemiology of chlamydia infection did not gain positive support from the present rough comparison of antibody and tick frequencies. This of course does not preclude the existence of such a mechanism as a minor pattern of maintenance and transmission.

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## PATHOLOGICAL STUDIES ON SHEEP AND GOATS PNEUMONIA IN THE SUDAN — PART I: NATURAL INFECTION.

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### SUMMARY

The pathology of different types of naturally acquired pneumonia of sheep and goats was studied. The causative agents were isolated and identified.

Three types of pneumonia, namely broncho-pneumonia, fibrinous pneumonia and chronic interstitial pneumonia were encountered. *Staphylococcus aureus*, *Streptococcus sp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Mycoplasma arginini* were isolated. No virus was isolated.

### INTRODUCTION

In the Sudan the majority of sheep and goats are reared under nomadic conditions. Respiratory diseases of sheep and goats are widely spread and responsible for considerable financial losses.

Pneumonia is one of the most damaging diseases, because a great number of sheep and goats are infected. Regardless of the low mortality rate, the animal performance is greatly affected in the form of poor growth, reduced productivity, debility and lowered resistance of lambs and kids.

In spite of the importance of sheep and goats pneumonia, in a country like the Sudan, and although the disease was reported as early as 1902, yet the studies conducted on this disease are to some extent scanty, specially in the field of the pathology of the disease. Most of the work done was directed towards the isolation and identification of the causative agents.

According to a report of the Sudan Veterinary Service (Sudan, 1958) attempts to isolate the causal organism of Contagious Caprine Pleuropneumonia had been largely unsuccessful. Karib (1958) succeeded in setting up lung lesions of Contagious Bovine Pleuropneumonia and Contagious Caprine Pleuropneumonia by spraying diluted lung fluid from infected animals. In 1960, Khan isolated Pleuropneumonia-like organisms from sheep. Nasri (1964) conducted some work to confirm that Contagious Caprin Pleuropneumonia is caused by a mycoplasma which he succeeded to isolate in 1967. Sheiba, in 1971, studied the symptomatology, macropathology and bacteriology of Contagious Caprine Pleuropneumonia. Sanousi *et al* (1971) isolated an organism resembling *Actinobacillus* from pneumonic lungs of sheep. Muna (1974) reported the isolation of some mycoplasmas from the lungs of apparently healthy and clinically diseased sheep. Nasri (1977) reported the isolation of mycoplasma other than *Mycoplasma mycoides* subsp. *mycoides* from man and animals. Harbi (1977) isolated and identified *Mycoplasma ovipneumonia* from pneumonia lungs of goats. Sanousi *et al* (1978) reported an association of *Pasteurella multocida* and *Actinobacillus lingiersii* with broncho-pneumonia in sheep.

From this short review of literature, it is evident that more research is needed especially in the field of the pathology of pneumonia in sheep and goats.

This paper (Part I) deals with the natural infection.

### MATERIALS AND METHODS

Portions of diseased lungs were aseptically collected from 140 sheep and 25 goats from provinces of Southern Darfur, Northern Darfur and Blue Nile between May 1977 and May 1978. These lungs were brought to the Central Veterinary Research Laboratory where they were subjected to pathological and microbiological studies.

Part of the affected lungs was collected in 10% formal-saline for histopathological studies.

Sections for microscopic examination were processed by the paraffin block method, cut at 5-6 microns and stained with Haematoxylin and Eosin. Portions of affected lungs intended for microbiological studies were aseptically collected and kept frozen. Culturing for bacteria was done on blood agar plates. Culturing for mycoplasma was done using mycoplasma agar, mycoplasma broth and heart infusion broth. Virus isolation was attempted using caprine kidney cell culture and embryonated chicken eggs.

The kinds of pneumonia encountered in sheep and goats under study were classified according to the principal constituents of the exudate and where known, the causative agent, as used by Runnells, Monlux and Monlux (1965).

### RESULTS

Gross lesions were equally distributed in both right and left sides of the lung affecting all lobes without any significant frequency. Nevertheless the lesions were usually encountered in the natero-ventral portions of the lobes (apical, cardiac and intermediate lobes) with the apical lobes on each side being more

extensively affected than the diaphragmatic lobe.

The gross lesions in pneumonic lungs were always nearly of the same type with some quantitative differences in extent and degree of severity depending mainly on the course of infection.

The affected lungs were voluminous, congested and oedematous. The colour of the affected area ranged from dark red, red to reddish-blue in colour. Serous, frothy and sometimes blood-stained fluid exuded from the cut surface of the affected lungs in different quantities. In some cases a frothy sometimes blood-stained fluid was present in the bronchial tree.

Histological studies revealed that sheep and goats pneumonia is mainly a bronchopneumonia (60%). Fibrinous pneumonia comprised 27% and chronic interstitial pneumonia comprised 10% of the cases.

### Bronchopneumonia

The histological appearances of bronchopneumonia were as varied as the gross appearance and depended too on the stage of inflammatory process. The different phases, especially the early ones, were seen histologically in adjacent alveoli. Bronchiolitis was the main lesion. The bronchial lumens were filled with leucocytes, mucous and desquamate cells (Fig. 1). Cellular infiltrations were present in all levels of the bronchial wall, in the peribronchial connective tissue and in the adjacent alveolar septa. The alveolar capillaries were dilated and diffused. A serous exudate, initially containing mononuclear leucocytes and a few alveolar macrophages, accumulated in the alveolar wall and the alveolar lumen. In some of the cases large numbers of neutrophils and some macrophages were seen in the alveoli. *Strepto-*

*coccus sp.* and *Staphylococcus aureus* were singly isolated.

### Fibrinous Pneumonia

In chronic cases of bronchopneumonia, where resolution was delayed, organization of the exudate resulted. Fibroblasts invaded the exudate in the

individual alveoli. In some areas, the exudate was completely replaced by fibroblasts resulting in large areas of organization (Fig. 2). In those areas there were a few alveoli devoid of exudate scattered through the newly formed connective tissue. *Proteus mirabilis* and *Pseudomonas aeruginosa* were singly isolated.

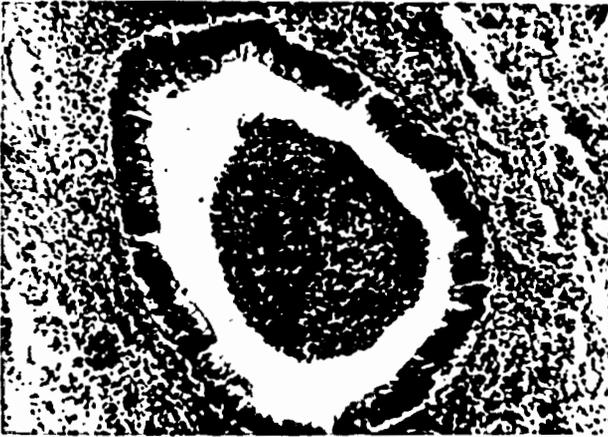


Fig. 1: Bronchopneumonia. The bronchial lumen is filled with leucocytes, mucous and desquamated cells. The peribronchial tissues is intensely infiltrated with cell masses. H. and E. Staining (X 100).

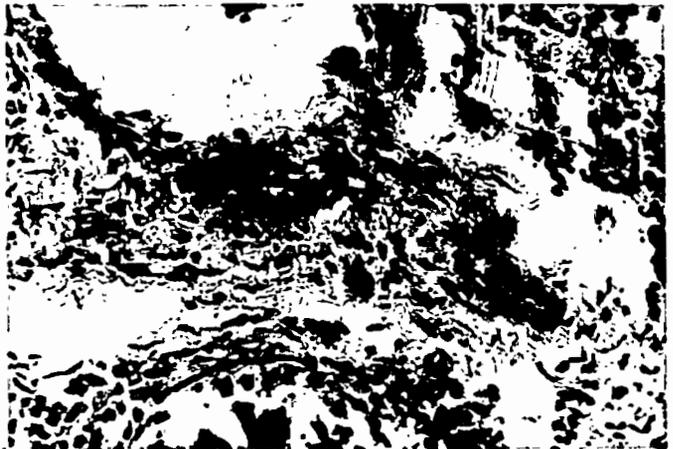


Fig. 2: Fibrinous Pneumonia. Note complete replacement of exudate by fibroblasts. H. and E. staining (X 100).

### Chronic Interstitial Pneumonia

In this type of pneumonia the alveolar walls were thickened by infiltrating neutrophils, monocytes and fibrin (Fig. 3). The alveolar lumen contained proteinaceous fluid and macrophages. *Staphylococcus aureus* and *Streptococcus sp.* were isolated.

In the lung sections prepared from animals from which *Mycoplasma arginini* was isolated, the lesions encountered were atypical for Mycoplasma pneumonia. They were very mild ones and were expressed in the form of congestion and

oedema of the lungs. *Mycoplasma arginini* was isolated from four cases associated with *Staphylococcus aureus* and *streptococcus sp.*

Diffuse alveolar emphysema accompanied by oedema and congestion was encountered in association with bronchopneumonia and interstitial pneumonia. Histologically, the alveoli and bronchioles were distended and knobs were formed at the tips of the broken alveolar septa (Fig. 4). Atelectasis was revealed as a complication of bronchopneumonia. No virus was isolated.

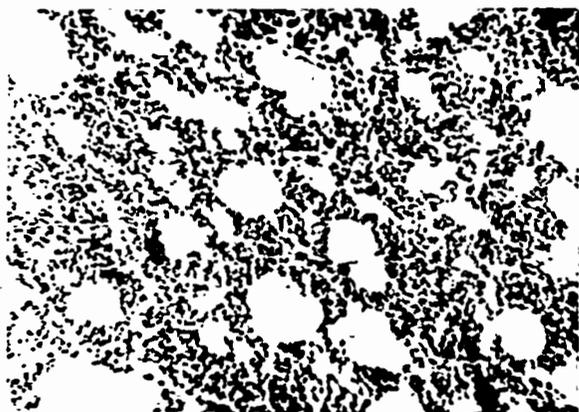
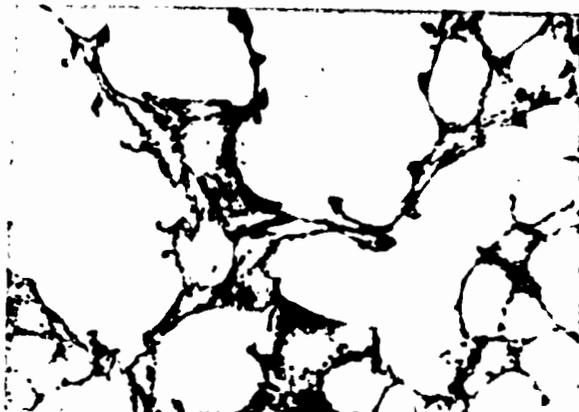


Fig. 3: Chronic Interstitial Pneumonia. Note thickening of the alveolar walls by infiltrating neutrophils, monocytes and fibrin. H. and E. staining (X 100).

Fig. 4: Diffuse Alveolar Emphysema. Note alveolar distension and knobs formation at the tips of broken alveolar septa. H. and E. staining (X 100).



## DISCUSSION

The classification used in this study is a combination of different methods for classification of pneumonia suggested by Runnells, Monlux and Monlux (1965). This method classified pneumonias according to principal constituents of the exudate, and where known, the aetiological agents. In our opinion the reason for the selective involvement of the anteroventral portions of the lungs is due to the shortness of their airways, to the shallowness of the respiratory excursion in these areas, and to the effect of gravity on exudates and oedema fluid.

The serous or fibrinous exudation into the alveolar walls denotes direct injury to the vascular endothelium.

The high incidence of pulmonary congestion and oedema in association with varying conditions reflects the lack of specificity. Apart from being a sequel to inflammation it may also be predisposing to it. This coincides with the findings of Ikede (1977).

The atelectasis encountered as a complication of bronchopneumonia follows the resolution of the parenchymal inflammation with persistence of obstructive bronchiolitis.

The four cases of pneumonia (two sheep and two goats) from which *Mycoplasma arginini* was isolated in association with *Staphylococcus aureus* and *Streptococcus sp.* did not show typical lesions of mycoplasma pneumonia.

It is generally recognised that bronchopneumonia is the commonest form of pneumonia in domestic animals (Davis, 1960; Smith and Johnes 1961, Jubb and Kennedy, 1963).

Our finding that bronchopneumonia is the commonest form of pneumonia encountered in sheep and goats (60%) confirms this general fact and coincides

with the finding of Ramachandran (1969) who reported 51.3% incidence of bronchopneumonia in sheep and 58% incidence in goats.

Our bacterial isolated from different type of pneumonia in sheep and goats confirm the findings of a number of authors. For example Ramachandran and Sharma (1969) isolated gram positive bacteria from cases of acute and subacute bronchopneumonia, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* from cases of interstitial pneumonia of sheep and goats. In 1971, Okot and Kagonyera isolated *Proteus sp.* and *Pseudomonas sp.* from cases of fibrinous pneumonia, and *Staphylococcus aureus*, *C. pyogenes* and *Streptococcus sp.* from cases of chronic interstitial pneumonia in cattle.

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## THE APPLICATION OF IMMUNODIFFUSION AND IMMUNOELECTRON MICROSCOPY IN THE DIAGNOSIS OF AFRICAN HORSE SICKNESS

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### SUMMARY

African horse sickness virus (AHSV) was isolated in suckling mice and was serologically detected by immunodiffusion which was shown to be a group specific test. The viral isolate was serotyped by serum neutralization test (SNT) as type 9. The use of immunoelectron microscopy (IEM) provided an immunological as well as a direct visual method for the characterization and diagnosis of the AHSV.

### INTRODUCTION

African horse sickness (AHS) is an acute or subacute viral disease of equines which prevails in Africa and Middle Eastern countries. The diagnosis of AHS is based on either the isolation of the virus in mice and/or cell cultures (Alexander, 1935; Mirchamsy & Taslimi, 1964; Ozawa *et al*, 1965) or demonstration of precipitating viral antigens in infected cell cultures or infected mouse brains (Hazrati *et al*, 1968; Hazrati & Mirchamsy, 1974), or retrospectively, by demonstration of antibodies against the virus in sera of previously infected animals by serum neutralization test (SNT) or complement fixation test (CFT). CFT is used as a group specific test whereas SNT is used to serotype AHSV isolates (McIntosh, 1956, 1958).

CFT, however, can only detect antibodies in sera of animals that had been recently infected with or vaccinated against AHSV (Parker, 1974; Parker *et al*, 1977). This report describes the detection of AHSV by immunodiffusion as a group specific test and its characterization by SNT and IEM.

### MATERIAL AND METHODS

#### The Virus

The original infective material was obtained from a spleen of a naturally infected horse showing symptoms of acute pulmonary form of AHS. This virus strain was designated Khartoum-2 AHSV isolate (AHSV-K2).

#### Isolation of the virus in suckling mice brains

The virus was isolated in suckling mice brains using essentially the same procedure as described by Alexander, 1935. The initial mouse inoculum was 0.04 ml of a 10% spleen suspension in phosphate buffered saline (PBS) from the naturally infected horse. 4-5 days old mice were inoculated intracerebrally (I/C). Five subsequent 1/C passages were carried out. On each passage the brains were harvested from mice in *extremis* on the fourth or fifth day post-inoculation (p.i.). A 10% brain suspension was prepared in PBS containing 100 I.U. of penicillin and 200 mg of streptomycin per ml. The brain sus-

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pensions were differentially centrifuged first at 3000xg for 15 minutes and secondly at 8000xg for 30 minutes. The second supernatant was tested for bacterial contamination by inoculation into thioglycolate medium. A group of 4, 4–5 days old suckling mice were inoculated with 0.04 ml each and were observed for disease signs. The virus titre was calculated according to the method of Reed and Muench, 1938.

An amount of 5 ml inoculum from the third mouse passage was intravenously inoculated into a one month old susceptible foal.

#### Antisera

Rabbit hyperimmune sera prepared against each of the 9 known serotypes of AHSV were kindly supplied by Dr. M. Hassamy of the Razi Institute (Iran).

Antisera against AHSV–K2 isolate were prepared by intramuscular inoculation of two rabbits three times at 2 weekly intervals. Each rabbit was inoculated with 0.5 ml from a 10% infected mouse brain on each occasion. Blood for serum was aspirated from the heart three weeks after the last dose.

#### Immunodiffusion

The micromethod of the agar gel precipitin test (Crowle, 1973) was employed for detection of viral antigens in infected mouse brain. Antigens were prepared by making a 20% infected mouse brain homogenates in Veronal buffer, pH 8.6. The brain homogenates were disrupted ultrasonically at a relative output of 100 watts for 30 seconds, and clarified by centrifugation at 3000xg for 15 minutes. Similar normal mouse brain suspensions were prepared and tested against antitype 3,7 and 9 hyperimmune sera. Type 3,7 and 9 rabbit hyperimmune sera were selectively

tested because their corresponding virus types were reportedly isolated in the Sudan (Eisa, 1974)

#### The serum neutralization test

Serial 2-fold dilutions of each of the 9 AHSV hyperimmune sera and anti–K2 serum were mixed with equal volumes of infected mouse brain inocula containing 100 MID 50% (mouse infective dose 50%). The serum virus mixtures were incubated at room temperature for one hour. Each mixture was then inoculated i.c. into four 4–5 days old suckling mice, so that each mouse received 0.04 ml. The inoculated mice were observed for 2 weeks. Parallel control titrations of the virus were carried out. The serum neutralization titre was expressed as the reciprocal of the highest serum dilution that produced complete protection.

#### Immunoelectron microscopy

Two per cent infected mouse brain homogenates were prepared in PBS and centrifuged at 3000xg for 15 minutes to sediment coarse particles. The supernatant and antitype 9 hyperimmune serum were separately centrifuged at 34800xg for 30 minutes in a Beckman L3–50 ultracentrifuge using type 40 fixed angle rotor to obtain a second supernatant from infected brain preparations. 1:10 and 1:20 dilutions were made from the serum supernatant in PBS, pH 7.4. An amount of 0.2 ml of each serum dilution was mixed with 0.8 ml of the second supernatant from infected brain preparations. After incubation for 60 minutes at room temperature the mixtures were centrifuged at 34800xg for 30 minutes and the resulting supernatant was discarded, and the pellet was reconstituted in 3 drops of distilled water. Drops from the recons-

tituted suspension were put onto 300 mesh formvar coated carbon stabilized copper grids and stained with 1.5% phosphotungstic acid (Hajer and Storz, 1978). The grids were examined with a Universal Electron Microscope Model E-100B operated at 75KV.

**RESULTS**

**Isolation of AHSV-K2**

The incubation period in suckling mice was 6-7 days in the first and second passages. The mortality rate was 100% in the last three passages and the incubation period was fixed at 4-5 days. The symptoms were indicative of nervous involvement and were classical (Alexander, 1935).

The inoculated foal reacted with elevated temperature of up to 39°C and respiratory distress at 25 days p.i. . It died five days later.

**Antigens detected by agar gel precipitin test**

Three very distinct precipitin lines were obtained from type 9 antiserum, whereas only one precipitin line could be discerned from both types 7 and 3 antisera. The type 9 precipitin line close to the antigen well merged with the line obtained from type 7 antiserum giving a line of complete identity on one side, whereas it formed a spur with the type 3 precipitin line giving a line of partial identity on the other side (Fig. 1--a and Fig. 1--b). No precipitin lines were obtained when normal mouse brains were reacted against type 3, 7 or 9 hyperimmune serum.

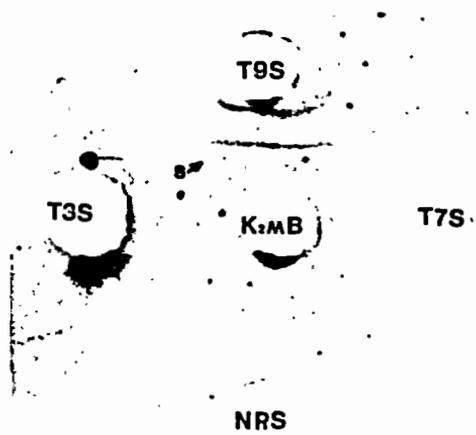


Fig. 1a: AHSV antigens detected by immunodiffusion  
 Central well (K2MB) = AHSV-K2 infected mouse brain.  
 Top well (T9S) = Type 9 hyperimmune serum.  
 Right well (T7S) = Type 7 hyperimmune serum.  
 Left well (T3S) = Type 3 hyperimmune serum.  
 Bottom well (NRS) = Normal Rabbit serum  
 S = Spur.

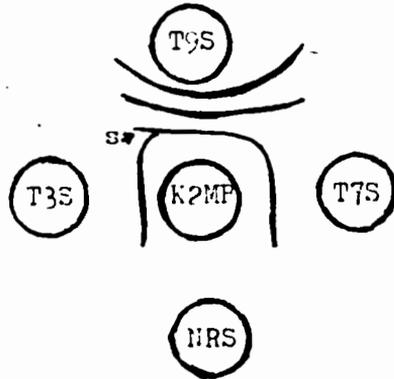


Fig. 1b: Diagrammatic representation of Fig. 1a.

Table 1: Neutralization titres of sera prepared against the 9 AHSV types and AHSV-K2 isolate.

Serum Type	1	2	3	4	5	6	7	8	9	AHSV-K2
Titre	64	64	128	64	64	64	256	128	512	1024

The titre was expressed as the reciprocal of the serum dilution that produced complete protection.

### Serotyping of AHSV-K2 strain

These results are summarized in Table I. Both the serum prepared against AHSV-K2 isolate and type 9 hyperimmune serum effectively neutralized the AHSV-K2 isolate in the cross protection test. Type 7 hyperimmune serum had a relatively high protection titre.

### Characterization by Immunoelectron microscopy

A few, usually 2 or 3, viral particles aggregated by antibody were visualized. The viral particles had an average diameter of 60 nm. Although antibody molecules masked the particle surface ultrastructure, they appeared to have a cubic symmetry and many capsomeres could be discerned (Fig. 2).

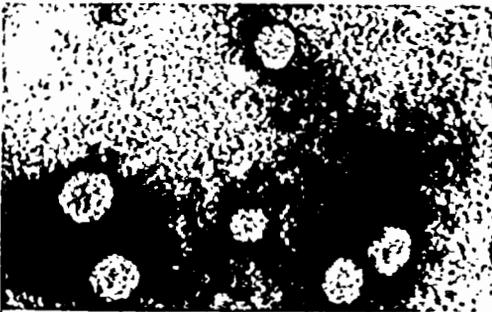


Fig.2: AHS virions aggregated by antibodies obtained by incubating 2% infected mouse brain preparations with 1:20 dilution of type 9 hyperimmune serum. The particles seem to have cubic symmetry. Even though the virion ultrastructure is somewhat obscured by antibodies viral capsomeres could be discerned. Stained with 1.5% PTA. Magnification X 160000.

### DISCUSSION

Neurotropic fixation by repeated I/C passage in mice is the most useful method of AHSV isolation.

The antigenic plurality of AHSV has been established (McIntosh, 1958) and cross protection or serum-virus neutralization test has been used for identification and serotyping of AHSV (McIntosh, 1956; Parker *et al*, 1977). The CFT is a group specific test that detects and quantitates factors common to all AHSV serotypes; but it can only detect antibodies in sera of recently infected or vaccinated animals (Parker, 1974; Parker *et al*, 1977). Immunodiffusion has been employed as a group specific test in the detection of homologous antibodies in sera prepared against different AHSV serotypes (Hazrati and Mirchamsy, 1974). The group specificity of the test is confirmed in this study by the line of complete identity between type 9 and type 7 antisera on the one hand, and the line of partial identity between type 9 and type 3 antisera on the other. The fact that no precipitin lines were obtained when normal mouse brains were reacted with types 3,7 and 9 hyperimmune sera indicate that the antigens detected in infected mouse brains were viral antigens.

The results of cross protection test indicated that AHSV-K2 isolate was antigenically similar to type 9. This result was not an unexpected one since

type 9 has been isolated from the same area (Eisa, 1974).

The use of immunoelectron microscopy provided an immunological as well as a direct visual method of AHSV characterization. The viral particle size reasonably corresponded with earlier measurements (Oellermann *et al*, 1970). The slight difference in size may be attributed to the antibody coat of the viral particles treated with hyperimmune serum. Although the antibodies partially obliterated the fine structure of the viral particles, the cubic symmetry of the virions is obvious and many capsomeres could be discerned.

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## ESCHERICHIA COLI AND DIARRHOEA IN KIDS, LAMBS AND PIGLETS

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### SUMMARY

In an investigation conducted in Oyo State of Nigeria on the aetiological agents of diarrhoea in kids, lambs and piglets, *E. coli* was isolated from all the animals investigated. In the kids, Ok groups 26/B6 (20 isolates, 114 (4 isolates), 128/B12 (1 isolates), in lambs OK groups 126 (10 isolates), 124 (2 isolates) while in the piglets OK groups 149 (4 isolates), 139 (8 isolates), 141 (1 isolate), G7 (6 isolates), 8 and 45; (one isolate each). Three cultures from *E. coli* strains isolated from kids dilated the ligated intestinal segment of rabbit while 2 cultures from *E. coli* strains isolated from lambs dilated the ligated intestinal segment of rabbits when overnight *E. coli* broth cultures in Trypticase soy broth were used, whereas 31 *E. coli* strains consisting of 19, 8 and 4 *E. coli* strains from kids, piglets and lambs respectively were positive when prepared heat labile enterotoxins were used. The *E. coli* strains isolated from lambs and piglets were highly resistant to sulphadimidine, streptomycin and Oxytetracycline in decreasing order. Eleven resistant *E. coli* strains, 3 from kids and 6 from piglets transferred their OT and S resistances to *E. coli* K<sub>12</sub>. Two *E. coli* isolates each from lambs and piglets and 15 isolates from kids were colicinogenic.

### INTRODUCTION

Published reports from workers (Moon, 1965, 1969; Moon and Whip 1970, Sojka 1965, 1970, 1971, Smith H. Williams and Jones 1963; Terlecki and

Sojka 1965) on *Escherichia coli* infections in farm animals have shown that *Escherichia coli*, a normal inhabitant of the intestinal tract can be associated with many pathological conditions in man and animals. For sometime this organism has been considered a potential aetiological agent of diarrhoea of infants, lambs, calves and piglets.

For the past two years it has been observed that neonatal diarrhoea affecting several kids, piglets and lambs occurred in many government farms and farm institutes in Oyo State. This condition led to several deaths and great economic losses in the state. This investigation was undertaken to establish the aetiological agents responsible for diarrhoea in kids, piglets and lambs in the state.

### MATERIALS AND METHODS

Faecal swabs were obtained aseptically from 46, 1–60–days old kids; 33, 3–weeks old piglets, 14, 2–weeks old lambs with diarrhoea. The faecal swabs were inoculated into selenite F broth, and onto MacConkey agar and sheep blood agar plates. The plates were incubated aerobically and anaerobically at 37° C for 24 h. After 24 h of incubation the results were recorded, all the lactose fermenting colonies on MacConkey agar and haemolytic colonies on the sheep blood agar plates were subcultured onto MacConkey agar and plated to obtain discrete colonies. They were biochemically studied for *E. coli* identification. All the lactose fermenting colonies which produced acid and gas from

glucose and which were indol positive were further characterized.

The selenite F broth medium culture was incubated aerobically for 48 hours and later subcultured onto MacConkey agar and DCA for *Salmonella* spp. and other non lactose fermenting organisms. Faecal samples from every animal covered in this investigation were microscopically studied for worm eggs.

### Serology

Rapid slide agglutination test was carried out on each strain according to the method described by Sojka Erskine and Lloyd (1957), using Polyvalent\* 2,3 and 4 and Monospecific\* antisera, 08, 024, 026, 044, 055, 086, 0111, 0112, 0119, 0124, 0125, 0126, 0127, 0128 and 0142.

### Antibiotic Sensitivity

Each *Escherichia coli* strain was tested for antibiotic sensitivity according to the method described by Walton (1972), using Multidisc\*\* code 3857 E containing oxytetracycline (OT30), chloramphenicol (C30), Ampicillin (PN25), Neomycin (Ne30), Nalidixic acid (Na50), Furazolidone (FR10), Streptomycin (S10) and Triple sulpham (S3300). The Minimum Inhibition Concentration (MIC) of 6 antibiotics (Table 1) were determined for *E. coli* isolates, from kids, lambs and piglets which showed resistance to one or more than 3 of the antibiotics shown above. The minimum inhibition concentration (MIC) was determined using the method described by Adetosoye, Willinger and Awad (1977). Plasmid transfer experiments were performed according to Walton (1966).

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\*\* Oxoid Ltd., London.

### Colicinogenicity of the *E. coli* strains.

Each of the strains was tested for colicine production according to the method described by Tracka and Willinger (1973).

### Enteropathogenicity

In order to know whether the *E. coli* strains were enteropathogenic two methods were used.

(a) For the first set of experiments 20 rabbits weighing 2 kg each were used. The rabbits were deprived of feed for 48 h prior to gut ligation test. Each haemolytic *E. coli* strain and *Vibrio cholerae* biotype *cholerae* serotype INABA were grown in 10 ml Trypticase soy broth (TSB)\*\* at 37° C overnight.

### The test

The gut ligation test was performed according to the method described by Moon, Sorensen and Saunter (1960). At a point 10 cm from the pylorus a ligation was made.

A 5 cm loop was inoculated with 0.5 ml culture of Trypticase soy broth. This served as a negative control. The second loop which was 2 cm long, served as an empty control. The third loop was 5 cm long and 0.5 ml culture of *Vibrio cholerae* biotype *cholerae* serotype INABA was inoculated, this served as a positive control. The next loop was 2 cm long and it again served as an empty control. Five loops were made in each rabbit. This procedure ensured that all the 62 haemolytic *E. coli* cultures were used in the 20 rabbits. The ligated intestinal loop was put back into the abdominal cavity and the incision was closed. The rabbits were kept in a warm place overnight. They were killed 19h post inoculation and the intestines were carefully dissected out of

Table 1

Chemotherapeutic agent	Source	Code
Ampicillin	Mast Laboratories Ltd.,	53-70-23
Neomycin	"	"
Chloramphenicol	"	"
Oxytetracycline	Mast Laboratories Ltd.,	53-70-23
Sulphadimidine	"	"
Streptomycin	Mast Laboratories Ltd.,	53-70-23

Table 2: Percentage resistance of *E. coli* strains to individual chemotherapeutic agents

Chemotherapeutic agent	animal isolates	No. of resistant	No. of resistant strains	% resistance	MIC
Ampicillin (PN <sub>25</sub> )	Piglets	33	9	27.2	250mcg/ml
	Lamb	14	14	100	250mcg/ml
	Kids	46	0	0	4mcg/ml
Chloramphenicol (C <sub>30</sub> )	Piglets	33	2	6	32mcg/ml
	Lamb	14	0	0	4mcg/ml
	Kids	46	0	0	4mcg/ml
Furazolidone (fr <sub>10</sub> )	Piglets	33	0	0	NOT DONE
	Lamb	14	0	0	" "
	Kids	46	0	0	" "
Nalidixic acid (Na <sub>50</sub> )	Piglets	33	1	3	" "
	Lamb	14	0	0	" "
	Kids	46	0	0	" "
Neomycin (Ne <sub>30</sub> )	Piglets	33	3	0	32mcg/ml
	Lamb	14	0	0	4 " "
	Kids	46	0	0	4 " "
Oxytetracycline (OT <sub>30</sub> )	Piglets	33	25	75.7	500mcg/ml
	Lamb	14	15	100	500mcg/ml
	Kids	46	21	45.6	500mcg/ml
Streptomycin (S <sub>10</sub> )	Piglets	33	30	91.0	500mcg/ml
	Lamb	14	14	100	500mcg/ml
	Kids	46	9	19.5	500mcg/ml
Triple sulpha (S <sub>300</sub> )	Piglets	33	33	100	NOT DONE
	Lamb	14	14	100	" "
	Kids	46	11	28.0	" "
Sulphadimidine	Piglets	30	33	100	500mcg/ml
	Lamb	14	14	100	500mcg/ml
	Kids	46	11	28.0	500mcg/ml

the abdomen. The lengths and fluid contents of each ligated loop of rabbit intestine were measured.

(b) For the second sets of experiments heat labile (LT) enterotoxins were prepared by centrifuging *E. coli* cultures grown in Donta medium (Holmgren, personal communication) at 1400 revolutions per minute in an MSE centrifuge (Gallempkamp London Ltd.). The Donta medium was incubated at 37°C in a water bath for 20 h. The filtrate obtained from every culture was used for determining the enteropathogenicity ability of the heat labile enterotoxin obtained from each *E. coli* strain in rabbit ileal loops.

Ten to 12 weeks old New Zealand rabbits were used for the experiment. The ligation test was performed according to the method described by Moon *et al* (1966). The procedure was as described for the first set of experiments, except that the caecum was used as landmark. Ten centimetres away from the caecum a ligature was put. One millilitre of sterile nutrient broth which served as negative control was injected into the first 5 cm loop. The next 2 cm loop was empty and served as empty control. The rest 5 cm loops were respectively injected with 1 ml of a particular prepared heat labile enterotoxin. Six rabbits were used for these tests. The ligated intestinal loops were put back into the abdominal cavity and the

incision was closed. The rabbits were then treated as described above in the first experiment.

## RESULTS

Haemolytic *E. coli* was isolated from all the faecal samples obtained from all the animals covered in this investigation. No *Salmonella* spp or other enteric bacteria were isolated. The microscopic examinations of the faecal samples were negative for worm eggs. The *E. coli* isolated from the kids belonged to OK group 26/B6, (20 isolates), 144 (4 isolates), 128/B12 (1 isolate). The *E. coli* isolated from lambs belonged to OK group 126, (10 isolates) and 124, (2 isolates), while those strains isolated from piglets belonged to OK groups 149 (4 isolates), 139 (8 isolates), 141 (11 isolates) G7 (6 isolates), 45 (1 isolate) and 8 (1 isolate).

The antibiotic resistance pattern of the *E. coli* strains isolated from piglets, lambs and kids is summarized in Table 2. Colicine production was shown by clear zones around the bacterial colonies (Fig. 1). Two *E. coli* strains each from piglets and lambs, 15 *E. coli* strains from kids were colicinogenic at 37°C. Cultures from 3 *E. coli* strains from kids and 2 *E. coli* strains from lambs dilated the ligated segment of intestine of rabbits when broth cultures grown overnight in trypticase soy broth were injected into the ligated intestinal loop.

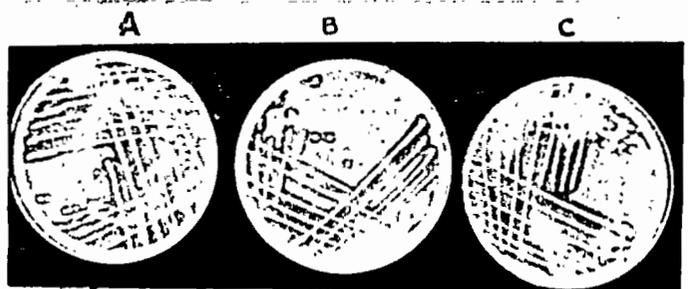


Fig. 1: Shows colicinogenic *Escherichia coli* from (A) Kid (B) Lamb (C) Piglets.

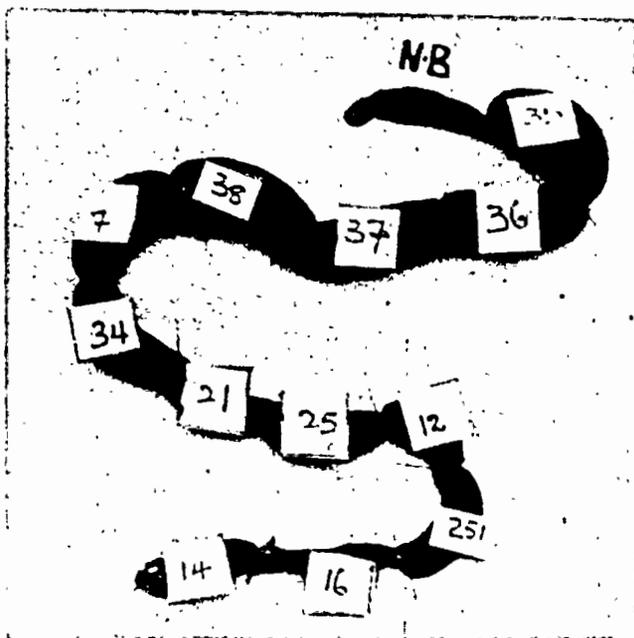


Fig. 2: Shows response of heat labile enterotoxin of *E. coli* from kids tested in Ligated ileal loop of rabbit intestine. Heat labile enterotoxin from *E. coli* strain Nos. 35, 36, 37, 38, 7, 34, 21, 25, 12, 251 and 14 dilated the ligated ileal loop with accumulation of fluid while Heat labile enterotoxin from *E. coli* strain No. 16 did not dilate the Ligated ileal loop.

On the other hand when heat labile enterotoxins prepared by growing *E. coli* in Donta medium, and centrifuged at 14000 revolutions per minute in MSE centrifuge were respectively injected into ligated ileal loops of rabbits, 19, 8 and 4 *E. coli* from kids, piglets and lambs respectively produced heat labile enterotoxins and dilated the ligated ileal loop of rabbit intestine with accumulation of fluid. (Fig. 2). Five *E. coli* strains from kids transferred their oxtetracycline (OT) and streptomycin (s) resistances to sensitive *E. coli* K12. Six *E. coli* strains from piglets transferred their (OT) and (S) resistances to sensitive *E. coli* K12 while none of the resistant *E. coli* strains isolated from lambs transferred their resistances to sensitive *E. coli* K12. None of the two *E. coli* isolates from piglets which were resistant to chloramphenicol transferred the chloramphenicol resistance to *E. coli* K12.

#### DISCUSSION

Many reports have been published in which *E. coli* was claimed as potential agent of diarrhoea in lambs, calves and piglets (Sojka 1965, 1970, 1971). Besides *E. coli*, *Salmonella* spp have been found to cause diarrhoea in animals. Falade (1976) isolated *Salmonella poona* from 7 kids with diarrhoea in Ibadan while Ojo (1972) reported cases of diarrhoea caused by *Salmonella* sp in domestic animals in Ibadan. From the results of this investigation it was seen that *E. coli* was involved in diarrhoea in the animals covered in this investigation. For *E. coli* to be enteropathogenic it must be able to proliferate in the small intestine, it must be capable of producing enterotoxin which in this situation and in the presence of intact epithelium causes a massive outpouring of fluid into the gut lumen which results into diarrhoea (Sojka 1971). When heat

labile enterotoxin prepared by centrifuging in MSE centrifuge at 14000 rpm. Cultures of *E. coli* grown in Donta medium for 20 h. was inoculated into ligated ileal loop of rabbit intestines it was seen that a total of 31 *E. coli* cultures out of 62 dilated the ligated ileal loops of rabbit intestines with accumulation of fluid.

This finding agreed with the finding of Landwall and Mollby (1977) who determined heat labile enterotoxin activity of *E. coli* 0149 K. 88ac a pig strain and *E. coli* 0126 H12 a human strain by injecting ligated jejunal loop of rabbit with prepared heat labile enterotoxins.

When the broth culture of *E. coli* grown overnight in trypticase soy broth was injected into the ligated intestinal loop of rabbit only 3 *E. coli* strains from kids and 2 *E. coli* strains from lambs dilated segments of rabbit intestine. This poor result agreed with those of Smith and Halls (1967), Smith and Jones (1963) who obtained poor results when they injected overnight broth cultures of *E. coli* from diarrhoeic animals into rabbit ligated intestinal loop. They concluded that rabbit intestinal loop was not satisfactory for demonstrating enteropathogenicity of strains of *E. coli* isolated from animals with diarrhoea. From this investigation it became apparent that overnight broth cultures of *E. coli* strains isolated from diarrhoeic animals should not be tested in rabbit intestinal loop to determine the enteropathogenicity of the *E. coli* strains. The prepared heat labile enterotoxin of the *E. coli* strains which gave good results should be used in rabbit ileal loop to demonstrate accumulation of fluid. That the heat labile enterotoxins prepared in Donta medium produced accumulation of fluid supports

the findings of Soderlind *et al.* (1974) Evans, Evans and Pierce (1973), Landwall and Mollby (1977) *E. coli* OK groups 149, 141, 139 G7, 8, 45 were isolated from piglets in this investigation. Other workers Sojka (1971) and Adetsoye (1975) isolated the same OK groups from piglets with diarrhoea in Britain and Austria respectively. That these OK groups are common in Europe and in Africa is an indication that there is movement of pigs from Europe to Africa.

Antibiotic resistant bacterial populations can arise in farm animals by the use of antimicrobial agents whether for therapy, prophylaxis or as growth-promoting agents. The frequent use of these valuable chemotherapeutic agents either at recommended or subtherapeutic levels may encourage selective development of antimicrobial resistance in bacterial commonly found in the gut flora as well as in pathogenic bacteria in animals. The result of antibiotic resistance obtained in this investigation showed the relationship of the patterns of antibiotic resistance to the particular drug. In Oyo State sulphadimidine is used prophylactically to control coccidiosis infection; oxytetracycline is used as growth promoter (Ojo and Ahanihu (1974) and as a chemotherapeutic agent on the field while streptomycin is also used routinely by veterinarians to treat clinical infections in animals on the farms. Walter and Heilmeger (1969) gave the Minimum Inhibition Concentration (MIC) of oxytetracycline, chloramphenicol, neomycin, ampicillin, streptomycin and sulphadimidine as 20 mcg/ml, 16 mcg/ml, 16 mcg/ml, 10 mcg/ml, 10 mcg/ml and 8 mcg/ml respectively. Using these criteria it was seen that all *E. coli* isolates which were described as resistant according to the

standard disc test were found to have high MIC values of 250 mcg/ml to ampicillin for all *E. coli* isolates from lambs and piglets, 500 mcg/ml to sulphadimidine, streptomycin and oxytetracycline for *E. coli* strains isolated from piglets was 32 mcg/ml whereas 30 *E. coli* strains as well as those isolated from kids and lambs were sensitive to neomycin. Hence it was seen that *E. coli* strains isolated from lambs had 100% resistance to oxytetracycline, dihydrostreptomycin and sulphadimidine. Those *E. coli* strains isolated from piglets had high resistance to oxytetracycline (75.7%), streptomycin (90.9) and sulphadimidine (100%). These agents are not routinely used on the farm where goats are reared. It is suggested that an antimicrobial agent like furazolidone which has been found to be superior to other agents for livestock feed because of its tolerance by livestock and the fact that resistance to it is slow, and when it occurs, it is not infectious, (Ojo and Adetosoye 1977), Walton (1972b) should be used as feed additive. Of the resistant strains, 5 from kids and 6 from piglets transferred their oxytetracycline and streptomycin resistance to sensitive *E. coli* K12. There was no chloramphenicol resistance transfer to sensitive K<sub>12</sub>. Since chloramphenicol resistance was not transferable, it indicated little public health problem. Chloramphenicol is the drug of choice for human typhoid. It is thought that oxytetracycline, triple sulphur and streptomycin resistance will decline once these drugs can be withdrawn and replaced by furazolidone to which *E. coli* from animals do not develop resistance readily (Ojo and Ahanihu 1974).

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## STAPHYLOCOCCUS AUREUS OF HUMAN ORIGIN IN UDDER INFECTION OF COW

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### SUMMARY

Forty-six isolates of *Staphylococcus aureus* were recovered from clinical and subclinical udder infections in 28 Holstein cows. *In vitro* sensitivity tests showed 26 of the isolates to be multiple-resistant to either two, three or four antibiotics including penicillin, ampicillin, streptomycin and tetracycline. On phage-typing, 42 isolates from the cows and 2 from the dairy attendants were found to be typable with W.H.O. set of staphylococcal phages for typing human strains. Of these, 14 isolates from cows and the 2 from the dairy attendants belonged to lytic group I including phage type 80/81 complex suggesting the possible source of infection to be humans.

### INTRODUCTION

The University of Nigeria maintains a small farm of Holstein cows for teaching animal science and veterinary courses. Some 25 — 30 animals usually remain in lactation at a time. During the last one year the farm experienced several sporadic cases of mastitis which led to isolation of multiple drug resistant strains of *Staphylococcus aureus*. An investigation was, therefore, mounted in an attempt to trace the possible source of infection. This paper deals with the results of isolation, phage typing and antibiogram of the *Staph. aureus* strains isolated from the cows and their attendants.

### MATERIAL AND METHODS

Udder milk samples were collected separately from each quarter of 28 animals before the morning milking. Materials from dairy attendants included swabs from nasopharynx and washings from finger tips collected before the morning milking. Materials from dairy attendants included swabs from nasopharynx and washings from finger tips collected before they milked the cows. Standard procedures were employed for collection of the materials, isolation and identification of the organisms (Schalm *et al*, 1971, Cowan and Steel, 1977 and Lenette *et al*, 1974). The milk samples were examined for *Staph. aureus*, corynebacteria and haemolytic streptococci, while the samples from attendants were screened only for *Staph. aureus*. The prime determinant in identifying a strain of staphylococci as *Staph. aureus* was its coagulase activity. Further confirmation was achieved by employing tests for anaerobic utilisation of glucose and mannitol. Phage typing of the strains was carried out with the W.H.O. set of 23 phages for human strains at RTD and X100 RTD. Antibiogram of the strains was determined by disk diffusion method, employing single disks (BBL) of penicillin, ampicillin, tetracycline, erythromycin and streptomycin.

### RESULTS

Sixty-two quarter milk samples were collected from 28 cows. Three randomly selected cows were resampled after six

weeks. Of these, nine cows had one or two fibrosed quarters and four were under active infection showing blood tinged and clotted milk. Eight dairy attendants were screened for *Staph. aureus*.

Forty-six isolates of *Staph. aureus* were recovered from the 62 milk samples showing that 74.0% of the quarters were infected. The three resampled cows were found to be sustaining the *Staph. aureus* infection. Neither corynebacteria nor haemolytic streptococci could be isolated from any of the samples.

Of the eight attendants three were carrying *Staph. aureus* in their nasopharynx and one on his finger tips. The *Staph. aureus* recovered from the nasopharynx of the attendants showed phage pattern identical with some of the animal strains belonging to lytic group I.

On phage typing, 42 isolates from cows and 2 from the attendants were found to be typable. Of the strains from cows, 14 belonged to lytic group I, 6 to group II, 4 to group III and the remaining 18 showed mixed phage reaction. Several isolates belonged to phage types 80/81 complex.

Six of the strains were found to be resistant to penicillin alone while other 26 strains showed multiple resistance either to two, three or four antibiotics but none was found refractory to erythromycin. The majority of the multiple resistant strains belonged to lytic group I.

## DISCUSSION

Staphylococcosis among animals and humans, under certain circumstances, is precipitated due to cross infections (Moeller *et al*, 1963; Schawbe, 1969 and Mohan and Pal, 1973). But staphy-

lococcal mastitis of cows is more commonly caused by bovine strains usually identifiable by employing Davidson's set of phages (Rep. of International Subcommittee, 1970). Nevertheless, workers have employed WHO set of staphylococcal phages to type strains of *Staph. aureus* from cows (Coles & Eisenstark, 1959; and Holmberg, 1975).

The results of this investigation are significant not only because of a high percentage of typability (90.0%) with W.H.O. set of phages but also because of predominance of strains belonging to lytic group I, including phage types 80/81 which have been associated with epidemic *Staph. aureus* strains of human origin. Consequently, it is possible that the infection was contracted by the cows sampled, as a result of cross infection from humans. Isolation of multiple drug resistant strains, several of which were resistant to ampicillin and streptomycin further confirms the human origin of the strains as these two drugs had not been used for the treatment in the cows. Although identical phage types were isolated from the cows and their attendants, the result does not, however, confirm the direction of transmission which might have been either way.

Of the three cows which were re-sampled, two continued to excrete the same strains while the third cow acquired a strain belonging to phage type 29/52/52A/80. A strain of this phage type was also carried by one attendant. This cow during the first sampling was found shedding a non-typable strain. Other authors have also commented on the possible human origin of *Staph. aureus* isolates from bovine mastitis that were typable with W.H.O. set of phages (Wallace *et al*, 1962, Slanetz & Bartley, 1962 and Havelka *et al*, 1975). In an attempt to experimentally infect

healthy udders of cows with *Staph. aureus* phage types 80/81, Drury and San Clemente (1962) reported no abnormality with the milk nor any gross or histologic lesion of significance in the udder. In our study, however several cows with clinical mastitis were found to be shedding strains of lytic group I including phage types 80/81. But still, incidence of bovine mastitis due to *Staph. aureus* strains possibly originating from humans should be considered as of occasional occurrence. Nevertheless the veterinarians of developing countries of Africa where dairy farming is still in its infancy should bear this aspect of staphylococcal mastitis in mind.

#### ACKNOWLEDGEMENT

My thanks are due to Dr. (Mrs.) Mauren de Saxe, Secretary, International Committee on Phage Typing of Staphylococci for typing the strains at the Central Public Health Laboratory, Colindale, London.

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A COMPARATIVE STUDY ON THE DISTRIBUTION OF *TRYPANOSOMA BRUCEI* AND *T. CONGOLENSE* IN TISSUES OF MICE AND RATS

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SUMMARY

The distribution of *Trypanosoma brucei* and *T. congolense* in tissues and body fluids of mice and rats was studied systematically by histological examination of organs and tissues taken from mice killed at intervals of 5 days or less, throughout the course of infection lasting for two or more months.

*T. brucei* was found both intra- and extravascularly in connective tissue spaces and fluids of the body cavities as early as 8 days after infection. The number of trypanosomes in the interstitial tissue spaces increased rapidly and remained high throughout the course of infection in contrast to the fluctuating parasitaemia. Organs containing much loose connective tissue such as the heart and epididymis showed a higher degree of extravascular localization with *T. brucei* than cellular organs like the liver and adrenal glands. The distribution of trypanosomes in most tissues was patchy and irregular in that some areas of the same tissues were more affected than others. The common form of trypanosomes seen in tissues was the trypomastigote similar to that seen in blood.

The localization of trypanosomes in tissue spaces was accompanied by oedema formation, mononuclear cell infiltration and tissue damage. These changes became pronounced as the infection progressed.

By contrast, *T. congolense* was confined to the blood vessels particularly

the capillaries and was not found in the fluids of the body cavities nor in lymph. The number of trypanosomes seen in the capillaries correspond with the number in tail blood, i.e. in sections made from mice killed at high parasitaemia, the capillaries contained more trypanosomes than in those taken at low parasitaemia.

Unlike in *T. brucei* infection, the extensive mononuclear cell infiltration in the interstitial tissues and the associated tissue damage were not observed in *T. congolense* infection.

INTRODUCTION

When reviewing the literature of the last 35 years on African pathogenic trypanosomes, there is a tendency to regard these organisms as primarily blood parasites. However, recently Ormerod (1970), Goodwin (1970a), Losos and Ikede (1970, 1972), have drawn attention to the fact that the *brucei* group trypanosomes in contrast to *T. congolense* and *T. vivax* are tissue parasites as indeed was shown by the earlier investigators (Yorke, 1910; Wolbach and Binger, 1912, Peruzzi 1928). It is unfortunate that this valuable early work has been overlooked and generally not accepted as judged by the current citations (Losos and Ikede, 1972). However, the majority of these earlier workers were mostly interested in the end product of the infection and most of their observations were made on tissues taken from the animals after

death or killed *in extremis*. The tissue distribution of trypanosomes at different stages of infection has not been systematically investigated.

It was, therefore, the aim of this study to examine in detail the comparative distribution of *T. brucei* and *T. congolense* in selected tissues of mice and rats and to relate their occurrences in the tissues to the numbers present in the blood during the course of an infection lasting two or more months.

## MATERIALS AND METHODS

### Laboratory Animals

*Mice* — Two inbred strains CBA and C57 black weighing approximately 20 g and over 12 weeks old, were used. They were bred and reared at Zoology Department, Edinburgh University.

*Rats* — Wistar Albino rats weighing about 250 g were obtained from the Small Breeding Station of Edinburgh University.

### Parasites

*Trypanosoma brucei* KBI (King's Building I) is a derivative of TREU 667. This stock gives rise to a chronic infection, the parasitaemia occurs in several peaks and death follows after about 2 or more months in mice, 40–50 days in rats and 30–40 days in rabbits.

*T. congolense* — stock Z 692 is derived from TREU 692. This stock runs a chronic infection of about 3-5 months in C57 black mice and about 40-50 days in Winstar rats but kills CBA mice in about 7-9 days. So in this study C57 black mice were used.

Both parasites were obtained from the Centre for Tropical Veterinary Medicine (CTVM), Edinburgh.

### Preparation of the stabilates

The stabilates were prepared following the method of Cunningham M.P. *et al* (1963).

Each stabilate was inoculated into six mice and then pooled infected blood was restabilated in 7.5% glycerol and stored in liquid Nitrogen.

The estimated numbers of *T. brucei* in the stabilated blood was  $5 \times 10^8$  /ml and of *T. congolense* —  $7.6 \times 10^8$  /ml.

### Infection of animals

Stabilates were removed from the liquid Nitrogen, placed in water at room temperature for 30 minutes. After this period, the thawed blood was diluted 1 part to 9 parts in citrated saline. Mice received 0.1 ml intraperitoneally (IP) and rats 0.5 ml IP. after examining a drop of blood under a microscope to make sure that the trypanosomes were still viable.

### Preparation of the tissues

Most of the histological investigations were carried out on tissues of infected mice killed at 5-day intervals or in some instances the interval was less. Rats were used occasionally and were killed at different stages post infection.

Mice and rats were killed using ether and immediately an animal was dead, the following tissues were removed for study: heart, brain, trigeminal nerve, skin and underlying muscles, epididymis, liver, urinary bladder, adrenal and pituitary glands and the eye.

The first six tissues were studied using both frozen and paraffin sections and the rest using only paraffin sections.

In preparing frozen sections, thin tissues were suspended in O.C.T. compound (an embedding medium for frozen tissues Ames Co. El-Khart Indi-

ana, U.S.A) placed on top of the chucks and quickly frozen with the help of two blocks of dry ice. They were transferred to the cryostat kept at  $-20^{\circ}$  and sections were cut at  $8-10\mu$ .

As for paraffin sections, thin tissues were quickly removed and fixed in precooled Acidified alcohol at  $4^{\circ}\text{C}$  (Wolman and Behar, 1952) for 4-6 hours, washed and dehydrated in absolute alcohol for 12 hours and cleared in Methylbenzoate for 4-8 hours. Then they were embedded in an oven at  $56^{\circ}\text{C}$  using filtered paraffin wax of  $54^{\circ}\text{C}$  and  $56^{\circ}\text{C}$  Melting Point (George T. Gurr Ltd. London). The tissues were embedded in watch glasses and serial sections were cut on a cambridge rocker microtome (Cambridge Instrument Co. Ltd.) at a thickness of 6-8 u. The sections were stained by the giemsa Colophonium technique (Shortt and Cooper, 1948).

Before the animals were killed, the numbers of trypanosomes in tail blood were estimated by haemocytometer method. In addition, daily counts of trypanosomes in tail blood of three mice and three rats were made in order to see the pattern of parasitaemia throughout the infection.

## RESULTS

The daily fluctuations in the numbers of trypanosomes in peripheral blood are shown in Fig. 1.

Note that in *T. brucei* infection, the number of trypanosomes rose to a peak and fell again to zero at about the eighth day. This was followed by the second peak which was higher but, thereafter, the numbers fluctuated irregularly.

With *T. congolense*, however the number of trypanosomes were lower, and fluctuated irregularly throughout the infection.

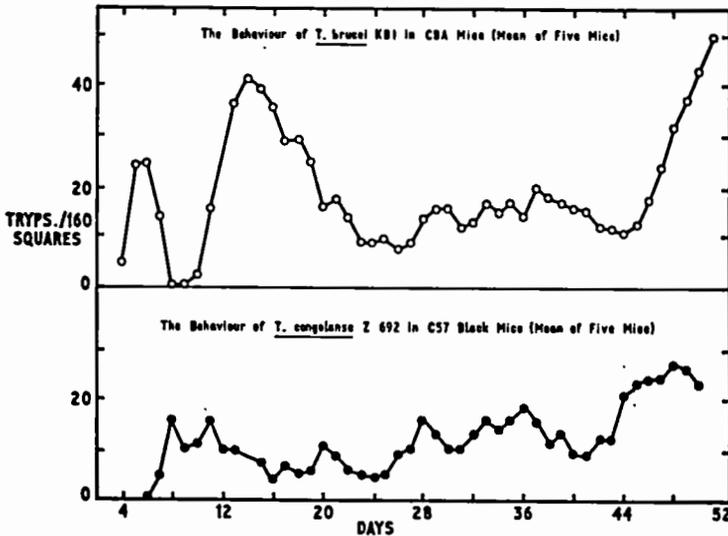


Fig. 1: *T. brucei* and *T. congolense*. The daily fluctuations in the numbers of trypanosomes in peripheral blood. Note that in *T. brucei* infection, the number of trypanosomes rose to a peak and fell again to zero at about the 8th day; this was followed by a second higher peak. Thereafter, the numbers fluctuated irregularly. In contrast to *T. congolense*, the trypanosome numbers were lower and fluctuated irregularly through the infection.

### Distribution of *T. Brucei* in tissues

Frozen sections were used initially for the preliminary study of the distribution of trypanosomes in tissues. The interpretation of these sections was difficult because it was not possible to see whether the trypanosomes were in the blood vessels or in tissue spaces owing to some degree of disruption of the tissues.

The following description refers to paraffin sections.

In most organs examined, *T. brucei* localized extravascularly in connective tissues and body fluids. The time after infection at which trypanosomes were detected outside the vessels varied with the organ.

Following intraperitoneal infection, trypanosomes were detected in the blood vessels in sections from mice killed early in the infection, a few days after they were seen in the peripheral blood samples. Extravascular localization was detected in some tissues, namely the epididymis, trigeminal nerve, peri-

cardium, at about the 8th day after infection just following the first peak parasitaemia (fig. 1.). In other tissues examined, tissue invasion was evident after about 10 days.

The organs containing much loose connective tissue showed a higher degree of extravascular localization than cellular organs. The localization of trypanosomes in tissues was accompanied by oedema formation, inflammatory cell infiltration and tissue damage. These changes became more pronounced as the infection progressed. Now using the heart as a representative organ, the changes in tissues as seen in serial paraffin sections taken at regular intervals for two or more months are as follows: fresh areas of the myocardium are invaded by trypanosomes followed by oedema which seems to favour their multiplication (Fig. 2) then inflammatory cells, mainly lymphocytes, macrophages and plasma cells appear in the infected areas. To start with the cells are few in the area but they gradually increase. As they

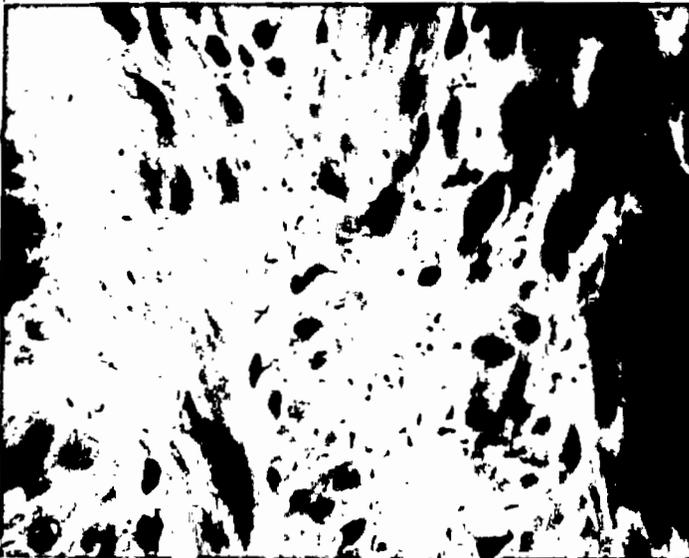


Fig. 2: *T. brucei* Myocardium 15 days after infection x 560. Area of myocardium freshly invaded by trypanosomes followed by oedema which seems to favour their multiplication.

Note the widening of the interstitial spaces (Giemsa Colophonium).

accumulate the architecture of the surrounding tissues tends to get distorted and the trypanosomes tend to disappear. Other areas are invaded and the same process continues so that in examining the sections of the heart at any one time after the infection is well established, the picture appears as follows: some areas of the myocardium appear freshly invaded by many trypanosomes and only a few inflammatory cells; (Fig. 3); some show trypanosomes and inflammatory cells in roughly equal numbers; in others, numerous inflammatory cells and hardly any trypanosomes or very few appearing as aberrant forms, are seen (Fig 4).

However, many areas of the myocardium still appear healthy and unaffected. The same processes and histological changes as described in the heart were observed in the urinary bladder, trigeminal nerve, skin and underlying muscles. However, neither the degree of trypanosomes infiltration nor the extent of the lesions was as great in these tissues as in the heart.

In the skin, no trypanosomes were detected in the epidermis at any time throughout the infection. Trypanosomes were found in the deep layers of the dermis adjacent to the subcutaneous muscles.

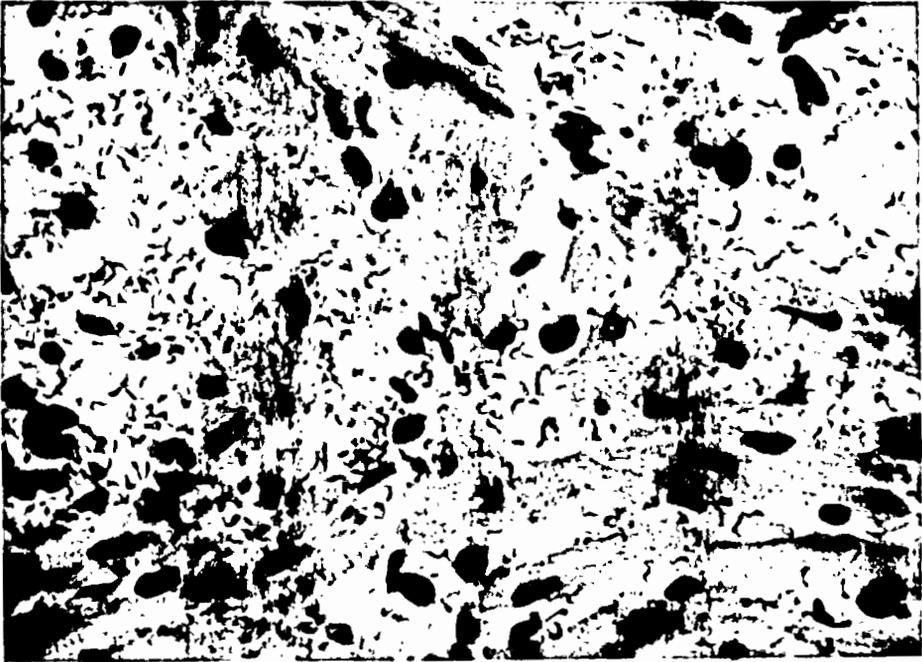


Fig. 3: *T. brucei*. Myocardium 40 days after infection x 560. Areas of the myocardium invaded by numerous trypanosomes and only a few inflammatory cells. Note that some heart muscles have been destroyed in some areas and replaced by numerous trypanosomes. (Giemsa Colophonium).

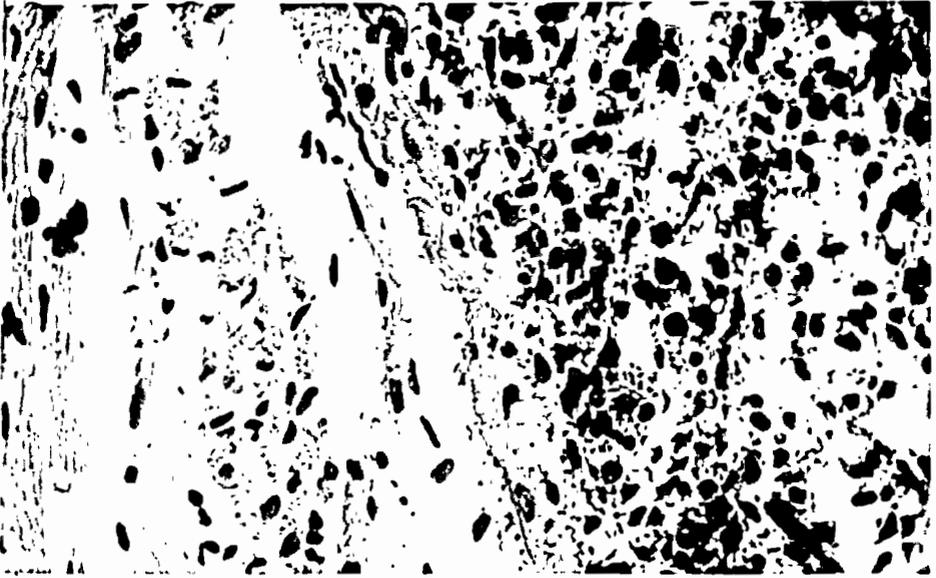


Fig. 4: *T. brucei*. Myocardium 63 days after infection x 280. Note the extensive destruction of the myocardium on the left by the numerous infiltrating trypanosomes and inflammatory cells. The myocardium on the right still appears normal. (Giemsa Colophonium).

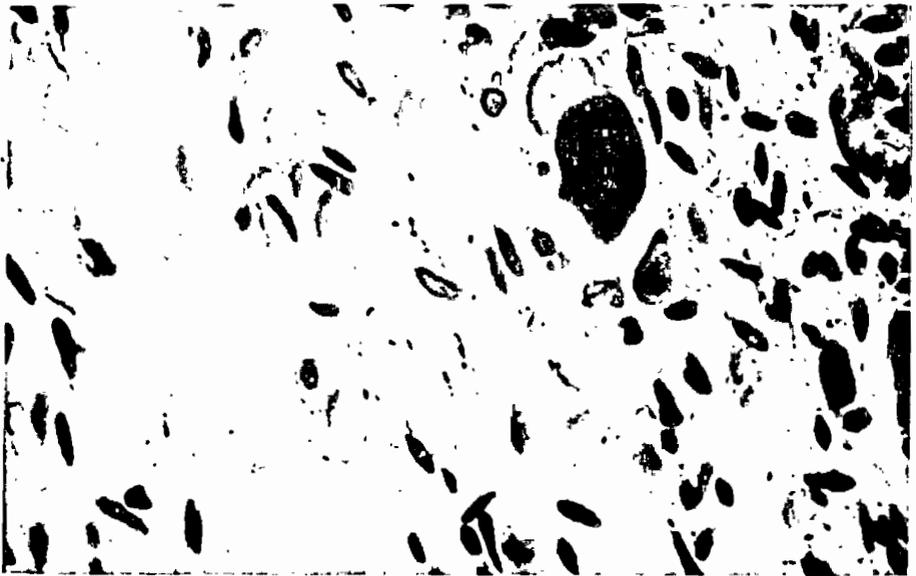


Fig. 5: *T. brucei*. Trigeminal nerve 8 days after infection x 560. Many trypanosomes can be seen lying in the nerve fibres. Note some inflammatory cells on the other side. (Giemsa Colophonium).

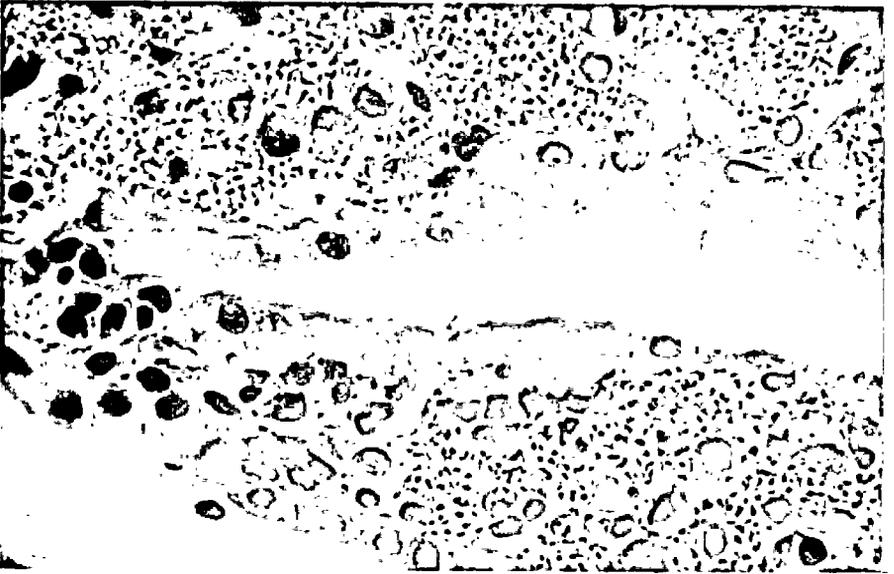


Fig. 6: *T. brucei*. Section of the brain showing the choroid plexus 20 days after infection, x 560. Masses of trypanosomes appearing as round forms are packed in the choroid plexus. (Giemsa Colophonium).

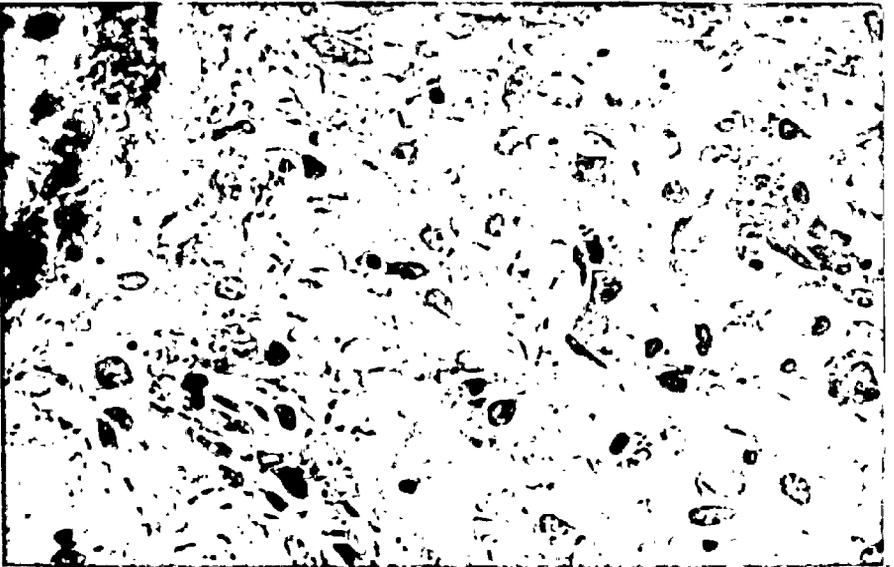


Fig. 7: *T. brucei*. Pituitary gland — 15 days after infection x 560. Section through the *pars nervosa* showing numerous trypanosomes (Giemsa Colophonium).

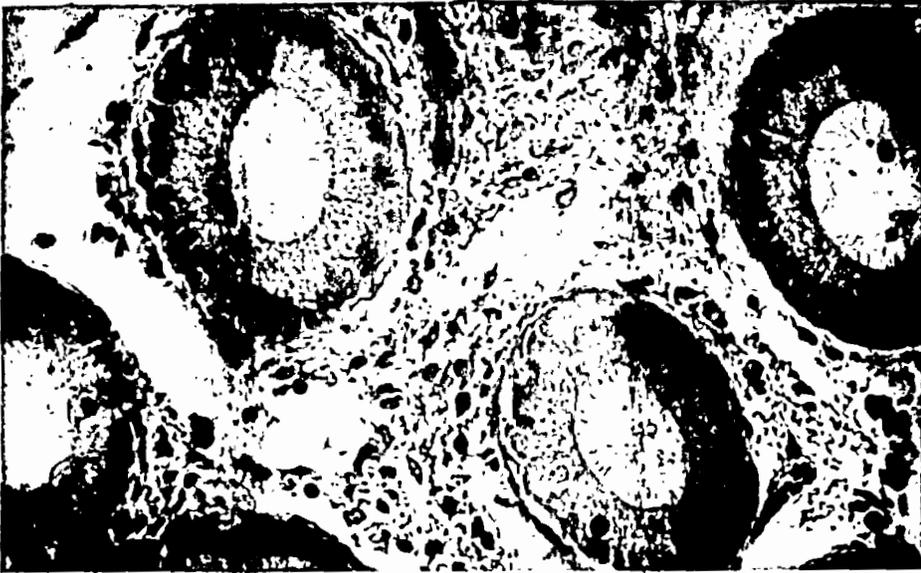


Fig. 8: Epididymis: *T. brucei* — 15 days after infection x 280. Numerous trypanosomes and some inflammatory cells are seen in the interstitial tissues (Giemsa Colophonium).

In the trigeminal nerve early extravascular localization of trypanosomes occurred in the distal portion of the nerve and around the ganglia. Trypanosomes then spread towards the proximal part as the infection progressed (Fig 5).

In the brain the most striking lesions were in the choroid plexus where numerous trypanosomes accumulated in the connective tissues and capillaries (Fig 6). Only a few trypanosomes were found in the brain substance and in the meninges.

In the pituitary gland, numerous trypanosomes localized in blood vessels and connective tissues of *pars nervosa* and *plexus intermedius* from the early stage of infection (Fig 7). No extravascular trypanosomes were seen in the distal lobe.

In the epididymis, trypanosomes localized extravascularly in the loose connective tissue early in the infection and

their numbers remained high throughout the infection (Fig 8). Later in the infection there was a progressive development of interstitial fibrosis and a gradual decrease in the number of trypanosomes. Only a small number of trypanosomes were found in the interstitial tissues of the testicles.

In the liver, no extravascular trypanosomes were observed; as few trypanosomes were seen in the sinusoids but only in section of mice killed at high parasitaemia.

In the adrenal gland, apart from the capsule, trypanosomes were present only in the blood vessels, and consequently were evident only at times of high parasitaemia.

In the eye of mice, trypanosomes were found in the smears made from the aqueous humour of only three mice and in the cornea, sclera and ciliary muscles of only one mouse

killed late in the infection. In all these cases, the eyes did not show any gross lesions like conjunctivitis, lacrimation or corneal opacity which were regularly seen in the rabbits (unpublished results).

#### Distribution of *T. congolense* in tissues

In all tissues of mice and rats examined, *T. congolense* in contrast to *T. brucei*, was confined to blood vessels, in particular to the capillaries. Following intraperitoneal infection, trypanosomes were found in the capillaries of most organs by about the 10th day and the number increased gradually as the infection progressed. Throughout the infection, the capillaries of the myocardium (Fig 9), brain (Fig 10), skeletal muscles and epididymis (Fig 11) contained more

trypanosomes than those of other organs. The numbers of trypanosomes observed in the blood vessels corresponded with the numbers of trypanosomes in the peripheral blood before mice were killed, in that sections of mice killed at high parasitaemia contained many more trypanosomes in the blood vessels than those of mice killed at low parasitaemia. In sections of organs taken at high parasitaemia, most of the capillaries were packed with clumps of trypanosomes (Fig 9) while only a few trypanosomes were seen in some of the big blood vessels. On the other hand, in sections taken at low parasitaemia, only a few trypanosomes were seen and only in a small number of capillaries mainly of the heart (Fig 12) brain and skeletal muscles.

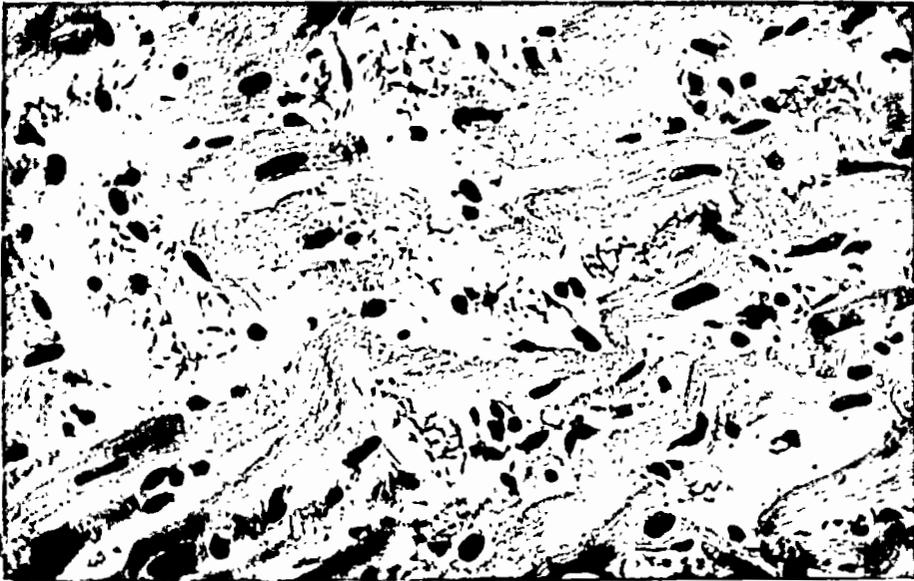


Fig. 9: *T. congolense*. Section of the myocardium 67 days after infection x 560. Giemsa Colophonium. Note clumps of trypanosomes in the distended capillaries. Some capillaries are so much distended that they deform the tissues around them.



Fig. 10: *T. congolense*. Section of the brain 20 days after infection x 560. Note that capillaries are blocked by clumps of trypanosomes and inflammatory cells.

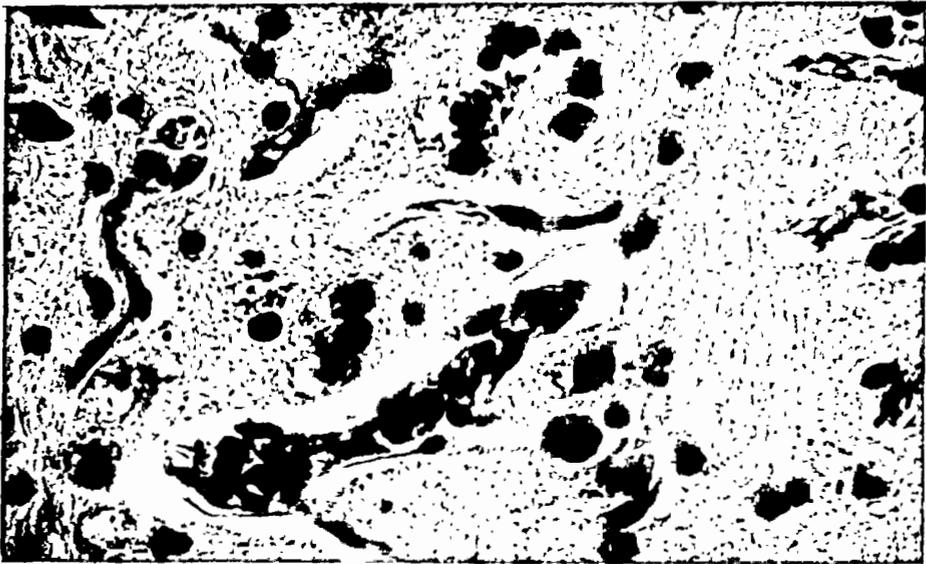


Fig. 11: *T. congolense*. Section of the epididymis 20 days x 280. Giemsa Colophonium. Trypanosomes are confined inside the capillaries and interstitial spaces are free.



Fig. 12: *T. congolense*. Section of the myocardium 15 days after infection x 560. Long form trypanosomes can be seen clearly inside the capillaries. This mouse was killed at low parasitaemia and only a few trypanosomes are seen inside the capillaries. Giemsa Colophonium.

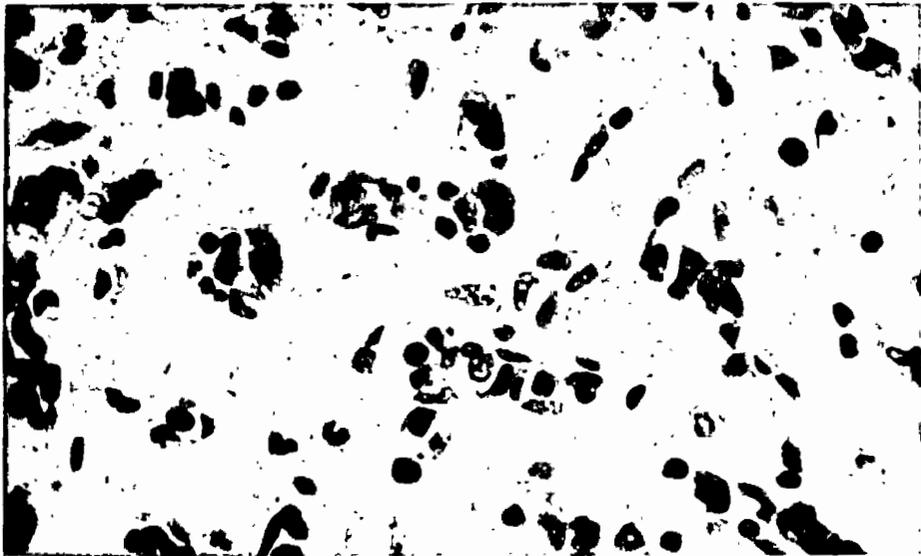


Fig. 13: *T. congolense*. Section through the *pars nervosa* of the pituitary gland 25 days after infection x 560. Giemsa Colophonium. The inflammatory cells are confined inside the capillaries and some capillaries are partially blocked.

From the early stage of infection, the capillaries were dilated and prominent and this feature became more pronounced as the infection progressed (Fig 9).

The most characteristic gross lesion observed in mice and rats was marked enlargement of the spleen and occasionally of the liver and the development of the anemia. These lesions were observed from the early stage of infection particularly the splenomegaly and were more marked in *T. congolense* than in *T. brucei* infection. The extensive mononuclear cell infiltration in the interstitial tissues and the associated tissue damage were not observed in *T. congolense* infection. Only minimal inflammatory reaction was observed and the cells were mostly confined to the blood vessels (Fig. 10) and (Fig. 13). The trypanosomes were clumped together and in some tissues like the myocardium and the brain, they blocked the capillaries (Fig. 9 and 10).

#### DISCUSSION AND CONCLUSION

The observations that *T. brucei* localizes extravascularly in connective tissues and body fluids support and agree with those of earlier workers made on various species of animals infected with *T. brucei* group trypanosomes (Morax, 1907; Yorke, 1910; Wolbach and Binger, 1912; Peruzzi, 1928) and the more recent observations of Goodwin (1970) a & b; 1971; Losos and Ikede (1972 a & b) and Murray *et al* (1974). Though trypanosomes were detected extravascularly in tissues at about the 8th day after infection in paraffin sections, tissue localization seems to occur earlier in the infection as could be judged from the thoracic lymph duct cannulation experiments where many trypanosomes were found in lymph,

48 hours after intravenous injection (Ssenyonga and Adam, 1975). Owing to the small number of trypanosomes in tissues at this time, it was difficult to distinguish with certainty in the paraffin sections whether the trypanosomes were in the small capillaries or in the tissue spaces. The same difficulty was pointed out by Peruzzi (1928). The extensive localization of trypanosomes of *brucei* group in organs containing much loose connective tissue suggests that the loose connective tissue provided a suitable environment for the growth and multiplication of trypanosomes (Yorke 1910; Goodwin 1970; 1973). Also Yorke (1910) suggested that the levels of antibodies in tissue fluids is lower than in blood so that the trypanosomes escape from the higher levels of antibodies in blood. However, Goodwin (1973) found that humoral anti-trypanosomal antibodies rapidly appeared in tissue fluids after infection although the titre was lower than that of serum. In my study (Ssenyonga 1974), using the agglutination test between serum and lymph from the same rats and the stabilates prepared at different intervals, I found that lymph from the same rats and the stabilates prepared at different intervals, found that lymph from the same rats and the stabilates prepared at different intervals, contained much less agglutinating antibodies than serum.

Since the ground substance of the loose connective tissues plays a vital role as a mechanical barrier to the spread of the invading organisms, it may be possible that initially the trypanosomes are trapped in this tissue. Probably, as the trapped trypanosomes accumulate, they produce some enzymes like hyaluronidase which then break down the tissue ground substance and make

the environment suitable for their multiplication and spreading. Although hyaluronidase production has never been demonstrated in trypanosomes, it is known to be produced by some protozoan organisms like *Balantidium coli* (Tempelis & Lysenko, 1957), *Entamoeba histolytica* (Bradin, 1953) and *Trichomonas vaginalis* (Boni and Orsi 1958). (Quoted from Muller (1967).

The characteristic feature of the lesion is the extensive extravascular localization of numerous trypanosomes in tissues and infiltration by mononuclear inflammatory cells, mainly lymphocytes, macrophages, and plasma cells. The large number of trypanosomes in tissues suggest that multiplication occurs at the sites of localization (Losos & Ikede, 1972). The response by mononuclear cells particularly lymphocytes and macrophages suggest that these cells play a major role in destroying trypanosomes in tissues and the presence of plasma cells suggests that humoral antibodies may be produced locally at the site of infection. The presence of many different sites in tissues infiltrated by varying numbers of trypanosomes and inflammatory cells may indicate that the trypanosome populations in these various sites are antigenically different.

This was also suggested by Seed *et al* (1973), who, using agglutination test, found that trypanosomes isolated from different parts of the brain of *Microtus montanus* (sic) infected with *T. gambiense* were serologically different from the original inoculum and also from each other.

The wide-spread distribution of *T. brucei* in the tissues of mice and rats from the early stage of infection explains the pathological changes observed in different tissues during the infection and supports the view of multiple

disturbance of normal physiological processes.

#### Morphology of *T. brucei* in tissues

Throughout this study, the most common form of the trypanosomes seen in the tissues was the trypomastigote, similar to those seen in blood. This observation agrees with those of most other workers. The amastigotes and sphaeromastigotes described by Ormerod and Venkatesan (1971 a & b) were not seen. However, trypanosomes which I have described as "aberrant" and which were seen in some tissues particularly the choroid plexus were believed to be normal trypomastigotes so closely packed in a small tissue that their cytoplasm seems to merge together and it is difficult to see the individual organisms. What can be seen are the prominently stained structures i.e. the nuclei and Kinetoplasts, which give the impression that the trypanosomes are in the amastigote form. Also the fact that the choroid plexus is much folded and invaginated into the ventricles rather than being flat contributes to this appearance of the trypanosomes. Also it is possible that some of these trypanosomes in the choroid plexus are degenerating and only the nuclei and kinetoplast remain stainable. This would explain the appearance of rounded (amastigote?) forms of trypanosomes in interstitial tissues of the neurohypophysis of sheep studied by Ikede and Losos (1975).

In Many other tissues, trypanosomes lose their cytoplasm and appear as nuclei and kinetoplasts when inflammatory cells invade the same areas. This has been demonstrated by the work of Takayanagi *et al.* (1974), in their *in vitro* experiments on the various stages of attachment and ingestion

of *T. gambiense* by the rat macrophages in the presence of a specific antiserum. After 90 minutes of the reaction, all the attached trypanosomes had been engulfed and only their nuclei were clearly seen when smears were stained with Giemsa. Furthermore by sampling lymph, one might expect to see forms representative of those in tissue spaces. However, no amastigotes or sphaeromastigotes were found (Ssenyonga and Adam 1975).

#### *T. Congolense* in tissues

In all tissues of mice and rats examined, *T. congolense* was confined to blood vessels, in particular to the capillaries. This observation agrees with those of other workers (Hornby 1929; 1932; 1952; Hornby and Bailey 1930; 1931; 1933; Lavier 1940; Fiennes 1952; Arnold 1956; Losos and Ikede 1970; Losos *et al* 1973). Also this observation was supported by the thoracic duct cannulation experiments where by *T. congolense* was not found in lymph of 4 out of 6 rats cannulated (Ssenyonga and Adam 1975) and the most recent work of Tizard *et al* (1978). This is in contrast to *T. brucei* which localizes extravascularly in various tissues and in fluids of body cavities.

The similarity between the picture observed in mice and rats and remission and in cattle, clearly confirms the observations of Fiennes (1950) who described some cryptic foci of the parasites in the heart muscles of cattle and suggested that these organisms were the active cause of the disease symptoms and that the pathology of the disease was dependent on the presence of these organisms in the capillaries.

The distribution of *T. brucei* and *T. congolense* in tissues and body fluids and the importance of trypanosomes

in tissues have been demonstrated in this work. Although these observations are based on the picture in laboratory animals, similar lesions have been demonstrated in large animals like sheep and cattle by Ikede and Losos (1972). The difference between the distribution of *T. brucei* and *T. congolense* in tissues and body fluids of mammalian host strengthens and justifies the observations of Hornby (1929) that trypanosomiasis is a group of diseases and not a single entity. Since *T. brucei*, *T. congolense* and *T. vivax* are regarded as distinct species so the diseases that they cause may be expected to differ.

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INFLUENCE OF SEASONALITY ON AESTIVATION AND BEHAVIOUR OF THE FOREST AFRICAN GIANT SNAIL *ARCHACHATINA (CALACHATINA) MARGINATA*, SWAISON

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SUMMARY

Climatic influence on behavioural patterns was studied in 21 snails kept in dry or wet apartments. Observations were made every six hours during 4 months (Nov 29 — March 31) dry period of the year. Relative humidity (R.H.), rainfall and vapour pressure deficits (V.P.D.) were the principal climatic factors influencing snail movement and aestivation.

Average distances moved by snails were significantly greater ( $P < 0.01$ ) in the wet apartment than in the dry, while numbers of snails aestivating in the dry apartment were significantly greater ( $P < 0.01$ ) than those in the wet apartment. Highly significant correlations were also observed between snail movement and aestivation. R.H. and V.P.D. accounted for 49% of observed variation in total snail movement. V.P.D. at night gave the only significant regression step ( $P < 0.05$ ) for the aestivation patterns. Snails tended to aestivate at peak periods for two months — January and February in both apartments.

A non-significantly ( $P < 0.05$ ) higher body weight loss was observed in the dry apartment as compared to the wet. There was also a general decrease in body weight with increase in aestivation. Snail mortality was similar in both apartments. Correlations between body weight loss and mortality were also insignificant ( $P < 0.05$ ).

Analysis of snail epiphragm produced during aestivation, revealed that calcium accounted for over 90% of the total mean value of 21% of all minerals analysed.

INTRODUCTION

The African giant snail *Archachatina marginata* is an edible large gastropod mollusc which can weigh up to 750 g. Its habitat ranges from dense tropical high forest in Southern Nigeria to the fringing riparian forests of the derived Guinea Savanna in Nigeria. It also inhabits marine and fresh-water swamps (Segun 1975). It is omnivorous, movement and feeding taking place mostly at night (Ajai *et al.* 1978). During the hot dry season (about November-March) in Nigeria, when climatic conditions are too severe for the snail, it goes into a state of inactivity, called "aestivation". During aestivation, the aperture is temporarily closed by a calcified material known as epiphragm. The present study examined some climatic conditions that lead to aestivation, snail behaviour during this period, the period of epiphragm formation and its chemical composition. As far as is known, there is no published information on this aspect of the biology of *Archachatina marginata*.

MATERIALS AND METHODS

The study was carried out at the University of Ibadan, 7° 23' N, 3° 53' E. Two snail compartments, each of 4m x 4m base and 2.5m high, were construc-

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ted with ½" mesh chicken wire netting. Each compartment had banana plant cover, to simulate the shady, cool natural environment of a snail. The tip of the fence was bent inwards to prevent climbing snails from escaping. No roof was provided to allow free movement of air.

Climatic observations were made every alternate day on a 24-hour basis as follows, using the wet and dry swirling hygrometre:

0700 — 0800 hrs  
 1400 — 1500 "  
 1800 — 1900 "  
 23.00 — 2400 "

Climatic studies were carried out throughout the dry season, starting at the end of the 1977 rains (November 29), and ending at the beginning of the 1978 rains (March 31).

Twenty one snails were obtained, and weighed. Numbers were painted on them with white enamel to enhance identification. One compartment, which was kept wet during the study period (by spraying water on the ground) contained 10 snails, while the other compartment, kept dry throughout the study, contained 11 snails. The wet and dry compartments thus provided contrasting conditions with regards to the effect of moisture on the behaviour of snails.

Aestivation was recognized by the presence of epiphragm, a whitish, fragile material covering the aperture.

At each observation period, snail positions were recorded using two of the sides of each apartment as the X and Y axis. The distance moved was recorded as the straight line joining the snail positions at one observation time to its position at the previous observation time. Measurements were made to the nearest centimetre.

Snails were weighed fortnightly throughout the course of study. Samples of epiphragms were collected in the apartments. They were either seen to be perfectly shaped like their respective apertures or partly broken giving an idea of the sizes and shape of their apertures. Thus the different epiphragms for each snail could be identified. Samples collected were weighed and used for mineral analysis on Atomic Absorption Spectrophotometre after wet washing with nitric and perchloric acid. The phosphovanadomolybdate method of the A.O.A.C. (1970) was used for phosphorous determination.

## RESULTS

### Comparison of behaviour between dry and wet apartments

The distances moved and numbers aestivating in each apartment at different periods of the day are shown in Table 1. Average distances moved by snails were greater in the wet apartment than in the dry apartment. These differences were highly significant ( $P < 0.01$ ) for the day as a whole and also for the night and morning periods. Distances moved during the evening periods were also significantly different ( $P < 0.05$ ), though distances moved during the afternoon were not significantly different. The average numbers of snails aestivating in the dry apartment were significantly greater than the number aestivating in the wet apartment ( $P < 0.01$ ) for all period of the day.

Correlation coefficients between average distance moved and the numbers aestivating for the four time periods of the day and for the day taken as a whole are given in Table 2. These are separated into the dry (Table 2) and wet (Table 3) apartments.

Table 1: Comparison of snail behaviour in dry and wet apartments.

	DRY APARTMENT		WET APARTMENT		
<b>Ave. Dist. Moved (M)</b>	<b>Mean</b>	<b>SE</b>	<b>Mean</b>	<b>SE</b>	<b>"t" test</b>
<b>Morning</b>	<b>0.3984</b>	<b>0.0713</b>	<b>0.8986</b>	<b>0.0879</b>	<b>4.419**</b>
<b>Afternoon</b>	<b>0.0155</b>	<b>0.0101</b>	<b>0.0452</b>	<b>0.0156</b>	<b>1.597</b>
<b>Evening</b>	<b>0.0412</b>	<b>0.0218</b>	<b>0.1911</b>	<b>0.0665</b>	<b>2.142*</b>
<b>Night</b>	<b>0.2239</b>	<b>0.0603</b>	<b>0.6087</b>	<b>0.0861</b>	<b>3.663*</b>
<b>Day</b>	<b>0.7029</b>	<b>0.1157</b>	<b>1.7432</b>	<b>0.1859</b>	<b>4.752**</b>
<b>Ave. No. Ast.</b>	<b>Mean</b>	<b>SE</b>	<b>Mean</b>	<b>SE</b>	<b>"t" test</b>
<b>Morning</b>	<b>0.5482</b>	<b>0.0587</b>	<b>0.2170</b>	<b>0.0371</b>	<b>4.770**</b>
<b>Afternoon</b>	<b>0.5323</b>	<b>0.0598</b>	<b>0.1705</b>	<b>0.0304</b>	<b>5.392**</b>
<b>Evening</b>	<b>0.5136</b>	<b>0.0603</b>	<b>0.1443</b>	<b>0.0292</b>	<b>5.514**</b>
<b>Night</b>	<b>0.5184</b>	<b>0.0610</b>	<b>0.1479</b>	<b>0.0287</b>	<b>5.500**</b>
<b>Day</b>	<b>0.5284</b>	<b>0.0583</b>	<b>0.1718</b>	<b>0.0305</b>	<b>5.416**</b>

\* Significant at 95% (1.960)

\*\* Significant at 99% (2.576)

Table 2: Correlations between average distance moved and average number aestivating in the dry apartment

		AVERAGE DISTANCE MOVED					AVERAGE NUMBER AESTIVATING				
Variables		Morning	Afternoon	Evening	Night	Day	Morning	Afternoon	Evening	Night	Day
Av. Dist. Moved	Morning	1.00000									
	Afternoon	0.20648	1.00000								
	Evening	0.20233	-0.02684	1.00000							
	Night	0.22198	0.42538**	0.25835	1.00000						
	Day	0.77352**	0.42522**	0.43296**	0.72776**	1.00000					
Av. No. Aestivating	Morning	-0.74150**	-0.23522	-0.29705*	-0.45900**	-0.74109**	1.00000				
	Afternoon	-0.72808**	-0.24911	-0.28224*	-0.45325**	-0.72786**	0.98273**	1.00000			
	Evening	-0.64328**	-0.22544	-0.17185	-0.53543**	-0.69456**	0.89998**	0.91634**	1.00000		
	Night	-0.62986**	-0.22371	-0.16022	-0.53788**	-0.68597**	0.88162**	0.89653**	0.99655**	1.00000	
	Day	-0.70457**	-0.24031	-0.23371	-0.51014**	-0.73202**	0.96703**	0.97509**	0.97970**	0.97006**	1.00000

$r = 0.2650$  — at 85% (Sig. Level)\*

$r = 0.3400$  — at 99% (Sig. Level)\*\*

Table 3: Correlations between average distance moved and average number aestivating in the wet apartment

		AVERAGE DISTANCE MOVED					AVERAGE NUMBER AESTIVATING				
Variables		Morning	Afternoon	Evening	Night	Day	Morning	Afternoon	Evening	Night	Day
No. Distance Moved	Morning	1.00000									
	Afternoon	-0.25103	1.000000								
	Evening	0.23597	-1.06814	1.00000							
	Night	0.48866**	-0.07773	0.49261**	1.00000						
	Day	0.76222**	-0.09503	0.69183**	0.86375	1.00000					
Av. No. Aestivating	Morning	-55951	0.10767	-0.16855	-0.46848**	-0.53060**	1.00000				
	Afternoon	-0.52300**	0.06284	-0.12804	-0.45416**	-0.49809**	0.91000**	1.00000			
	Evening	-0.47551**	0.02389	-0.23402	-0.47853**	-0.52816**	0.84067**	-0.91152**	1.00000		
	Night	-0.49970**	0.05050	-0.24320	-0.50788**	-0.55313**	0.90054	0.90064**	0.96846**	1.00000	
	Day	-0.53653**	0.07690	-0.19900	-0.49530**	-0.54861**	0.95089**	0.96694**	0.96168**	0.97400**	1.00000

$r = 0.2650$  — at 95% (Sig. Level)\*

$r = 0.3400$  — at 99% (Sig. Level)\*\*

### *The dry apartment*

There was a negative correlation between the average distances moved and the number aestivating during the five time intervals. Correlations were highly significant ( $P < 0.01$ ) for the distances moved during the morning, night and day.

### *The wet apartment*

There was a highly significant correlation ( $P < 0.01$ ) between the average distance moved in a day and the average numbers aestivating during the four time periods of the day and the day taken as a whole. The average distances moved in the morning and night followed this daily pattern.

### Relationships between climate and behaviour

#### *The dry apartment*

Correlations were carried out between climate and behaviour. This is presented in Table 4. Considering average daily conditions, relative humidity (RH), vapour pressure deficit (VPD) and rainfall (R) were significantly related to the average distance moved by the snails, while temperature was apparently unrelated. Climatic conditions in the morning and afternoon were not significantly correlated to the average daily distance moved, whereas, climatic conditions during the evening and night were significantly related to the daily movements.

The average number of snails aestivating followed these same general patterns of significance in relation to daily climatic conditions, with a marked difference between morning/afternoon and evening/night climatic conditions. Temperature conditions appeared to be an exception, with night temperatures

correlated ( $P < 0.01$ ) to the daily number aestivating while evening temperatures were apparently unrelated.

Significant correlations were also obtained between the behaviour patterns of the snails during each of the different time periods of the day as shown in Table 4.

A stepwise multiple regression of climate against behaviour of snails in the dry apartment is shown in Table 5. The table shows the significant steps in the multiple regression, together with the resulting regression formula, percentage of variation explained and the standard error.

The average daily distance move by a snail could be significantly predicted by two regression steps; vapour pressure deficit at night and daily rainfall; these two variables accounting for 49% of the observed variation in the movement pattern. A similar relationship existed for the night movements, though in this case, the two climatic variables explained 51% of the variation. Distances moved in the morning could only be significantly predicted by one regression step; the average daily vapour pressure deficit — and this accounted for 14% of the variation. Distance moved during the afternoon could not be significantly predicted by any of the climate variables while distances moved in the evening were significantly predicted by three climatic variables; daily rainfall, evening and morning relative humidity, which together accounted for 42% of the variation.

With regards to the average number aestivating, during the different periods of the day, and for the day as a whole, vapour pressure deficit at night seemed to be the controlling factor, appearing as the only significant regression step for morning, afternoon and average

Table 4: Correlations between climate and behaviour dry apartment

Climatic Conditions	AVERAGE DISTANCE MOVED					AVERAGE NO. AESTIVATING				
	Morning	Afternoon	Evening	Night	Day	Morning	Afternoon	Evening	Nigh	Day
<b>I. TEMPERATURE</b>										
Morning										
Afternoon										
Evening			—	—	—			*	*	
Night				—	—			*	*	*
Day										
<b>II. REL. HUMIDITY</b>										
Morning	*									
Afternoon	*									
Evening	*		**	*	**	—	—	—	—	—
Night	**		*	**	**	—	—	—	—	—
Day			*		**	—	—	—	—	—
<b>III. VAP. PRESS DEFICIT</b>										
Morning	—									
Afternoon	—									
Evening	—					*	*	**	*	*
Night	—		—	—	—	**	**	**	**	**
Day	—		—			—	**	**	**	**
<b>IV. RAINFALL</b>										
	*		**	**	**	—	—	—	—	—
* +ve Sig. at 95%										
** +ve Sig. at 99%										
— -ve Sig. at 95%										
— -ve Sig. at 99%										

r = 0.26500 at 95%

r = 0.3400 at 99%

Table 5: Stepwise multiple regression of climate against behaviour for the day apartment.

AVE. DIST. TIME	VPD TOTAL	RAIN- FALL	RH (EVE)	RH (MORN)	VPD (NIGHT)	TEMP (NIGHT)	RH (NIGHT)	CONST	R%	STD. ERROR
I. AV. DIST. (MORN)	-0.0679	--	--	--	--	--	--	1.04295	14	0.50065
AV. DIST. (AFT)		--	--	--	--	--	--	--	--	--
AV.DIST. (EVE)	--	0.00490(1)	0.00744(2)	-0.00563(3)	--	--	--	-0.0563	42	0.12792
AV.DIST. (NIGHT)	--	0.01708(1)	--	--	-0.06631(2)	--	--	0.50408	51	0.32282
AV.DIST (DAY)	--	0.02728(2)	--	--	-0.15323(1)	--	--	1.40486	49	0.63070
II. AV. NO. ASST(MORN)	--	--	--	--	-0.08417(1)	--	--	0.09533	21	0.39464
AV. NO. AST(AFT)	--	--	--	--	0.08551(1)	--	--	0.07224	21	0.40269
AV. NO. AST(EVE)	--	--	--	--	-0.022350(1)	0.08862(2)	-0.03658(3)	1.45683	38	0.36599
AV. NO. AST.(NIGHT)	--	--	--	--	0.096520(1)	0.04957	--	1.15134	34	0.37884
AV. NO. AST.(DAY)	--	--	--	--	0.09264(1)	--	--	0.02993	25	0.38049

F ratio 95 = 4.02

-- not significant.

daily aestivation patterns. Night temperature was also a significant determinant of the numbers of snails aestivating during the evening and night while night relative humidity was also significant for the numbers aestivating in the evening.

#### *The wet apartment*

Significant correlations between the climatic conditions and the behaviour patterns during the five periods of the day are presented in Table 6. The average number of snails aestivating during the five-time periods were only correlated to a few of the climatic variables, compared to the correlations observed between the average distance moved and the climatic variables. Considering the average daily conditions, relative humidity, vapour pressure deficit and rainfall were significantly correlated to the average distance moved by the snails, while temperature was apparently unrelated.

Stepwise multiple regression of climate against behaviour (Table 7) show that the average distance moved could be significantly predicted by two regression steps; vapour pressure deficit at night and evening temperature, which together accounted for 58% of the variations observed. The average distance moved during the night and morning, could also be predicted from vapour pressure deficit at night, though night movements were also predicted by the relative humidity and temperatures at night. Distances moved in the afternoon, could best be predicted by vapour pressure deficit in the morning, though this regression only accounted for 7% of total variation. Average distances moved in the evening could best be predicted by daily rainfall, evening temperature and night relative humidity.

The number of snails aestivating during the day as a whole, night and evening could be predicted by the nightly vapour pressure deficit, though numbers aestivating at night were also apparently determined by night temperatures. The average number aestivating during the morning could be predicted by relative humidity during the morning, while average number aestivating during the afternoon could be predicted by morning vapour pressure deficit.

#### *Changes in Body Weight*

The changes in body weight of snails during the experimental period are given in Table 8. While loss in body weight was higher in the dry apartment, this was not statistically significant ( $P < 0.05$ ).

In the dry apartment from the inception of the experiment to late December, with increasing numbers of snails going into aestivation, there was a general loss of body weight from the population as a whole. By the time most snails were aestivating, loss of body weight occurred at between 5-7% per fortnight approximately, reaching a maximum weight loss of about 28% of the original body weight. When the population broke aestivation, the rate of gain in body weight was rapid compared to the rate of loss during aestivation. During the month of March, all snails broke aestivation and by the end of March, the population body weight was back to the original weight before aestivation.

In the wet apartment, the situation did not exactly follow the pattern in the dry apartment. There was a general decrease in body weight with increase in number aestivating at a rate of 5% per fortnight, reaching a peak of about 15% in mid February. During the peak

Table 6: Correlations between climate and behaviour wet apartment

CLIMATIC CONDITIONS	AVERAGE DISTANCE MOVED					AVERAGE NO. AESTIVATING				
	Morning	Afternoon	Evening	Night	Day	Morning	Afternoon	Evening	Night	Day
<b>I. TEMPERATURE</b>										
Morning										
Afternoon										
Evening			—	—	—			*		
Night				—	—			*		
Day										
<b>II. REL. HUMIDITY</b>										
Morning	*				*	—				—
Afternoon										
Evening			**	**	**					
Night	**		**	**	**	—				
Day	**		**	**	**	—				
<b>III. VAP PRESS DEFICIT</b>										
Morning		*				*	*			*
Afternoon										
Evening			—	—	—					
Night	—		—	—	—	*	*	*	**	*
Day	—		**	**	**					
<b>IV. RAINFAIL</b>										
* +ve Sig. at 95%										
** 3ve Sig. at 99%										
— -ve Sig. at 95%										
— -ve Sig. at 99%										

r = 0.2656 at 95%

r = 0.3400 at 99%

Table 7: Stepwise multiple regression of climate against behaviour for wet apartment

AV. DIST. (TIME)	VPD (NIGHT)	VPD (MORN)	RAIN- FALL	TEMP (EVE)	RH (NIGHT)	TEMPT. MONTH	RH MORN	CONST	R%	STD ERROR
I. AV.DIST. (MORN)	-0.17904	--	--	--	--	--	--	1.73109	24	0.58054
AV.DIST. (AFT)	--	0.01321	--	--	--	--	--	-0.01601	7	0.11363
AV.DIST. (EVE)	--	--	0.01146	-0.09733	0.02209	--	--	0.89079	55	0.34385
AV.DIST. (NIGHT)	-0.05337	--	--	--	0.07144	-0.20239	--	-0.64529	54	0.44933
AV.DIST. (DAY)	-0.50711	--	--	-0.16367	--	--	--	8.50568	58	0.92424
II. AV.NO. AST(MORN)	--	--	--	--	--	--	-0.01231	1.25988	17	0.25559
AV.NO. AST(AFT)	--	0.02626	--	--	--	--	--	0.04886	8	0.22042
AV.NO. AV.NO.	0.03622	--	--	--	--	0.02930	--	-0.75257	19	0.20041
AV.NO. AST.(NIGHT)	0.09216	--	--	--	--	--	--	-0.05285	13	0.20196
AV.NO. AST.(DAY)	0.04161	--	--	--	--	--	--	-0.02169	11	0.21785

Table 8: Body weight change and mortality of snails kept in dry and wet apartments.

PARAMETERS	DRY		WET	
Average Initial Body wt., g.	266.41±21.90		291.02±20.37	
Average Body weight change g:				
18/12/77	-9.37±1.63	(0)	-5.27±4.08	(0)
3/1/78	-14.19±1.56	(0)	-9.94±3.68	(0)
17/1/78	-20.54±1.40	(0)	-14.18±3.56	(0)
31/1/78	-23.50±1.34	(9)	-13.25±4.26	(0)
14/2/78	-25.04±1.57	(18)	-14.98±4.42	(10)
1/3/78	-27.31±1.57	(27)	-9.85±0.97	(20)
16/3/78	-6.55±3.46	(36)	-6.51±2.14	(20)

Figures in parenthesis represent percentage mortality per group ± Standard Error of Mean

period, at no one time, was the percentage of the number of snails aestivating greater than 50. When aestivation was fully broken, the rate of body weight gain was slower than in the dry apartment.

#### Mortality

The mortality in each group is shown in Table 8. Correlations between body weight loss with mortality showed that the obtained values were not significant ( $P < 0.05$ ) either in the wet apartment or dry apartment. All estimates of snail movements and aestivation were calculated as average values per snail to take into account any bias due to sample number.

#### Relationships between average weight and mineral composition of epiphragms

The average weight and mineral composition of snail epiphragms are presented in Table 9. The nine elements analysed for in the snail epiphragm accounted for about 21% of the average weight of the epiphragms of which calcium accounted for over 90% of the total mean value of the 21.

Table 9: Average weight (q) and mineral composition of epiphragm

Av. weight Minerals	Mean 0.9362	Standard Error 0.090
% Calcium	19.2092	2.529
% Sodium	0.3483	0.062
% Phosphorous	0.2793	0.062
% Magnesium	0.3080	0.034
% Potassium	0.0718	0.016
% Iron	0.0111	0.018
% Zinc	0.0520	0.015
% Copper	0.0026	0.000
% Manganese	0.0058	0.001

However, reference to the correlation matrix, presented in Table 9, indicated that differences in the weights of epiphragms between the individual snails were not related to the variation in the percentage of calcium. The only minerals which were significantly correlated to epiphragm weight were sodium and phosphorous and in both cases, the correlation was negative.

The percentage manganese was positively correlated to the percentage sodium present, while percentage zinc was

Table 10: Correlations between average weight and mineral composition of epiphragm

Variables										
1. Weight	1.00000									
2. % Calcium	-0.03633	1.00000								
3. % Sodium	-0.68022**	0.56954**	1.00000							
4. % Phosphorus	-0.65563**	0.18583	0.55484*	1.00000						
5. % Magnesium	0.19103	0.66049***	0.47468	0.01306	1.00000					
6. % Potassium	-0.51307	-0.02939	0.47651	0.03573	-0.08992	1.00000				
7. % Iron	-0.30822	0.09423	0.41832	0.15241	0.21270	0.21397	1.00000			
8. % Zinc	-0.40480	-0.03315	0.40268	0.60577**	0.08109	0.07392	0.38025	1.00000		
9. % Copper	-0.11867	0.21054	0.16815	0.40532	0.02916	-0.12599	0.00818	0.40093	1.00000	
10. % Manganese	-0.35212	0.14855	0.56467*	0.35303	0.23560	0.13929	0.77132**	0.75174**	0.24929	1.00000

correlated to the percentage of phosphorous. A highly positive correlation exists between the percentage of manganese and those of Iron and Zinc.

### DISCUSSION

Considering the day and night observations of climatic variation and snail behaviour, it can be inferred that, like most living organisms, the daily behavioural pattern of the African giant snail, *Archachatina marginata*, could be greatly influenced by climate. The climatic variables most related to snail movements and aestivation were vapour pressure deficit (especially at night), relative humidity and rainfall.

A comparison of the climatic variable readings for the dry and wet apartments showed that both the temperature and vapour pressure deficit of the dry apartment were higher than those of the wet apartment. However, the relative humidity readings of the wet apartment were higher than those of the dry apartment. The daily addition of 8-litre volume of water to the wet apartment could possibly make for such a difference.

It was interesting to observe that, when compared on a mean daily, evening and night basis, the average distance moved by the snails in the dry apartment was significantly correlated to relative humidity and rainfall while it is negatively correlated to both vapour pressure deficit and temperature. Pollard (1975), found that the activities of *Helix pomata* were closely related to humidity and rainfall and that during the dry season, these snails could be completely inactive for long periods. He also observed individual snails remaining on the same spot consecutively for up to 14 and 11 days in June and July respectively. Observations during the course of the present study revealed similar facts and

further showed that if there were no interruptions to the snails' aestivations, a record of 20-25 days of snail remaining at the same spot in the dry apartment was common in the months of January and February.

In very hot weather, Pollard (1975) also noted that snails frequently climbed vegetations. During the present study, especially, immediately after rains in hot weather, snails were often seen to have climbed the chicken wire netting fence provided. Pollard (1975) suggested that the main reason for such actions was probably to escape the hottest layer of still air close to the ground. Wells (1943), working on factors determining activity in *Helix pomata* concluded that the pace-maker controlling snail activity was environmental moisture, acting as a stimulus to feed or by hydrating tissues.

One of the several determinants of the amount of moisture in the environment is temperature, because the higher the temperature, the less humid the atmosphere. It was quite revealing to note during the present study that night vapour pressure deficit and rainfall appeared to be the most important factors predicting the average distance moved and together explained about 50% of the observed variations. When the dry and wet apartments were compared, there were no significant differences in the snail behaviour of both apartments in the afternoon — when temperature was most high. This was because, around this period, the snails generally did not move in either apartments, and no differences were noted in the snails behaviour in either of the two apartments. A study with different temperature gradients as the major variable may reveal more remarkable relationships between this climatic factor and

snail movement.

Snail aestivation is generally regarded as a response to unfavourable environmental conditions (Wells, 1943). Results in the present study showed that climatic factors were important in controlling both the number of snails aestivating and the duration of aestivation.

Vapour Pressure Deficit and temperature were shown to be positively correlated to the average number of snails aestivating while relative humidity and rainfall were negatively correlated. Average daily vapour pressure deficit and also the deficits recorded in the evening and night clearly indicated that with increase in vapour pressure deficit appeared to account for the largest proportion of the observed variations in snail aestivation. In most cases, this was the primary influential factor, with other subordinates like the night temperature and relative humidity also being important.

During the course of the present study there was no occasion when all the snails went completely into aestivation in the wet apartment. The maximum of about 50% went into aestivation during the periods from January to mid February. Thus, the effect of watering was obviously critical in controlling the formation of the epiphragms, but all the same, it could not prevent aestivation completely from amongst the snails.

The percent decrease in body weight was higher in the dry apartment, about 5-10% per fortnight reaching a maximum of about 28%; while it was 5% with a maximum of about 15% in the wet apartment. The main reason for the few snail aestivating in the wet apartment could be due to the interruption during the wetting process and the moist conditions itself. With the advent of the

rainy season, there was rapid increase in the weight of snails in the dry apartment. Most of the snails in the dry apartment broke aestivation at this time to feed on the plant materials in their environment and of course experience a more humid condition. The slower rate of increase in weights of snails in the wet apartment may be due to the fact that they had been experiencing some moist climate throughout the period of study.

Considering the mineral analysis of the epiphragm, calcium which was clearly the most abundant accounted for 19% of total weight of the epiphragm. This fact has been confirmed by other recent studies (Segun, 1975 and Mead 1961). Calcium is also the most abundant mineral in the snail shell (Ajayi *et al.* 1978), but in this case, it accounts for 86% of the weight. Others died of what appeared to be a more complex bacterial and viral infection — as shown by the results of some post-mortem examinations. However, this is a subject for further investigation.

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## OCULAR LESIONS ASSOCIATED WITH *TRYPANOSOMA (TRYPANOZOON) BRUCEI* INFECTION IN THE RABBITS

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### SUMMARY

The ocular lesions associated with *Trypanosoma brucei* infection in the rabbits have been described.

Numerous trypanosomes were found in aqueous and vitreous humour, cornea, sclera, iris and ciliary processes. These were associated with extensive infiltration of the *substantia propria* of the cornea by mononuclear cells, extensive vascularization, extravasation of red blood cells from newly formed capillaries and proliferation of some connective tissue cells. All these changes were confined to the anterior two thirds of the *propria* and the posterior third was not affected.

Ocular lesions associated with *brucei*-group trypanosomes seem to be more common in rabbits than in other animals.

### INTRODUCTION

The occurrence of trypanosomes in different parts of the eye of various species of animals infected with trypanosomes of subgenus *Trypanozoon* has been widely observed. Trypanosomes were found in eyes of dogs infected with *T. brucei* and *T. equiperdum* (Morax 1907; Leber 1908; Woods and de Schweintz 1917; Neame 1927) dogs and rabbits infected with *T. evansi* (Goel and Singh 1970); goats infected with *T. brucei*, *T. rhodesiense* and *T. equiperdum* (Morax 1907; Yorke 1910), and in the aqueous and vitreous humour of sheep infected with *T. brucei* (Ikede and Losos 1972).

Daniels 1918 observed keratitis in about 35.7% of the humans suffering from sleeping sickness. In most of the above reports numerous trypanosomes were found in the *substantia propria* of the cornea, in the iris, ciliary processes and in the aqueous and vitreous humour. The occurrence of the trypanosomes in these various parts of the eye was also associated with interstitial keratitis and iritis.

In this paper, ocular lesions in rabbits infected with *T. brucei* in which numerous trypanosomes were found in the aqueous and vitreous humour, in the *substantia propria* of the cornea, in the iris and ciliary processes are reported.

### MATERIALS AND METHODS

Three rabbits, 2 males and a female of different breeds and weighing between 3 to 3½ kg were supplied by the Small Animal Breeding Station of Edinburgh University. They were infected with *Trypanosome brucei* KBI (Kings Building I) which is a derivative of TREU 667 as described previously (Ssenyonga 1974). Originally these rabbits were used for preparation of antitrypanosomal sera. Blood for serum was collected from the ear vein at 5 days' interval for 25—30 days.

They were killed at different intervals and tissues particularly the eyes, brain, heart, epididymis, trigeminal nerve, skin around the testicles and face pituitary and adrenal glands were fixed

in acidified alcohol and Zenker's fluid for histological sections.

Before the eyes were fixed, samples of the aqueous and vitreous humours were withdrawn with a 23 — gauge needle attached to 5 ml syringe, put on a clean slide and examined wet under a microscope for trypanosomes. Each eye was divided into two by a longitudinal incision and the aqueous and vitreous humours was collected and was also examined for the presence of trypanosomes. Half of the eye was fixed in acidified alcohol and the other half into Zenker's fluid sectioned at 6 $\mu$ , and stained by Giemsa colophonium technique.

## RESULTS

In the course of the infection, the rabbits developed conjunctivitis, corneal apacity, photophobia and blindness in either one or both eyes. Two rabbits became scabby around the muzzle, face, ears and scrotum. One of them became markedly emaciated.

The most striking lesions were found in the eyes. The frequency of distribution of trypanosomes in the aqueous and vitreous humours is shown in the table below:

No. of Rabbit	Duration of Infection	Right Eye Aqueous	Vitreous	Left Eye aqueous	vitreous
1	47 days	++	+	+++	+
2	27 days	+	—	+++	+
3	35 days	+++	+	+++	—

+++ = very numerous (teeming)

+ = few

++ = Numerous

— = Absent.

## Distribution of trypanosomes in the eye

Numerous trypanosomes were found in the *Substantia propria* of the cornea (Fig.1), in the iris, ciliary process and sclera. The localization of trypanosomes in these tissues was accompanied by extensive infiltration with mononuclear cells and a few polymorphs. In the cornea this was also accompanied by extensive vascularization of the propria, extravasation of red blood cells from the newly formed capillaries and proliferation of some connective tissue cells (Fig. 2). All these reactions were confined to the anterior two thirds of the propria and the posterior third was not affected.

Three distinct areas of reaction could be observed in the cornea. Starting at the limbus (cornea-sclera junction) the first area showed numerous inflammatory cells and no trypanosomes. The second area contained many inflammatory cells and few trypanosomes. The third area which was far away from the limbus and near to the centre of the cornea was characterized by the presence of numerous trypanosomes and the cellular infiltration was generally absent.

The same picture was observed with the blood-vessels. The area adjacent to the limbus contained bigger and more

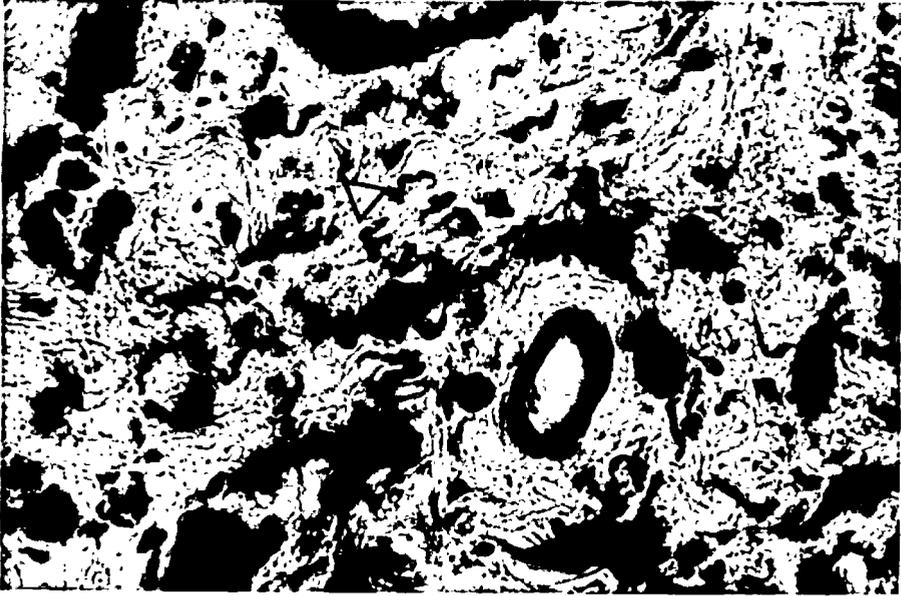


Fig. 1: *Substantia propria of the cornea*. 35 days after infection with *T. brucei* x 560.

Numerous trypanosomes and inflammatory cells can be seen in the area. Note also the extensive vascularization of the propria. Giemsa colophonium.

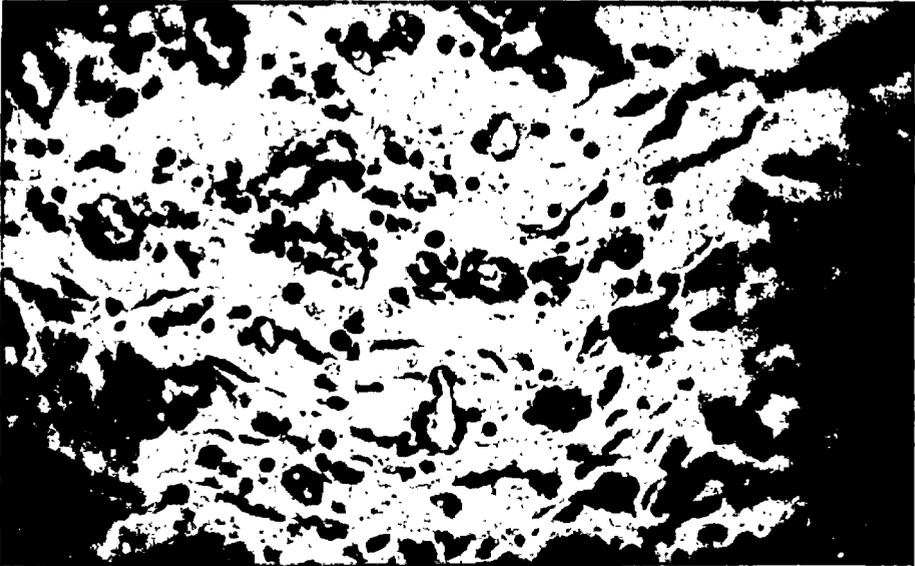


Fig. 2: *The entire cornea* — 47 days after infection x 280. The section shows extensive cellular infiltration of the *substantia propria* of the cornea, extensive vascularization and extravasation of red blood cells from newly formed capillaries. The section was mixed in Zenker's fluid stained by Giemsa colophonium technique.

blood vessels while that area of the cornea far removed from limbus showed fewer and smaller vessels.

The eyes of the rabbits which were severely affected during life showed more extensive infiltration with trypanosomes and inflammatory cells.

In other tissues examined, numerous trypanosomes were found in epididymis and the skin, few trypanosomes were found in the heart and pituitary gland and no trypanosomes were found in the brain, trigeminal nerve and adrenal gland.

## DISCUSSION

The description of the occurrence of trypanosomes in the cornea in relation to the inflammatory cells and the blood vessels agrees very well with the observations of Morax (1907). The picture described above suggests that since the cornea is physiologically avascular, trypanosomes invade the cornea from the limbus. As they multiply in the *substantia propria*, they provoke an inflammatory reaction with the formation of oedema followed by invasion with inflammatory cells from the limbal vessels in the affected area. This is then followed by proliferation of the capillaries from the limbus into the affected part of the cornea. The trypanosomes keep on spreading to new areas of the cornea, followed by inflammatory cells and picture is accentuated by the vascularization of the cornea.

It is interesting to note that all these changes were confirmed to the anterior two thirds of the propria; the posterior third was not affected. This is possibly because of the laminated structure of the corneal stroma which tends to con-

fine the reaction almost entirely between those layers in which the invading organisms are multiplying. It is also interesting to note that the aqueous humour contained on the whole more trypanosomes than the vitreous humour.

It is unlikely that the trypanosomes invade the cornea from the aqueous humour because there is no evidence of the area adjacent to the posterior epithelium showing lesions.

The iris and ciliary processes being highly vascular, were uniformly infiltrated with trypanosomes and inflammatory cells throughout the whole interstitial tissues.

Unlike in mice and rats where the eye lesions were not prominent (Ssenyonga, 1974), in rabbits the eyes appeared to be highly preferred by the trypanosomes.

Though some investigators thought that eye lesions were due to toxic substances liberated by trypanosomes rather than the actual presence of trypanosomes in eye tissue (Leber 1908), it is quite evident from this work and those of others (Morax 1907; Yorke 1910; Woods and de Schweintz 1917; Neame 1928) that the eye lesions are caused by the actual presence of trypanosomes in the tissues.

The affinity of trypanosomes for the aqueous humour of dogs and rabbits experimentally infected with *T. evansi* has been suggested as a possible means of diagnosis of Surra in animals (Goel and Singh 1970). This work also supports this suggestion.

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## SERUM MINERAL STATUS OF NORMAL AND *DERMATOPHILUS CONGO-LENSIS* INFECTED FRIESIAN CALVES

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### SUMMARY

Serum magnesium, copper, calcium, zinc, sodium and potassium levels were estimated in normal and streptothricosis infected Friesian calves. There was no statistically significant difference in all the minerals determined between normal and infected animals although slightly higher and slightly lower mean serum copper and sodium were obtained respectively for infected animals. It was concluded that apart from mineral deficiencies speculated to predispose the animals to streptothricosis, other factors are involved.

### INTRODUCTION

Bovine cutaneous streptothricosis caused by *Dermatophilus congolensis* (Van Saceghem 1915), a filamentous, septate and branching mycelial organism with coccoid elements, is an important disease of cattle in West Africa as a whole and in Nigeria in particular where it accounts for a major part (usually 50%) of all bovine skin diseases (Oduye and Lloyd 1971). The importance of this disease lies in the spoilage of hides, loss of condition of the animal from toxæmia and difficulties in movement and prehension and death when the disease is severe and generalized (Jubb *et al* 1970).

Mineral elements found in the body of animals are of great importance in a number of physiological activities and may be found in either increased or decreased quantities in association with certain diseases (Coles 1974). Friot

and Calvet (1971) speculated that bovine cutaneous streptothricosis might be bound up with zinc deficiencies. Amakiri (1976) found that serum zinc level of *Dermatophilus congolensis*-infected white Fulani cattle was significantly lower than in healthy white Fulani cattle. Kapu (1975) found that the mean serum Zn, Ca, and Mg levels in Zebu cattle with dermatophilosis under grazing conditions were significantly lower than in normal cattle and the converse was true of serum Cu and K.

The present report describes six mineral values in normal and streptothricosis infected Friesian calves.

### MATERIALS AND METHODS

Thirty seven animals with no physical evidence of infection with *D. congolensis* and 15 animals shown bacteriologically to be infected with *D. congolensis* were used. The animals were from Live-stock Investigation Centre (L.I.C.) of the Veterinary Research Institute, Vom, during the rainy season. They were pure-bred Friesian calves and with age ranging from three to twelve months.

The cattle at L.I.C. normally receive according to age, milk, hay and concentrates, groundnut cake, cotton seed cake, maize, churn mineral, vitamin mixture, meat and bone meal.

Bleeding was from the jugular vein using vacutainer tubes. The blood was allowed to clot at room temperature, centrifuged and serum separated within three hours after collection. The serum samples were stored at -25° C until assayed. Calcium, copper, magne-

sium and zinc were determined by atomic absorption spectrophotometry using Pye Unicam SP 1900 as described in the Pye Unicam method sheet. Calcium absorption was measured at a wavelength of 422.7 nm, magnesium at 285.2 nm, copper at 324.7 nm and zinc at 213.86 nm.

Sodium and potassium were estimated by flame photometry as described by Varley (1969).

## RESULTS

Table 1 shows the results of the evaluated serum mineral values with the range, mean and standard deviation for both normal and infected calves. There was no significant difference ( $P > 0.05$ ) in all the minerals evaluated between normal and infected animals.

## DISCUSSION

Table 1 shows that there was no significant statistical difference in mean serum mineral levels of normal and infected animals, but a slightly higher and slightly lower mean serum copper and sodium respectively were obtained for infected animals. Since Friot and Calvet (1971) speculated that Bovine cutaneous streptothricosis might be bound up with zinc deficiencies, some workers

have tried to substantiate this speculation by estimating serum zinc in particular and some other serum minerals in streptothricosis infected animals. Among these is the work of Kapu (1975) who found that the mean serum Zn, Ca, and Mg levels in Zebu cattle with streptothricosis were significantly lower than in normal animals.

Of great interest are the findings of Amakiri (1976) who found that zinc ( $\mu/100\text{ml}$  serum) was significantly higher ( $P > 0.01$ ) in the White Fulani (419.64) than in the N'Dama cattle (343.00) and significantly higher in the healthy than in the streptothricosis infected White Fulani Cattle (375.00). The White Fulani is known to be susceptible whereas the N'Dama is resistant to Dermatophilus infection (Amakiri 1976). Amakiri's report shows that the serum zinc level of the N'Dama which is resistant is lower than that of the infected White Fulani. This confirms the well known fact of differential breeds nutrient requirements. The results of Kapu (1975) and Amakiri (1976) does not agree with the present findings. The question that readily comes to mind with regard to the mineral work in streptothricosis animals is, did the reduction in serum mineral occur as a result of the infection or did the reduction predis-

Table 1: Summary of Serum Mineral Values in Normal and Streptothricosis Infected Calves

		Magnesium mmol/l	Copper mmol/l	Calcium mmol/l	Zinc mg/l	Sodium mmol/l	Potassium mmol/l
Streptothricosis Infected Animals	Range	0.90-0.99	8.79-19.47	2.25-2.75	1.40-1.88	123.75-145.75	3.80-6.96
	Mean	0.95	13.82	2.47	1.64	136.62	5.65
	Standard Deviation	0.03	3.36	0.21	0.19	8.44	1.17
Normal Animals	Range	0.61-1.15	7.85-18.84	2.00-2.75	1.24-2.22	140.25-145.75	4.64-5.98
	Mean	0.96	12.66	2.47	1.60	144.00	5.17
	Standard	0.10	2.16	0.19	0.24	2.70	0.50

pose the animals to infection? The findings of Kapu (1975) and Amakiri (1976) did not show that the serum mineral levels of Dermatophilus animals reached acceptable critical deficiency levels. Infact, the serum zinc level in both normal and infected animals in Amakiri's findings were above what is regarded as normal values, 150-180 mg/100ml serum (Underwood 1971). These facts tend to indicate that if mineral deficiency predisposed the animals to infection, it must be that even though the minerals were available in the system of the animals, for one reason or the other the animals were unable to metabolise them.

The present findings tend to indicate that it is not mineral deficiency that predisposed the animals to infection but that the drop in serum mineral levels might have arisen as a result of the effect of the disease, and thus there could be differential breed physiological reaction to the presence of the disease. This is further confirmed since it is known that profound changes in the zinc content of the plasma and cellular elements occur in various diseases like with chronic and acute infections (Vikbladh, 1950). This could explain the lack of any significant difference in serum minerals determined among the two groups in this work.

Thus, there is an indication from these studies that other factors apart from

mineral deficiency might be involved in predisposing the animals to infection. These may include cellular resistance factors as speculated by Amakiri (1977) and hereditary predisposition as shown by the work of Dumas *et al* (1971).

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## TRANSMISSIBLE ENTERITIS OF TURKEYS (BLUECOMB) IN NIGERIA

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### SUMMARY

A clinical outbreak of Transmissible enteritis (Bluecomb) of turkeys is described in a flock of commercial turkey poults imported as day olds from Britain. The signs of the disease were sudden onset and high morbidity rate within the flock. Sick birds chilled, chirped and warmed constantly. Other signs were depression, loose droppings, diarrhoea, dehydration, emaciation and high mortality. Typical gross lesions were transparent intestines with watery content, together with distended caeca with frothy material. The predisposing factors of the disease include the long periods the birds were kept before being collected at the port of entry, and adverse weather conditions.

### INTRODUCTION

Transmissible enteritis (TE) is a specific disease of turkeys of all ages particularly young birds. It was first described in the state of Washington in the USA in 1951 by Peterson and Hymas. Pomeroy and Sieburth (1953) reported that the disease occurred in Minnesota in the USA in 1951. In Virginia USA Sieburth and Johnson (1957) described it as a serious problem in young turkeys, while Ferguson (1961) made a similar report on the occurrence of TE of turkeys in Ontario, Canada.

The exact distribution of TE is unknown but published reports indicate that the disease is widely distributed in turkey-growing areas of the USA and Canada.

Truscott *et al.* (1960) isolated a small gram-negative anaerobic pleomorphic rod from the intestinal tract of affected birds which they considered to be the etiological agent. Truscott and Morin (1964) further characterised the agent as a member of the genus *Vibrio* and they reproduced the disease with vibrio cultures. Recent work by Deshmuk *et al.* (1969) suggests that probably a reovirus is the causative agent of TE. So far this group has been found to include nine viruses and all seem to produce the disease. These viruses have been found in the serum liver, and spleen of infected turkeys. Ritchie *et al.* (1973) in additional studies on the etiology reported that electron microscopy of the intestinal preparation revealed the presence of corona virus-like particles in turkey bluecomb disease. Dzuik *et al.* (1969) reported that it is possible to mimic the clinical signs of TE by feed and water deprivation, they concluded that any agent which establishes on infection that results in anorexia may produce clinical signs similar to TE.

Topp and Pettit (1972) reported that the etiological agent is spread through the faeces of infected birds, that it survives several years in frozen faecal matter in the laboratory and that its resistance to germicide is so far unknown. Topp and Pettit (1972) also observed that recovered turkeys excrete the agent for a long time and as such are carriers.

Various attempts to infect chickens were unsuccessful (Pomeroy and Sieburth and Johnson, 1951). Mortality of up to 50% of young turkeys has been

reported, Biester and Schwarte, (1972). The characteristic gross postmortem changes reported in the literature include, watery and gaseous content of duodenum, jejunum and caeca, the caeca are often distended and filled with watery, yellowish content; the breast muscle usually appear dehydrated and the carcass generally emaciated; urate deposits may occasionally be present in kidneys and ureters (Topp and Pettit 1972, Biester and Schwarte 1972).

This report describes a recent outbreak of TE, involving a flock of 760 commercial turkey poultts imported into Nigeria from Britain together with the clinical signs and pathology associated with the disease.

### Case History

The turkeys were imported by air when day old from a reputable commercial supplier in Britain, and comprised of broilers and some parent stock. Owing possibly to communication problems, they were not collected from the port of entry until the fourth day after landing. They were vaccinated at source against Newcastle Disease. The brooding management at the farm in Nigeria which received the birds was considered adequate. Starting from the seventh day, however, mortality in the flock rose from an average of 7 a day to over 50 a day, until in the third week all but 4 had died. The observable clinical signs were depression, loss of appetite weight loss, and wet sometimes frothy droppings sick birds tended to cuddle around the source of heat and made lots of noise. There was also marked diarrhoea dehydration and emaciation, birds became weak and their droppings contained mucus and urates.

### Necropsy Findings

The typical gross lesions were severe dehydration of the carcass, seen by the changes in the breast muscle and kidneys; and the accumulation of urates in the ureters. The intestines were transparent, dilated, flaccid and thin-walled with watery contents. The caeca were filled with characteristic frothy materials. The gross lesions were seen primarily in the intestinal tract. The other internal organs appeared normal.

### Microbiology

Pieces of liver, spleen, heart blood and caecum were collected as aseptically homogenised individually in nutrient broth and inoculated onto blood agar plates. Thin film of clear swarming growth and few large creamy white colonies were recovered from the liver, when the plates were examined 48hrs after inoculation. Gram positive rods identified as *Diphtheroids* were isolated from the liver. Bacteria identified on the basis of morphology, staining, biochemical and colony characteristics as *Proteus mirabilis* and *Pseudomonas putida* were isolated from caeca.

Portions of spleen and liver samples from the dead birds which were submitted to the Virology department of the Veterinary Institute for virus isolation yielded negative results.

### DISCUSSION

In the absence of any specific laboratory procedure for diagnosing the disease, the history of the outbreak, the course of the disease and the gross postmortem lesions were emphasised in the diagnosis of the condition. A clinical history in turkey poultts characterized by weight loss, frothy dropping, dehydration followed by death suggests possibilities of transmissible enteritis of turkeys,

hemorrhagic enteritis, coccidiosis, arizonosis of poult or black head. Hemorrhagic enteritis, coccidiosis and black head were easily eliminated at the post-mortem table. When epizootiology, clinical signs and gross pathology are considered arizonosis of poult remains outstanding as a differential diagnosis. Distended caeca filled with watery yellowish brown content having a foetid odour will according to Biester and Schwarte, (1972) suggest transmissible enteritis of turkeys, when a cheesy caecal core and eye lesions resulting in opacity, partial or complete blindness is presented this suggests arizonosis of poult. Neither cheesy caecal core nor eye lesions were found in the poult presently discussed. This finding together with clinical signs, mortality pattern and other postmortem lesions confirms the diagnosis of transmissible enteritis of turkeys.

In earlier outbreaks the disease was designated trichomoniasis of the lower intestine, because of the presence of increased number of trichomonads, in the caeca and rectum of affected birds, Biester and Schwarte, (1972). No trichomonads were recovered from the caecal content of this particular outbreak. There was no evidence from other workers that the disease is egg-transmitted, but the infection may have been introduced into started poult in a hatchery battery room and then transmitted to the poult shipped from the hatchery.

The most important consideration for treating affected birds was to increase

the pen temperature until it was approximately 85° F, or until the birds were comfortable and ceased to chirp. The temperature could be decreased gradually as the birds improved. Calf milk replacer was given to sick birds in drinking water to boost appetite and provide good source of nutrition. At present there is no vaccination against transmissible enteritis of turkey. When turkey poult are imported from abroad adequate arrangement should be made for their prompt collection from the port of entry to offset adverse effects of stress.

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## GESTATION PERIOD AND WEIGHT CHANGES DURING PREGNANCY IN THE NIGERIAN DWARF SHEEP

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### SUMMARY

The gestation period of 29 ewes of the Nigerian Dwarf Sheep were analysed in order to determine the effects of various influences upon it. Of the total number of ewes, five had a second pregnancy during the period of study and thus a total number of 34 gestation periods were studied.

The average length of the gestation periods was  $147.4 \pm 2.4$  (142-153) days. This was neither significantly affected by the liveweight and age of the ewe at breeding, nor by the sex of the lamb, nor by the birthweight nor by the number of lambs per parturition (singles or twins). During pregnancy, the ewes gained an average of 9.5 kg liveweight of which 3.8 kg were lost at parturition as products of conception (lamb birth weight, 2.03 kg; foetal membranes etc, (1.8 kg), indicating an anabolic tissue weight gain of 5.7 kg. Highest weight increases of the dams during pregnancy were recorded at the last trimestre. Pregnancy weight gains for ewes carrying twins were not significantly higher than those of single bearing ewes. Ewes did not show any oestrous symptoms during pregnancy.

### INTRODUCTION

Literature reports on pregnancy in various breeds of sheep (Terrill and Hazel, 1947, Terrill, 1968, Asdell, 1964; Quinlan and Mare, 1931; Hunter, 1957) have shown that the breed of sheep has an important effect on the length of the gestation period. There is to date no scientific report on studies on the gestation period of the Nigerian Dwarf Sheep. This experiment was therefore, set up to study as accurately as possible some important parameters of pregnancy in the Nigerian Dwarf Sheep namely;

- (i) the duration of the gestation;
- (ii) the pattern of weight changes during pregnancy;
- (iii) the effects of various other factors on the duration of gestation.

### MATERIALS AND METHODS

Twenty-nine (29) ewes aged between 1 and 3 years and 5 adult rams — 2 of which were vasectomized were used in the investigation. Of the 29 ewes, five had a second pregnancy during the period, bringing the total number of pregnancies studied to thirty four. Twenty one of these pregnancies were started during the dry season (January/February) while 13 others started during the rainy season (May to August); seven of the ewes were bred as ewe lambs at the 3rd heat after puberty. Throughout pregnancy the ewes received supplementary concentrate ration at the rate of 454 gm/day, in addition, they

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were allowed free grazing all day. Trees and shrubs along the fences provided shade and browsing for the animals during hot afternoons. Mineral lick, hay, cut green fodder of giant star grass mixed with *centrosema pubescens* legume and water were provided freely in the pens.

#### Mating system

Before breeding, the ewes were checked for heat with the two vasectomized rams, every morning and evening. All observations were made in the pens. An ewe detected on heat in the morning was hand bred in the evening to one of the 3 fertile rams. Those first detected in the evenings were bred the following morning. Checking of the ewes for heat continued throughout pregnancy with the vasectomized rams in the mornings and evenings.

#### Weighings

The ewes were weighed at breeding and once a week thereafter (on Saturdays) throughout pregnancy. Lamb birth weights were taken on a spring balance as soon as the dam had completed drying the lambs, except for lambings made during the night, where weighings were done first thing in the morning, before 08 hours. Dam weights were taken soon after dropping the afterbirth.

#### Duration of pregnancy

The duration of pregnancy was calculated as the interval between the day of breeding and the day of lambing. The day of breeding was counted as day 1.

#### RESULTS

##### Duration of pregnancy

The duration of the pregnancies is summarised in table 1.

Table 1: Length (days) of pregnancy in the Northern Dwarf Sheep

Variable	n	Mean (days)	SE	range (days)	mode
All pregnancies	34	147.4	0.38	142-153	148
First pregnancy	29	147.3	0.42	142-153	148
Second pregnancy	5	148.4	1.34	144-152	—

where n = no. of pregnancies

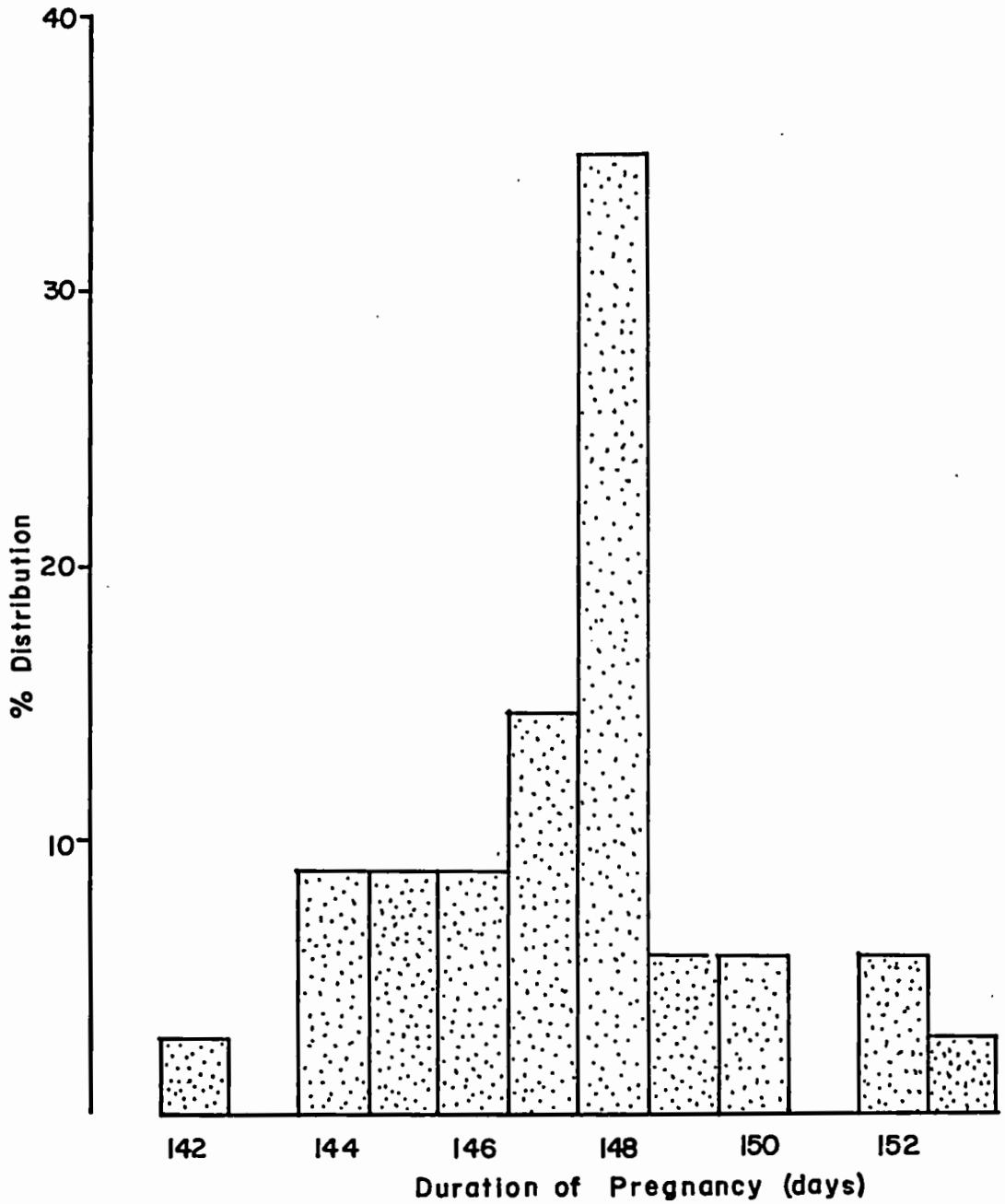


Fig. 1: Frequency distribution of the duration of pregnancy in the Nigerian Dwarf Sheep.

**Table 2: The influence of the type of birth and the sex of the lamb on duration of pregnancy.**

Variable		n	Duration of pregnancy (days)	t-value
Type of birth	Twins	6	148.00±0.52	0.63
	Singles	28	147.32±0.48	
Sex	Ram lambs	19	147.58±0.65	0.34
	Ewe lambs	15	147.29±0.48	

The average length of the gestation period was  $147.4 \pm 0.38$  days range 142-153 days. The pattern of distribution is presented on figure 1. The modal value is 148 days. This forms 35% of all the gestation periods. The differences between the length of the first and second gestation periods (Table 1); between those pregnancies initiated in the dry season ( $147.57 \pm 0.46$  days) and those in the rainy season ( $147.31 \pm 0.78$  days) were not significant. All subsequent analysis were therefore based on all the pregnancies. The age at breeding and the liveweight of the ewes at breeding had no significant effect on the length of gestation. The type of birth (twins Vs singles) and the sex of the lambs at birth had no significant effect on the length of gestation (Table 2). The birth weight of the lambs had also no significant effect on the length of gestation. There was however, significant side effect on the length of gestation ( $P < 0.05$ ).

#### Weight changes during pregnancy

The differences between the weights of the ewes at breeding and just before parturition in both pregnancies (first and second pregnancies) and in both seasons (wet season, April to September and dry season, October to March) were not significant. However, ewes

bearing twins were significantly heavier at breeding than ewes bearing single lambs. However, weight increases during pregnancy in the twin bearing ewes were not significantly different from weight increases in the single lamb-bearing ewes. The pattern of weight increases during pregnancy is shown in figure 2. The greatest increase was recorded from the 15th week to term — the last trimestre. The log-log growth constant for the ewes during pregnancy is shown in Table 3. The ewes gained on the average  $9.50 \pm 0.4$  kg range 4.0-14.5 kg, liveweight during pregnancy.

**Table 3: Growth constants (b) for the various stages of pregnancy (log-log constants)**

Phases of pregnancy	growth constant
1st Trimstre	0.04
2nd Trimestre	0.25
3rd Trimestre	0.34

Out of this liveweight gain,  $3.8 \pm 0.2$  kg, range 2.0-6.0 kg, were lost at parturition as products of conception namely;  $2.03 \pm 0.06$  kg, range 1.0-4.5 kg, as lamb birth weight and  $1.8 \pm 0.11$  kg, range 1.0-3.0 kg, as foetal membranes and fluids.

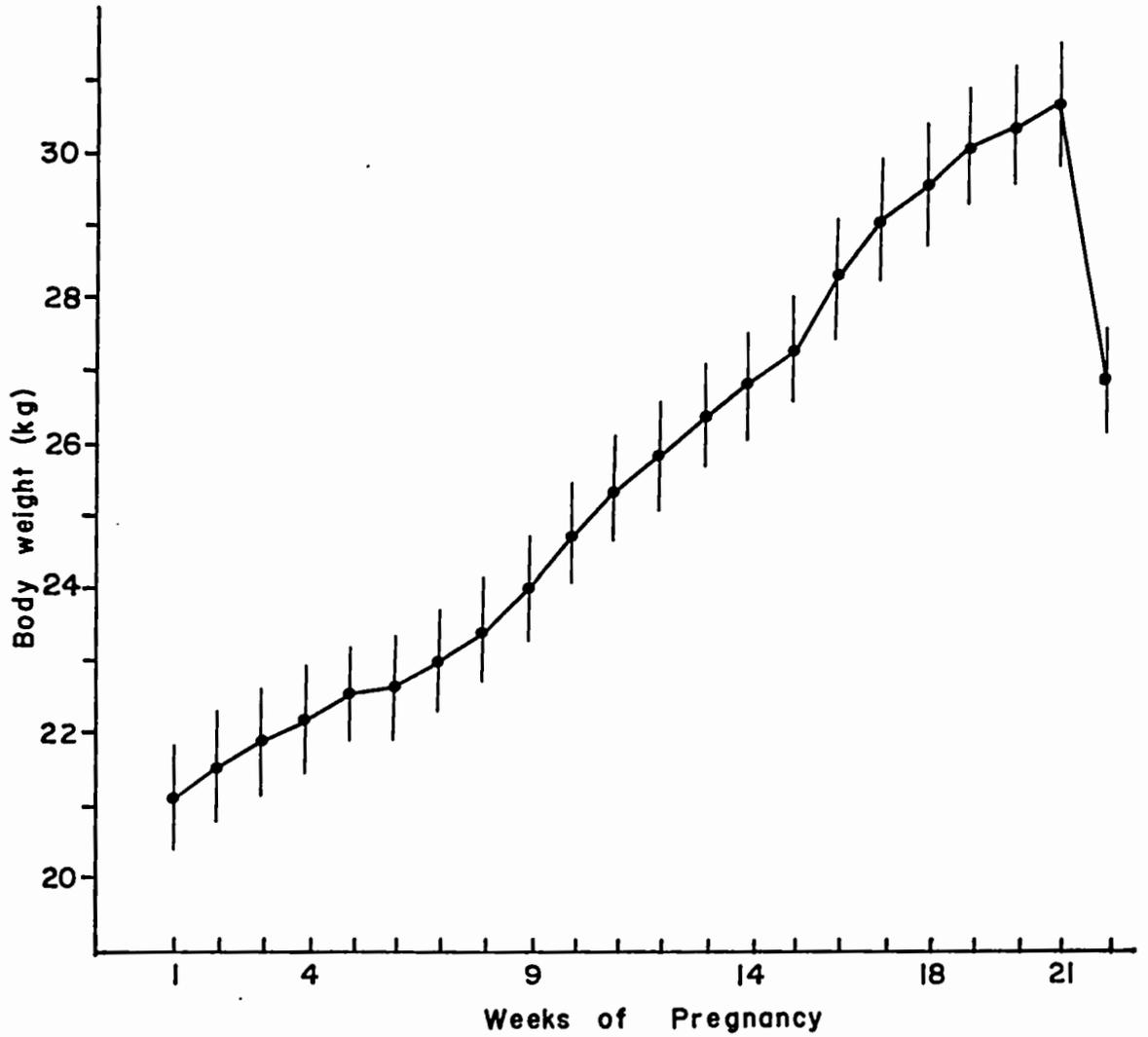


Fig. 2: Weight changes in the pregnant ewes of the Nigerian Dwarf Sheep.

The remaining  $5.7 \pm 0.4$  kg, range 2.0-10.5 kg, constitute the anabolic tissue gain and gain due to the young age of the ewes. Fluids and membrane weights from ewes carrying singles did not differ significantly from similar tissues from ewes carrying twins. However, the weights of products of conception before removing the lambs birth weight was significantly higher in the pregnancies that resulted in twins ( $5.4 \pm 0.16$  kg, range 5.0-6.0 kg) than in those that resulted in singles ( $3.4 \pm 0.15$  kg, range 2.0 to 5.0 kg).

#### Oestrous behaviour during pregnancy

None of the ewes either came on heat or expressed oestrous behaviour in the presence of the teaser rams during pregnancy. Instead, there was active avoidance of the teasing rams.

#### Lamb birth weight

The mean body weight of the lambs generally was  $2.03 \pm 0.06$  kg, range 1.0-2.75kg. The differences between the birth weights of the sexes were not significant (Table 4).

However, single ewe lambs were significantly heavier than twin ewe lambs while the birth weights of the single and twin ram lambs were not significantly different. In general, singles were 8% heavier than twins at birth, the differences between the singles and twins were wider (though not significantly) in the females than in the males.

When the births were regrouped according to the month they occurred, those lambs born in December/January were slightly lighter ( $1.6 \pm 0.23$  kg) than those born in June/July ( $2.13 \pm 0.09$  kg) and October/November ( $2.13 \pm 0.18$  kg).

Table 4: Birth weights of lambs of the Nigerian Dwarf Sheep

Variable	n	Means (kg)	SE	Range (kg)	t-value
Ram lambs	22	2.02	0.09	1.09-2.75	0.09
Ewe lambs	18	2.03	0.04	1.5-2.5	
Single ram lambs	17	2.01	0.12	1.0-2.75	0.94
Single ewe lambs	11	2.17	0.11	2.0-2.5	
Twin ram lambs	5	1.95	0.05	1.75-2.5	0.87
Twin ewe lambs	7	1.85	0.09	1.75-2.5	

**Table 5: Length of pregnancy (days) in selected breeds of sheep**

Breed	Location	Duration pregnancy (days)		Reference
		Mean	Range	
Boarder Leicester Dorset Horn	England	144.7	—	Hunter (1957)
	Australia	144.1	138-149	Daley & Eastoe (1948)
Hampshire	Missouri	144.6	140-147	Mckenzie & Philips (1932)
Merino	S. Africa	149.0	142-156	Quinlan & Mare (1931)
Romney	New Zealand	148.7	144-153	Dry (1933)
Welsh Mountain	England	147.1	—	Hunter (1957)
Nigerian Dwarf	Ibadan Nigeria	147.4	142-153	Present study

**Table 6: Birth weight of the lambs in relation to the weight at breeding of selected breeds of sheep**

Breed	Wt. at breeding (kg)	Average lamb birth weight (kg)	Birth wt. Dam wt. %	Reference
Boarder Leicester	60.55	5.45	9.00	Hunter (1957)
Welsh Mountain	33.82	4.00	11.83	
Yankassa	30.91	3.77	12.21	Ferguson (1964)
Ouda	30.00	3.75	12.50	
Nigerian Dwarf	21.12	2.03	9.61	Present study

## DISCUSSION

The length of gestation in the Nigerian Dwarf Sheep is within the range of values published in the scientific literature on other breeds of sheep (Table 5). Although in the present study the age of the ewes at breeding and the live weight at breeding had no significant effect on the length of gestation, earlier investigations (Terrill and Hazel 1947) had found some significant increase in

the length of gestation with advancing age of the ewes. This discrepancy could be partly attributed to the total number of pregnancies and the actual pregnancies studied. Besides the differences in the ages of the ewes used in the current study appeared too narrow to be able to give a clear separation of the effects of the age at breeding on the length of gestation unlike ewes varying in age from 2 to 9 years considered by the former authors.

The significant sire effect on the length of gestation in the Nigerian Dwarf Sheep is in conformity with the results of Terrill and Hazel (1947) in a flock of range sheep.

McLaughlin (1970) had observed that within breeds of Merino sheep, an increase in the body weight of the ewes at breeding is associated with an increase in multiple births. In the present study on the Nigerian Dwarf Sheep, ewes bearing twins were significantly heavier at breeding than ewes bearing single lambs. During pregnancy, the pattern of weight changes shown in figure 2 and the log-log growth constant in Table 3 tended to follow the pattern of growth of the foetus described by Hafez (1968), Hammond (1957) and Thomson and Aitken (1959) where the maximum growth of the foetus (about 70% of the final weight) was recorded at the last trimestre.

The Nigerian Dwarf Sheep is a small sized sheep ecotype whose female mature body weight is about 32 kilograms (Orji, 1976). The weights of the products of conception recorded in this investigation is therefore lower than those of the larger breeds of sheep published in the literature, for example, in the Hampshire sheep, Guyer and Dyer (1954 cited by Thomson and Aitken 1959) estimated the weights of the products of conception as 7.73 kilograms in the ewes bearing single lambs and 12.73 kg in the ewes bearing twin lambs. In Australia, Papadopoulos and Robinson (1957) recorded 11.36 kilograms for Merino ewes and their crosses with single lambs and 17.73 kilograms for those with twins. Thus these differences are largely due to the differences in the sizes of the breeds.

On oestrous behaviour during pregnancy, although the pregnant ewes

did not express any oestrous signs and there was active avoidance of the teasing rams by the pregnant ewes, earlier studies (Hafez, 1968; Hammond, 1962; Jollans, 1960) had found that pregnant ewes could sometimes accept tugging especially when a strange ram was introduced into a flock.

On the birth weights of the lambs, the pattern of the results (singles and males being heavier than twins and females) obtained in the present study is in agreement with earlier reports on the Suffolk sheep (Hammond, 1932). The birth weights were generally smaller than those of the larger breeds of sheep. However, this notwithstanding, the relationship between the birth weight of the lambs and the rams weight at breeding compares favourably with those of the larger breeds (Table 5).

The lighter birth weight recorded in the dry season (December/January) may be partly attributed to the low quality forage at the later part of the pregnancies that terminated during this season.

This study has therefore shown that the length of gestation in the Nigerian Dwarf Sheep was on the average 147.4 days though the modal value was 148 days. The length of gestation was neither significantly affected by the season of breeding nor by the type of birth (twins or singles). The twin bearing ewes were on the average heavier at breeding than single-bearing ewes. The overall weight increase of the ewes during pregnancy was highest in the last trimestre, while the average birth weight of the lambs was 2.03 kilograms and this was influenced by the type of birth, sex of the lambs, season of lambing and the weight of the ewes at breeding.

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## POST-PARTUM ANOESTROUS PERIOD AND LAMBING INTERVAL IN THE NIGERIAN DWARF SHEEP

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### SUMMARY

The incidence of post-partum oestrus was investigated in 27 ewes, of which five had two pregnancies. Thus, a total number of post-partum periods studied was 32. The ewes, 1 to 3 years old, at parturition were checked twice daily for standing heat with two vasectomised rams, both of which were used in the mornings and in the evenings, from the day of lambing until the third post-partum heat period. All surviving lambs were weaned at 100 days of age. The first post-partum oestrus occurred after  $54.91 \pm 3.5$  (22-106) days in all the ewes as follows:  $58.1 \pm 3.6$  (25-106) days in the ewes with a normal lactation (NL) and  $32.3 \pm 3.5$  (22-34) days in those with a short nursing period (SL). Thus lactation does not prevent the resumption of oestrous cycles but it appears to delay it. The duration of the first post-partum heat period of the NL-ewes was 38 hours as compared to 27 hours in the SL-ewes. The duration of the first heat was slightly shorter and more variable than subsequent heat periods. Normal oestrous cycles of about 17 (16-20) days were usually restored after the first post-partum oestrus but the duration of the first oestrous cycle was more variable than the duration of later cycles. All ewes that had the second

pregnancy were bred at the second post-partum heat. Their lambing interval was 221 (199 to 256) days.

### INTRODUCTION

In most temperate breeds of sheep where studies on the lambing interval and post-partum anoestrus were done, early weaning of the lambs were usually adopted in order to induce early rebreeding (Johnson, 1969; Copenhaver and Carter, 1966; Barker and Wiggins, 1964; Hafez, 1952; Hunter and Lishman, 1967). In the Nigerian Dwarf Sheep where natural rearing and mating mainly are adopted and the lambs are weaned at 4-5 months of age, the results on the lambing intervals (Orji 1976) indicate the possibility of the resumption of oestrus and successful rebreeding during nursing.

This study was therefore aimed at:

- (i) establishing the natural recommencement of oestrous cycle and its time of onset during lactation in the Nigerian Dwarf Sheep;
- (ii) determining the duration of the first oestrus and oestrous cycle;
- (iii) determining the duration of early oestrous cycle and factors associated with it, as there is no previous report on these in the Nigerian Dwarf Sheep.

### MATERIALS AND METHODS

Twenty-seven (27) ewes, aged between 1 and 3 years were used in this investigation. Five of the ewes had a second lambing during the period. Thus

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in all, 32 post-partum periods were studied. All surviving lambs were weaned at 100 days of age.

### Management and Feeding

The animals were housed at night and during the rains in semi-open concrete floored pens with saw dust litter but grazed in enclosed paddocks on *Cynodon nlemfuensis* (giant star grass) pastures all day. Trees and shrubs along the fences provided shade and browsing for the animals during hot afternoons. Supplemental concentrate ration, hay, cut green fodder of giant star grass mixed with *Centrosema pubescens* (legume), mineral salt lick and water were provided freely.

### Detection of Oestrus

The ewes were checked for behavioural oestrus twice daily, in the mornings and in the evenings, with two vasectomised rams. All trials were made in the pens starting from the day of lambing until the third post-partum heat period. Both vasectomised rams were used in each trial, each being allowed 20-30 minutes in each pen and then swapped. Shorter swapping interval was adopted towards the end of the oestrus to ensure as accurate a determination of the end of the oestrus as possible.

### Length of post-partum Anoestrus

This was calculated from the day of lambing to the last day prior to the date of the first oestrus.

### Duration of lactation

This was calculated from the day of lambing to the date of termination of nursing, either by death or by weaning of the lambs normally at 100 days. A lactation interval of 12 weeks had been used by earlier workers (Barnicoat *et al*,

1957; Bonsma, 1939) for studies on lactation characteristics in sheep. However, in the current study, lactation in the ewes is classified as either normal or short, based on the termination of lactation in relation to the recommencement of oestrous cycle. For example, ewes that died or lost their lambs before the resumption of oestrous cycle are classified as having short lactation, whereas all ewes that started cycling before the termination of lactation either through weaning or death of the dams or their lambs, are considered to have normal lactation.

### Interval between lambings

This was calculated from the day of the first lambing to the day prior to the date of the next lambing. The ewes (five in all) were hand-mated at the second post-partum heat.

## RESULTS

### Post-partum anoestrus

The duration of the post-partum anoestrus is summarized in Table 1 and the frequency distribution is shown in figure 1.

The first post-partum oestrus occurred after  $54.9 \pm 3.5$  days (Table 1). Only one out of the 32 post-partum heat periods started after weaning at 100 days of age. The difference between the duration of anoestrus in ewes with normal lactation and those with short lactation was significant ( $P < 0.05$ ). The anoestrus was longer in the former than in the later, where the four ewes lost their lambs before the resumption of oestrous cycle, when the lambs were  $13.0 \pm 6.7$  days of age.

### First post-mortem oestrous cycle

The patterns of the first post-partum oestrus and oestrous cycle are presented in Table 2. The duration of the first

Table 1: Length (days) of post-partum anoestrus

Ewes	n	Mean	SE	Range	Nursing Period	Range
All ewes	32	54.91	3.5	22-106	84.3	0-113
Ewes with short lactation	4	32.25	3.5	22-34	13.0	0-31
Ewes with normal lactation	28	58.14	3.6	25-106	97.3	54-113

n = Number of ewes used.

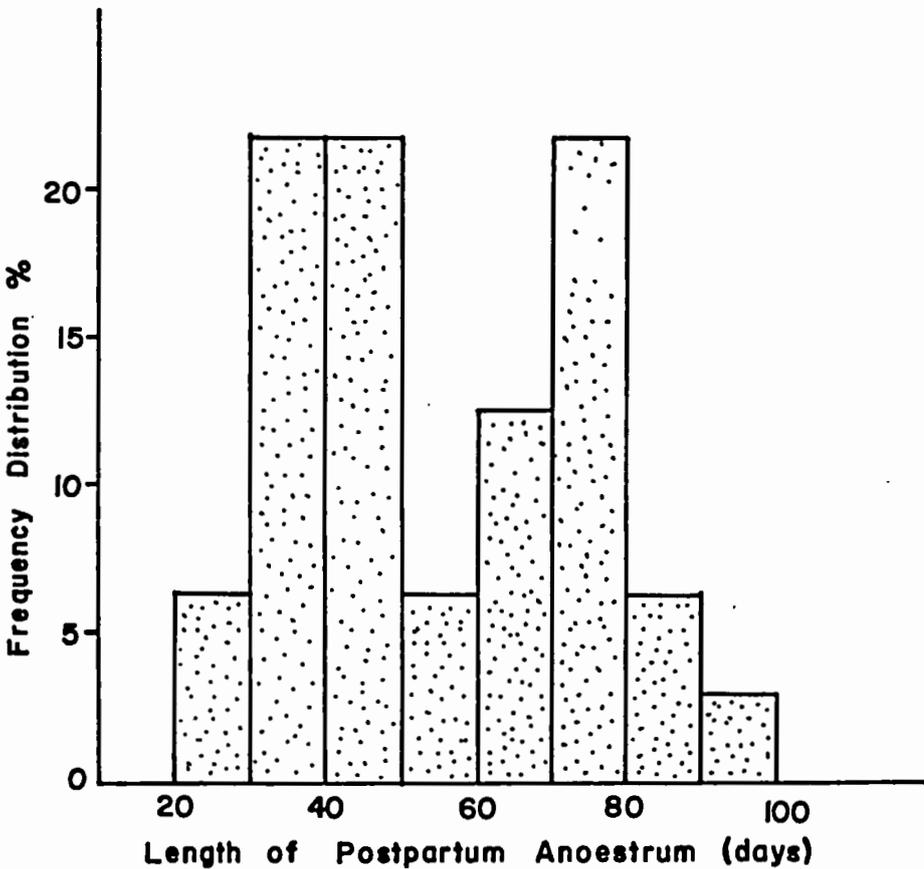


Fig. 1: Frequency distribution of the length of post partum Anoestrus in the Nigerian Dwarf Sheep

Table 2. First post-partum oestrus and oestrous cycle in the Nigerian Dwarf Sheep

(i) The first post-partum oestrus (hours)

Ewes	n	Mean	SE	Range
All ewes	32	37.13	2.6	12-72
Ewes with short lactation	4	27.00	3.0	24-36
Ewes with normal lactation	28	38.57	2.9	12-72

(ii) First post-partum oestrous cycle (days)

Ewes	n	Mean	SE	Range
All ewes	32	17.27	0.25	15-22
Ewes with short lactation	4	17.50	1.50	16-22
Ewes with normal lactation	28	17.23	0.22	15-20

post-partum oestrus was not significantly shorter in the ewes with short lactation (27 hours) than in the ewes with normal lactation (38 hours) ( $P < 0.05$ ). The difference in the oestrous cycle length between the ewes that had normal lactation and those that had short lactation was also not significant. The correlation between the duration of oestrus and the time of the onset of the oestrus approached significance.

Early post-partum oestrus and oestrous cycle

The first post-partum heat was significantly shorter (Table 3) and more

variable than subsequent heat periods. The variability of the first heat was 1.2 and 1.6 times that of the second and the third heats, respectively.

Normal cycles of about 17.6 (16-20) days were usually restored after the first oestrous cycle was slightly (though not significantly) shorter and also 1.4 times more variable than the second.

Lambing Interval

All the ewes retained service at the first tupping which was at the second post-partum oestrus. The mean lambing interval was  $221.4 \pm 12.96$  days, range 199 to 256 days.

**Table 3: Early post-partum oestrus and oestrous cycle in the Nigerian Dwarf Sheep**

**(i) Duration of early post-partum oestrus (hours)**

Heat Number	Mean	SE	Range	CV
1	37.13	2.6	12-72	41.89
2	47.20	3.0	24-96	33.94
3	46.20	2.7	24-72	25.67

**(ii) Length of early post-partum oestrous cycle (days)**

Oestrous cycle number	Mean	SE	Range	CV
1	17.27	0.25	16-22	8.3
2	17.57	0.24	16-20	6.1

**DISCUSSION**

Previous report (Orji, 1976) on the performance of the Nigerian Dwarf Sheep had shown that the ewes were capable of lambing all through the year under natural rearing conditions without showing any seasonality pattern. A comparison of the duration of nursing and the length of post-partum anoestrus (Table 1) shows that the resumption of oestrous cycle is possible during lactation in this sheep ecotype. However, the significant difference between the time of recommencement of oestrous cycles in the normal lactating and short lactating ewes strongly suggests that lactation delays the onset of normal oestrous cycle. These results are therefore in conformity with earlier reports (Cole and Miller, 1935; Baranov, 1941) that lactation did not hinder the onset of oestrus in sheep. On the other hand, they differ from the findings of others (Asdell, 1964; Hammond, 1944; Speedy and Owen, 1975) that lactation inhibits

the onset of oestrus in sheep. The observed delay in the resumption of oestrous cycle among ewes with normal lactation has also been reported in other breeds of sheep (Roux, 1936; Opperman, 1949). On the whole, the mean value of the length of the post-partum anoestrus obtained in the present study in the Nigerian Dwarf Sheep falls within the range of values published in the scientific literature on the other breeds of sheep (Table 4). The values in the various reports suggest strong breed and environmental influence on the post-partum anoestrus in sheep.

The significantly shorter and more variable first post-partum oestrus observed among the ewes appears to be normal. An earlier report on the early post-partum period in cows (Hafez and Jainudeen, 1974) had shown that the first cycle following the re-establishment of the ovulatory cycle tends to be shorter than subsequent cycles. It is not clear what precise factors might

Table 4: Duration of post-partum Anoestrus in selected Breeds of sheep

Breed	Location	Postpartum Anoestrus (days)		
		Mean	Range	Reference
German Merino	Natal	57.1±4.5	—	Hunter and Lishman, 1967
Persian	S/Africa	90.1	8-283	Joubert, 1962
Ausimi	Egypt	41	—	Hafez, 1952
Suffolk	England	35	2-60	Hafez, 1952
Merino	Australia	47.8±2.1	—	Fletcher, 1973
Nigerian Dwarf	Ibadan Nigeria	54.91±3.5	22-106	Present study

be responsible for this but the apparent increase in the duration of oestrus with the delay in the onset of oestrus and the decline in variability in oestrous cycle length with successive cycles, indicate a gradual return of the reproductive system to its normal physiological state.

The lambing interval of the ewes is within the range of values reported earlier (Orji, 1976) in this sheep ecotype. Thus since the mature ewes of the Nigerian Dwarf Sheep cycles all through the year (Orji, 1976) they can under good management produce three lamb crops in two years.

This study has therefore shown that the resumption of oestrous cycle is possible during lactation in the Nigerian Dwarf Sheep although a bit delayed by it.

#### ACKNOWLEDGEMENT

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## PORTLAND CEMENT AS A CALCIUM SUPPLEMENT IN LAYING DIETS

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### SUMMARY

The effects of five levels of portland cement and two levels of calcium on the performance of Yaffa layers in battery cages were investigated.

Feeding cement up to 1.0% of the diet had no adverse effect on egg production, feed efficiency, bone ash, egg weight, egg shell quality and body weight change.

Egg production, feed efficiency and shell quality increased ( $P < 0.01$ ) while bone ash decreased ( $P < 0.01$ ) with increase in dietary calcium level. Egg weight and body weight were not significantly affected by calcium levels.

Eggs production, shell quality and feed efficiency decreased ( $P < 0.01$ ) while egg weight increased ( $P < 0.01$ ) with increase in age of the hens.

### INTRODUCTION

Calcium sources for laying hens have been extensively and intensively investigated over the years. Peterson *et al.* (1960) observed that substitution of hen-sized oyster shell for an equal amount of shell flour up to 1.0% in a complete layer diet did not affect egg shell quality. Quisenberry and Walker (1970) and Scott *et al.* (1971) reported that substitution of oyster shell for a portion of limestone in a complete layer diet improved egg shell thickness and shell strength.

Oluyemi and Fowokan (1973) indicated that bone meal and oyster shell could be substituted for limestone without affecting egg production and feed consumption, irrespective of strain

of bird. Feeding either limestone, oyster shell, clam shells, aragonite or egg shell as calcium supplements had no effects on the overall hen-housed egg production, feed efficiency, livability, body weight, egg weight or egg shell thickness (Muir *et al.*, 1976).

These findings and the general drive to make maximum use of locally available feed materials have recently stimulated interest in portland cement as a source of calcium. Portland cement is a type of cement consisting of approximately 64% CaO, 21% SO<sub>2</sub>, 6% Al<sub>2</sub>O<sub>3</sub>, 3% FeO<sub>3</sub>, 3% SO<sub>3</sub> and 2% Mg O (Ferguson *et al.* 1974).

Available evidence so far seems to suggest that portland cement may have a beneficial effect on bone formation and shell quality. Ferguson *et al.* (1974) reported that addition of PC levels of 0.25, 0.5 and 1.0% to a basal diet already containing 3.5% calcium resulted in increased breaking load of the radius. Egg weight, shell weight, shell thickness, egg specific gravity and body weight were not affected by the treatment. There was no difference in egg production between the control and groups fed 0.5 and 1.0% cement.

Comparing 0.5, 1.0 and 2.0% cement levels at either 3.5 or 4.0% dietary calcium levels, Scott *et al.* (1975a) observed that increasing cement levels increased percent shell, shell thickness, and specific gravity. They, however, observed depressed egg production and depressed feed efficiency. In another study, Scott *et al.* (1975b) noted that feeding White Leghorn pullets, a diet containing either calcium carbonate

or cement as the major calcium source had no effect on egg production, feed efficiency, bone ash or bone breaking strength. However, they indicated that cement significantly improved egg size, percent shell, shell thickness and shell breaking strength.

Besides its potential as a calcium supplement in laying rations, portland cement is relatively cheaper per unit weight than the conventionally accepted calcium sources. Current prices for the commonly used calcium supplements are N566.00/ton for limestone, N451.00/ton for  $\text{CaCO}_3$ , N180.00/ton for oyster shell, N150.00/ton for bone meal as against about N85 per ton for portland cement.

The objective of this study was to further investigate the suitability of portland cement as a calcium supplement for laying hens.

#### MATERIALS AND METHODS

Two hundred and seventy high producing layers of the Yaffa breed, about thirty-two weeks old were used in this study. The birds were randomly divided into groups of twenty seven layers each. Each group was sub-divided into three replicates of nine layers each, ensuring that the initial body weights in these replicates were about equal. The birds were individually housed in battery cages in the same battery house leaving one cage between birds on different treatments.

All birds had previously been fed the standard University of Ife Teaching and Research Farm layer ration containing approximately 18.0% crude protein, 3.5% calcium and 0.75% total phosphorus for a period of twelve weeks. Thereafter, they were put on the test diets (Table 1) which contained factorial combination of two levels of calcium

(3.0 and 3.5%) and five levels of cement (0, 0.25, 0.50, 0.75 and 1.00%). The test diets were fed *ad libitum* for a preliminary period of two weeks, during which no data were collected and subsequently for five 28-day periods for data collection. Water was provided *ad libitum*.

Body weight of individual birds was taken at the beginning of the experiment. Weighing was subsequently carried out on the last day of each period between August 21, 1978 and January 22, 1979.

Daily egg production records were kept collectively for all birds in each replicate throughout the experimental period. The average percent hen-day egg production for each replicate was calculated on 28-day period basis.

Egg shell quality was determined every 28-days. On the last day of each 28-day period, a sample of ten eggs randomly selected out of all the eggs produced in each replicate on the previous three days was taken, weighed, broken and the shells containing the membranes washed in distilled water. The shells were dried for 24 hours at about  $40^\circ\text{C}$ , cooled, weighed and egg shell quality expressed as shell weight per unit area ( $\text{mg}/\text{cm}^2$ ). Surface area was calculated by using the formula,  $S = 4.67 W^{2/3}$  (Mueller and Scott, 1940) where W is the fresh weight of the egg. Egg shell quality was calculated on group basis.

Feed efficiency was expressed as kilogram feed per dozen eggs (kg feed/doz egg) for each replicate over the whole experimental period.

At the end of the last 28-day period, two birds from each replicate were randomly selected and slaughtered. The right tibia of each bird was removed for bone-ash determination using the

Table 1: Composition of the test diets

DIETS										
Ingredients (%)	1	2	3	4	5	6	7	8	9	10
Corn	61.19	61.24	61.30	61.35	61.40	59.87	59.93	59.98	60.04	60.09
Brewers' Dried Grain	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Groundnut cake	14.13	14.13	14.13	14.13	14.13	14.13	14.13	14.13	14.13	14.13
Fish meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Agricare premix*	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72
Oyster shell	5.91	5.61	5.30	5.00	4.70	7.23	6.92	6.62	6.31	6.01
Portland cement**	—	0.25	0.50	0.75	1.00	—	0.25	0.50	0.75	1.00
Amprolium***	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
<b>Total</b>	<b>100.00</b>									
<b>Calculated Analysis</b>										
M.E. (Kcal/kg)	2967.11	2968.83	2970.88	2972.60	2974.31	2921.84	2923.89	2925.61	2927.67	2929.38
Crude protein (%)	18.17	18.17	18.18	18.18	18.19	18.06	18.06	18.07	18.07	18.07
Ether Extract (%)	3.97	3.97	3.97	3.98	3.98	3.92	3.92	3.92	3.92	3.93
Crude fibre (%)	4.14	4.14	4.14	4.14	4.14	4.11	4.11	4.11	4.11	4.11
Methionine (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Cystine (%)	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Lysine (%)	0.82	0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81	0.81
<b>Determined Analysis</b>										
Calcium (%)	3.04	3.09	3.06	3.10	3.10	3.48	3.50	3.49	3.51	3.49
Calcium (%)	3.04	3.09	3.06	3.10	3.10	3.48	3.50	3.49	3.51	3.49

\*A Pfizer (Ikeja) laying vitamin-mineral premix supplying the following per kg of diet: Vit. A, 8000 I.U.; D<sub>3</sub>, 2000 I.U.; riboflavin, 4.20 mg pantothenic acid, 5.0 mg; nicotinic acid, 20.0 mg; Folic acid, 0.5 mg; choline, 300 mg; Vit. B<sub>12</sub>, 0.01 mg; Vit. K, 2.0 mg; Vit. E, 2.5 mg; Manganese, 56 mg; iodine, 1.0 mg; iron, 20.0 mg; copper, 10.0 mg; Zinc, 50.0 mg; cobalt, 1.25 mg; Methionine, 225 mg plus Etoxyquin (antioxidant).

\*\*Portland Cement (Elephant) made in Nigeria and contains about 46.34% calcium.

\*\*\*Commercial coccidiostat.

methods described by Kalango and Ademosun (1973). Mortality was recorded as and when it occurred.

All data were analysed using the analysis of variance and significant mean differences tested by the Duncans New Multiple Range test (Steel and Torrie, 1960).

## RESULTS

Samples of portland cement made in Poland, Turkey and Nigeria contained approximately 45.14, 45.93 and 46.34% calcium respectively. These values compare well with the 45.71% calculated from the cement composition reported by Ferguson *et al.* (1974). Based on these averages, portland cement contains more calcium per unit weight compared to the conventional calcium supplements such as calcium carbonate (40.0%), limestone (35.0%), Oyster shell (38.0%) steamed bone meal (24.0%) and feed grade dicalcium phosphate (23.0%) (Scott *et al.*, 1969).

Dietary cement had no significant effect on body weight change, feed efficiency, egg weight and egg shell quality but hens on the cement diets tended to be slightly superior to those on the non-cement diet (Tables 2 and 3). There were no differences in egg production between the control and groups on the 0.75 and 1.00% cement diets.

Dietary calcium had a significant effect on egg production, shell quality and tibia ash ( $P < 0.01$ ) and feed efficiency ( $P < 0.05$ ) irrespective of cement level or period of lay (Table 2). Individual mean comparisons showed that hens on the 3.5% calcium diets laid more eggs with better egg shell quality and converted their feed more efficiently into eggs than those on the 3.0% calcium diets (Table 4). Tibia

ash of hens on the 3.5% calcium was significantly ( $P < 0.01$ ) lower than that of hens on the 3.0% calcium diet. Dietary calcium had no significant effect on egg weight and body weight change.

Egg weight increased significantly ( $P < 0.01$ ) while shell quality, egg production and feed efficiency (kg feed/doz eggs) decreased significantly ( $P < 0.01$ ) with the age of the hens (Tables 2 and 5). Egg production during the first two periods was not significantly different. These were however significantly ( $P < 0.01$ ) higher than production during the third, fourth and fifth periods. Egg production during the third and fourth periods was significantly higher ( $P < 0.01$ ) than the production during the fifth period, which was in fact the lowest. Similarly, shell quality showed a significantly ( $P < 0.01$ ) progressive decrease with increase in age of the hens (Table 5). Shell quality during the first two periods of lay was significantly ( $P < 0.01$ ) higher than the rest of the laying periods. No significant difference existed between the third and fifth periods and between the fourth and fifth periods.

There was a general tendency for egg weight to increase with increase in laying age (Table 5). Mean egg weights of the first and second periods were significantly ( $P < 0.01$ ) lower than those for the third, fourth and fifth periods, differences among which were not significant.

Feed efficiency decreased ( $P < 0.01$ ) with increase in period of lay. Up to the second period of lay the decrease in efficiency was not significant. Thereafter, the decrease in efficiency became significant ( $P < 0.01$ ). The least efficiency was noted during the fifth period of lay.

Table 2: Mean squares of analyses of variance

Source	df	Hen-day egg prodn (%)	Egg wt (g)	Kg feed/ dozen eggs	Body weight change (g)	Shell-Quality (mg/cm <sup>2</sup> )	Tibia ash (%)
Period (A)	4	1178.37**	26.89**	0.8666**	+	44.5695**	+
Calcium (B)	1	313.05**	0.01	0.3361*	262.85	160.0427**	44.41**
Cement (C)	4	61.71*	7.29	0.1350	355.27	7.9594	1.62
A x B	4	20.42	2.83	0.1749	2363.88	3.5145	12.56
A x C	16	9.91	2.36	0.0318	+	2.9224	+
B x C	4	32.90	3.39	0.0947	+	5.4416	+
A x B x C	16	10.39	1.42	0.0334	+	2.3707	+
Residual	++	24.58	3.64	0.0830	4276.80	3.9813	61.03

\* P<0.05,      \*\* P<0.01

+ Factors not considered in model

++Residual degrees of freedom were 100 for egg production, egg weight, kg feed/dozen eggs and shell thickness and 20 for body weight change and tibia ash.

Table 3: Effect of portland cement level diet on laying performance

Cement level (%)	Hen-day egg production (%)	Egg weight (g)	Kg feed/ dozen eggs	Body wt. change (g)	Shell quality (mg/cm <sup>2</sup> )	Tibia Ash (%)
0	62.3 <sup>a</sup>	60.3	2.31	81.0	66.65	54.2
0.25	65.8 <sup>b</sup>	61.5	2.20	69.5	69.74	55.3
0.50	65.6 <sup>b</sup>	61.5	2.20	90.5	69.68	55.2
0.75	63.7 <sup>ab</sup>	60.9	2.34	35.1	69.62	54.3
1.00	64.3 <sup>ab</sup>	60.8	2.22	41.3	69.92	54.8
S.E.M	0.91	0.35	0.053	26.7	0.364	0.71

<sup>a,b</sup> Within each column means carrying different superscripts are significantly different at (P<0.05).

Table 4: Effect of dietary calcium level on laying house performance

Calcium level (%)	Hen-day egg production (%)	Egg Wt. (g)	Kg feed/ dozen eggs	Body wt. change (g)	Shell quality (mg/cm <sup>2</sup> )	Tibia ash (%)
3.00	62.9 <sup>A</sup>	61.0	2.30 <sup>a</sup>	60.5	68.49 <sup>a</sup>	56.0 <sup>b</sup>
3.50	65.8 <sup>B</sup>	61.0	2.21 <sup>b</sup>	66.4	70.55 <sup>b</sup>	53.5 <sup>a</sup>
S.E.M.	0.57	0.22	0.033	16.89	0.230	0.45

<sup>a,b</sup> Within each column means carrying different superscripts are significantly different. Lower case letter indicate 5% level of significance while capital letters indicate 1% level of significance.

Table 5: Effect of period of lay on the laying performance of the birds

Period	Hen-day egg production (%)	Egg weight (g)	Shell Quality (mg/cm <sup>2</sup> )	Feed Efficiency (Kg/feed/ dozen egg)
1	70.2 <sup>c</sup>	60.1 <sup>a</sup>	70.71 <sup>c</sup>	2.08 <sup>a</sup>
2	70.9 <sup>c</sup>	59.9 <sup>a</sup>	70.50 <sup>c</sup>	2.12 <sup>ab</sup>
3	63.2 <sup>b</sup>	61.3 <sup>b</sup>	69.21 <sup>b</sup>	2.27 <sup>bc</sup>
4	61.6 <sup>b</sup>	61.4 <sup>b</sup>	68.13 <sup>a</sup>	2.29 <sup>c</sup>
5	55.9 <sup>a</sup>	62.2 <sup>b</sup>	68.72 <sup>ab</sup>	2.51 <sup>d</sup>
S.E.M.	0.91	0.35	0.364	0.053

<sup>a,b,c,d</sup> Within each column, means carrying the same superscripts are not significantly different at (P<0.01).

## DISCUSSION

The non-significant effect of dietary cement levels used in this study on body weight change, feed efficiency, bone-ash, egg weight and shell quality confirms the earlier reports of Ferguson *et al.* (1974) and Scott *et al.* (1975b). It had earlier been observed that no difference existed in egg production between hens fed 0.50 or 1.00% cement diets and the control (Ferguson *et al.*, 1974). In this study, feeding cement up to 0.50% of the diet significantly ( $P < 0.05$ ) increased egg production. Cement levels of 0.75 and 1.00% had no adverse effect on egg production. The egg production of hens on these higher levels of cement appeared to be slightly but non-significantly better than the production of hens on the control diet. Contrary to these reports, Scott *et al.* (1975a) demonstrated a progressive decreasing effect on egg production and feed efficiency by increasing dietary cement level from 0.5 to 2.0%, irrespective of calcium level in the diet. This decreasing effect was significant only at the 2.0% cement level when compared to the control. Dietary cement level had no adverse effect on body weight, egg weight, egg shell quality, bone ash and mortality. In a subsequent study where they compared calcium carbonate and cement as the sole sources of calcium, Scott *et al.* (Personal Communication) observed that cement had no effect on egg production and feed efficiency. No attempt was made to explain these discrepancies. It is possible that the 20 week-old pullets used by Scott *et al.* (1975a) in their first study could have been less tolerant to the high cement levels compared to the hens used in the present study and that of Ferguson *et al.* (1974) which were already 3 and 6 months in lay, respectively, at the onset of the trials.

It is not certain by what mechanism portland cement affects laying performance. Its effect could not be attributed totally to its calcium content as the level of dietary calcium was kept constant in most studies. Also, the other sources of calcium used with cement are known to be highly available for hens, hence the problem of availability of calcium from the other sources does not arise. It has however been reported that silicon found in cement as  $S_1O_2$  is an essential mineral element for normal growth and development of the chick (Carlisle, 1972, 1974) and rats and mice (Carlisle, 1974, Schwarz, 1974). Carlisle (1974) postulated that silicon may be involved with calcium and phosphorus in bone calcification, and that at silicon-rich sites in the periosteal region of the young bone, silicon and calcium occur together. This has also led to the speculation that silicon may hasten the rate of bone mineralization. Since the hen depends partly on bone calcium for egg shell deposition, any factor aiding in the build-up of the labile calcium, is only enhancing egg production. Thus, the possible beneficial effect of silicon in portland cement could partially be explained by its involvement in bone calcification.

Increasing calcium level from 3.0 to 3.5% resulted in increased egg production, better bone calcification, better egg shell quality and better feed efficiency. Ademosun and Kalango (1973), Kalango and Ademosun (1973) and Oluyemi and Fowokan (1973) made similar observations after feeding diets containing about 3.50% calcium as compared with 3.0% calcium and lower levels in Nigeria. It is interesting, however, that the bone ash of hens on the 3.5% calcium diet was significantly lower than that of the hens on the 3.0% calcium diet. This response may be due to

the observed increased egg production and shell quality triggered by the higher level of dietary calcium, which is in turn, could have increased the demand on bone calcium, hence, the reduced bone ash.

Dietary calcium level had no significant effect on egg weight. It appears egg weight is more associated with stage of maturity or reproductive age and breed than dietary manipulations (Gilbert *et al*, 1978, Hamilton *et al*, 1979, Wolford and Tanaka, 1970, Roland *et al*, 1975). There is a general tendency for the hen to reduce the number of eggs produced and maintain egg weight under moderate deficiency conditions or cease laying, where severe deficiency conditions exist.

The hens on the 3.5% calcium diet seemed to have gained slightly more weight than those on the 3.0% calcium diet although the difference was not significant. This effect of increasing levels of calcium on body weight is in agreement with the observations of Ademosun and Kalango (1973) and Olu-yemi and Fowokan (1973). Paterson (1965) reported that increasing dietary calcium level from 2.25 to 3.75% increased body weight gain significantly. The lack of significant calcium effect in the present study may have been due to the higher levels of calcium used.

The increase in egg weight and decrease in shell quality, egg production and feed efficiency with the age of the hens was expected. It has been established that egg weight increases and shell quality declines as the hen ages (Peterson, 1965; Ademosun, 1969; Ademosun and Kalango, 1973; Wolford and Tanaka, 1970, Roland *et al*, 1975; Mamilton *et al*, 1979). It is also known that egg size increases more rapidly

than shell weight (Roland, 1976; Hamilton, 1978). It thus implies that the ratio of dry shell weight to egg weight decreases with chronological age, the rate of change dictated mainly by change in egg weight. This general decline in the performance of the hen with age may be due to possible reduced efficiency of feed utilization. It has been reported that calcium retention (Hurwitz and Griminger, 1962) and residual skeletal calcium (Tyler, 1940) decrease as hens become older. Since egg production depends largely on calcium availability, the decline observed in performance of the hen with age could be partly explained by the lowered efficiency of calcium utilization. Because of this irreversible decline in calcium utilization with age, it is certain that optimal egg production and egg shell quality of the aging hen cannot be maintained simply by increasing the calcium level in the diet.

Besides its beneficial effect on laying performance, portland cement has an added advantage over the conventionally used calcium supplements. Compared to the latter, portland cement has a higher concentration of calcium. It is also relatively cheaper.

The results of this study indicate that portland cement could be fed up to 1.0% of the diet to laying hens without any deleterious effect on performance. A dietary calcium level of 3.0% was not adequate for satisfactory productive performance of hens under the tropical Nigerian conditions. Egg production, shell quality and feed efficiency declined with the age of the hen while egg weight increased.

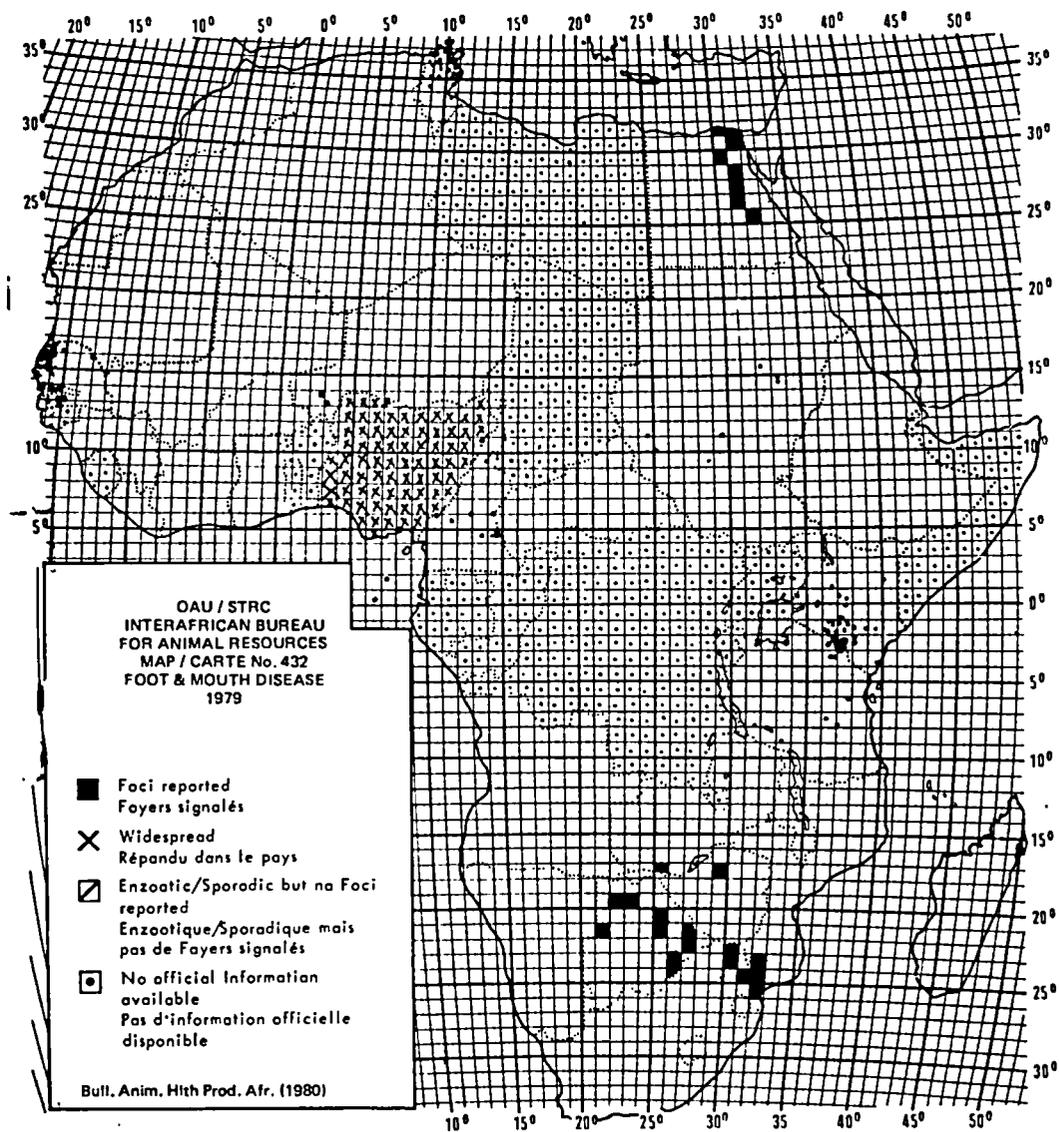
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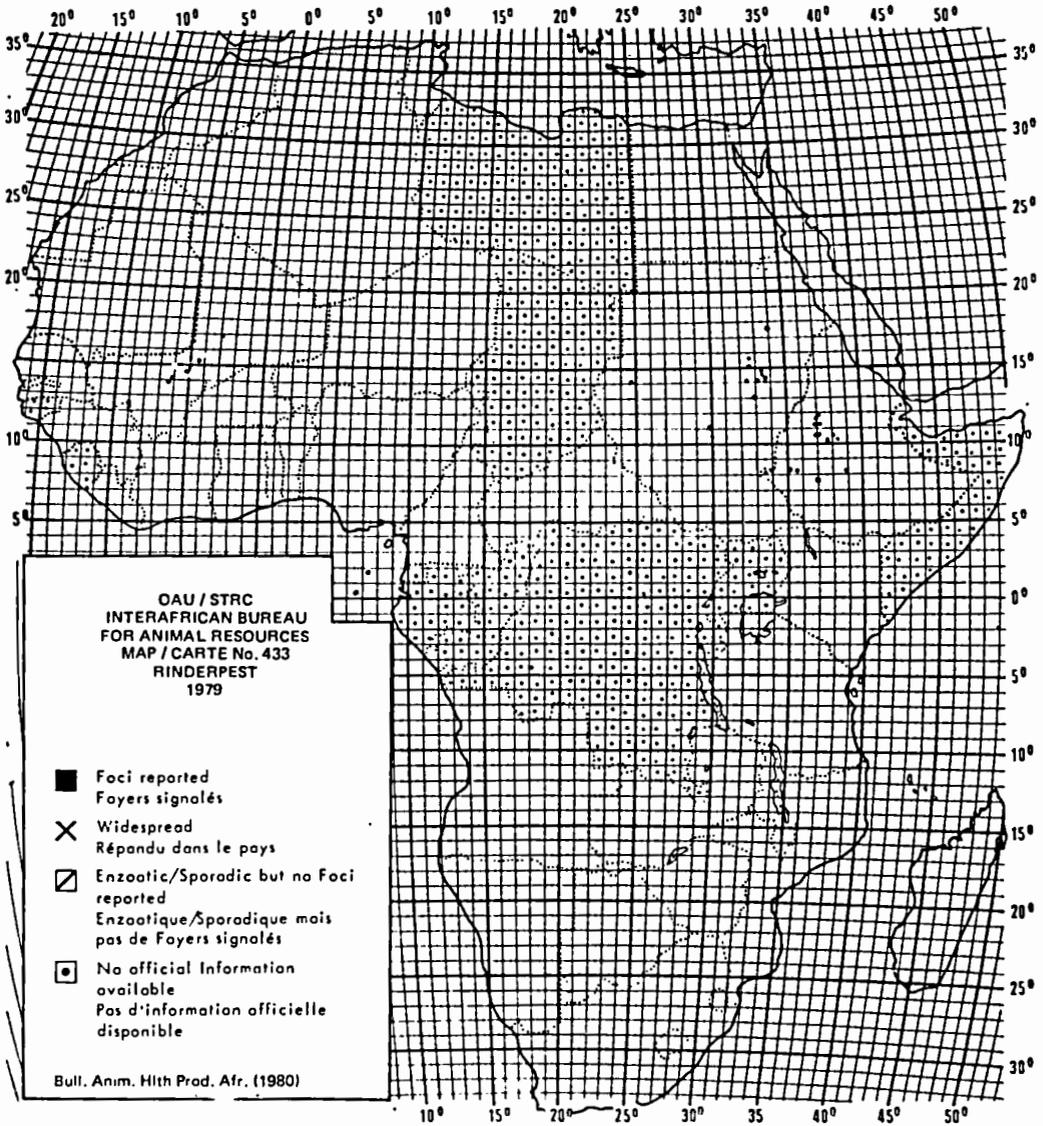
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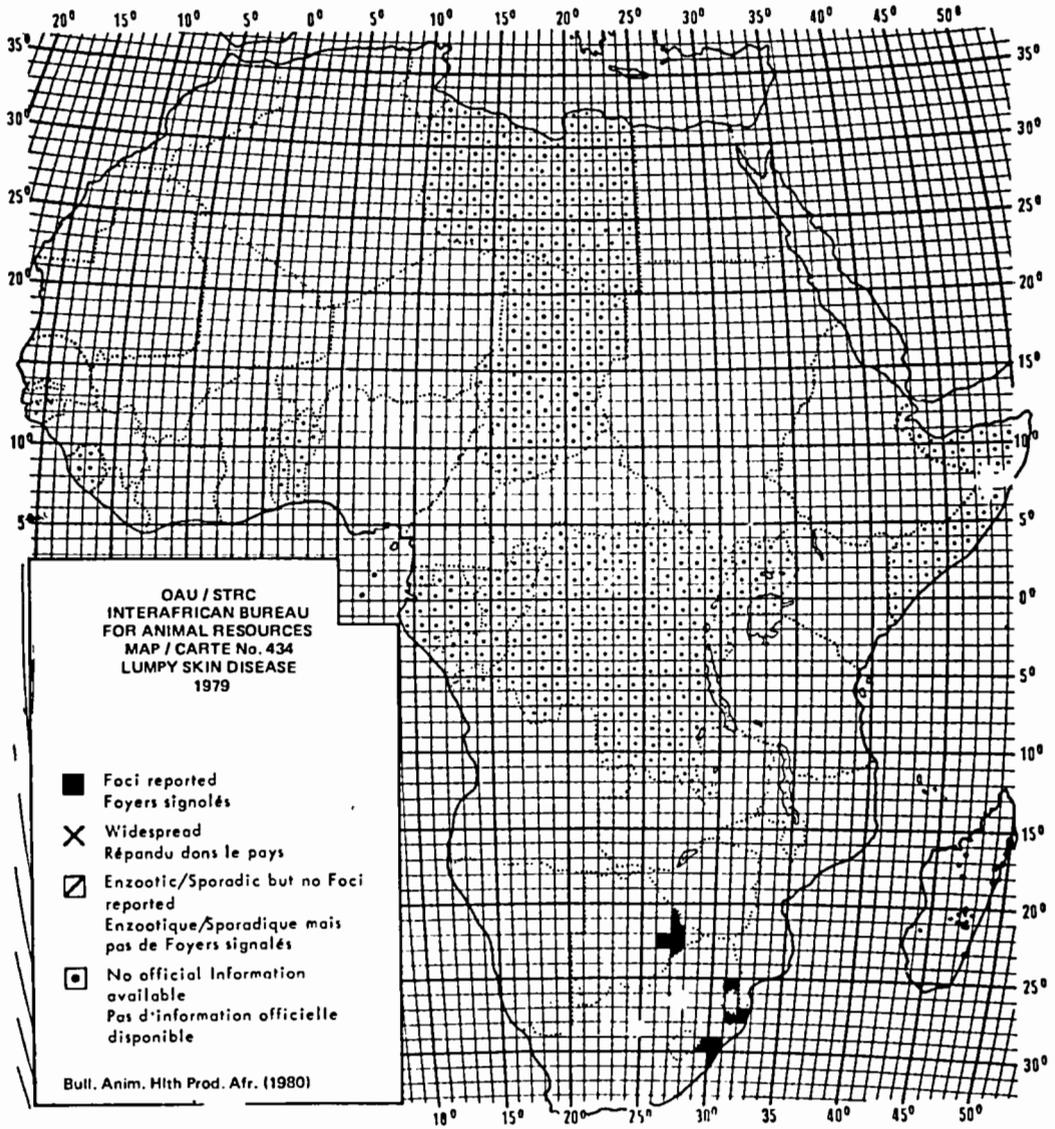
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Geographical Distribution of Animal Diseases /  
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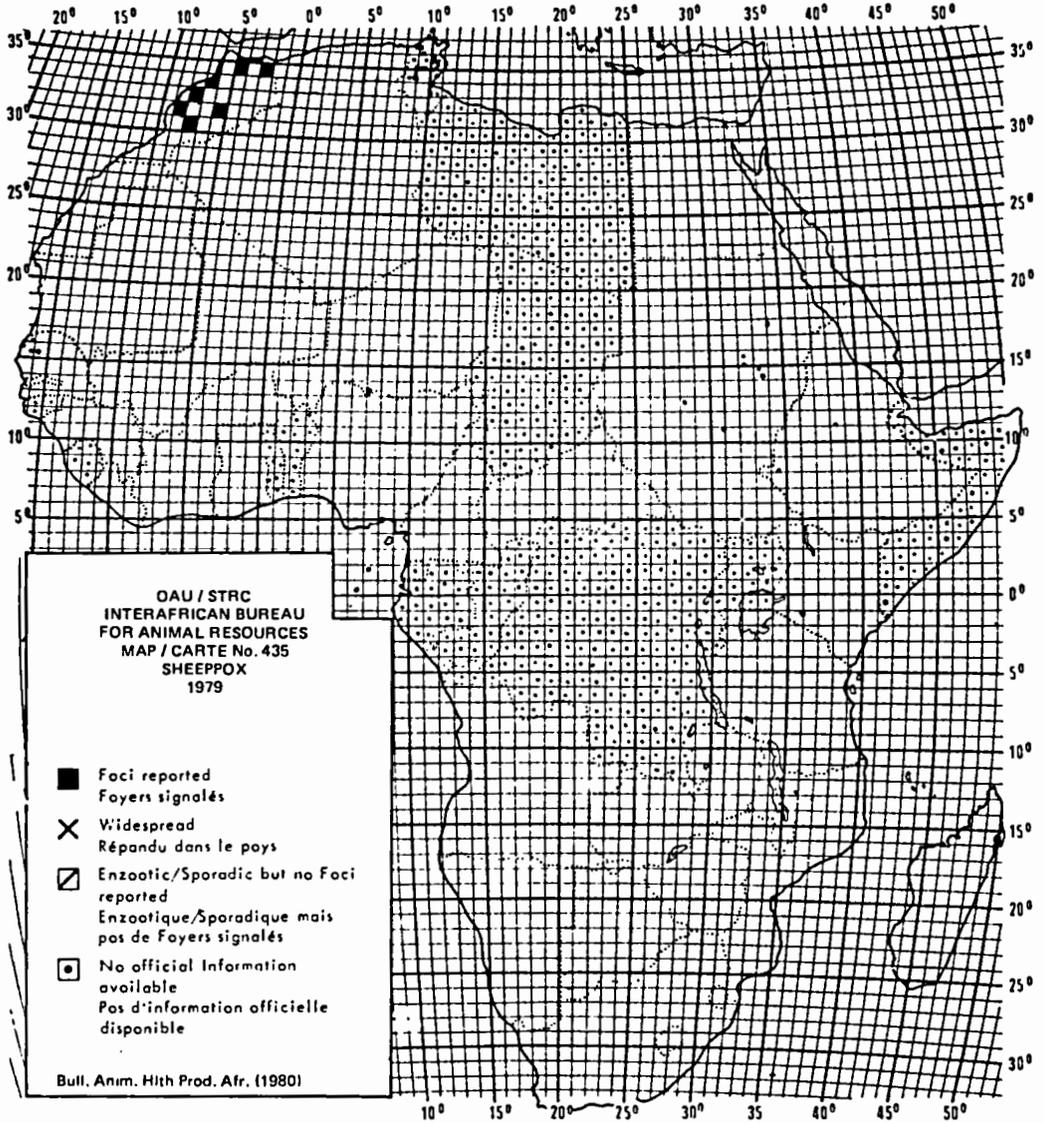
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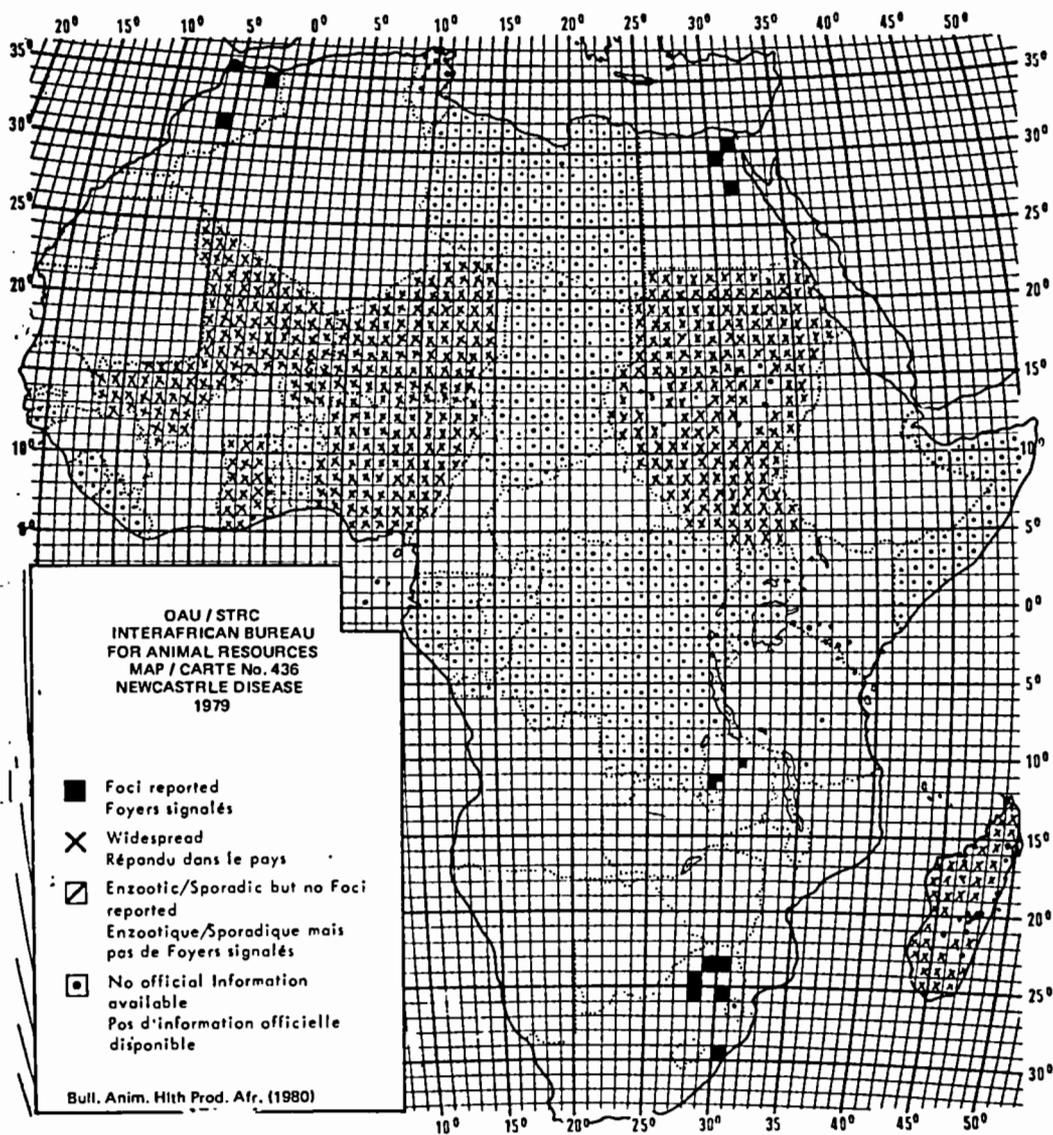
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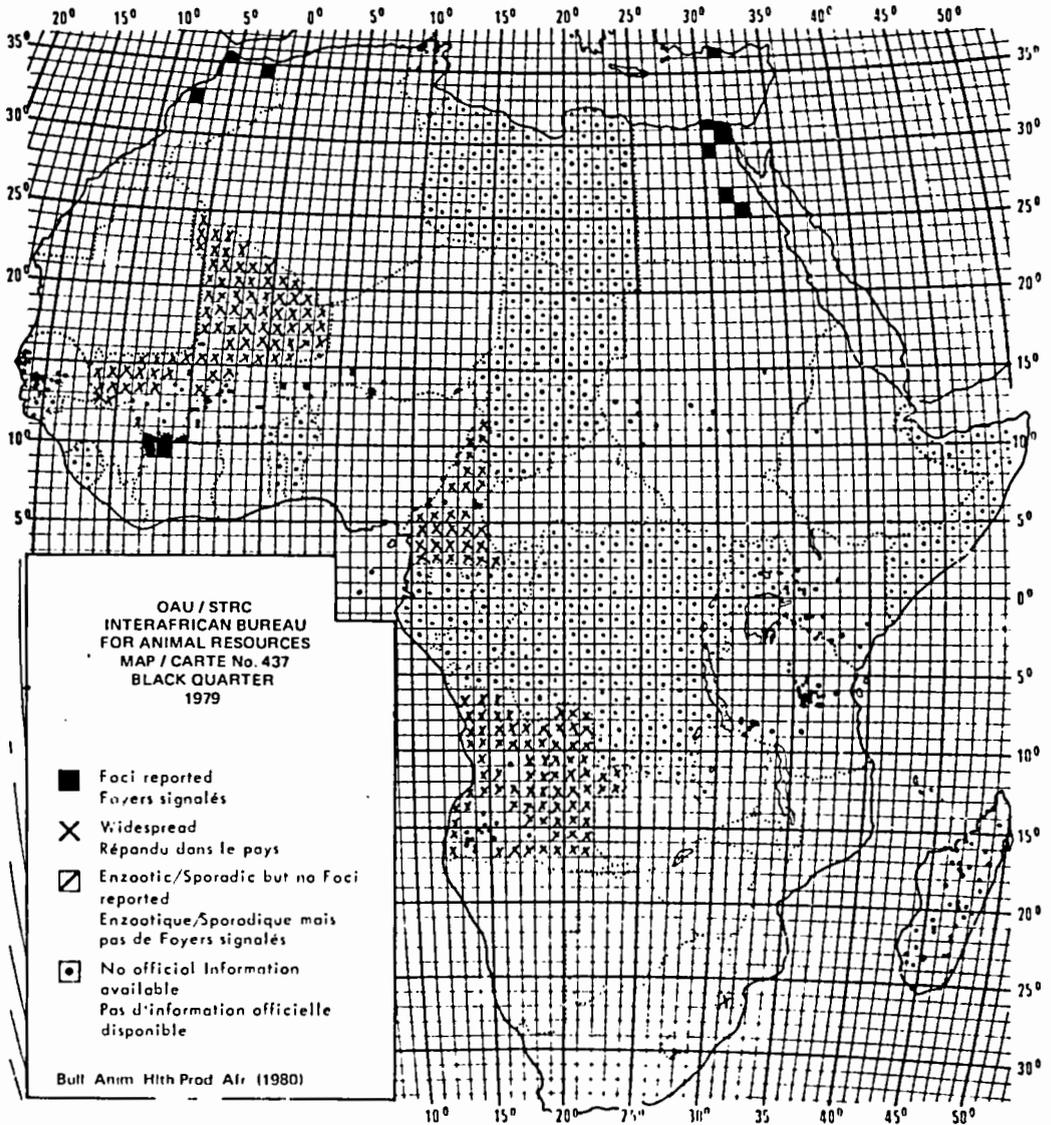
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Distribution géographique des Maladies animales

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IBAR/1980 WATSON, D.L. and CAMP-  
84 BELL, R.S.F.

Vaccination Against Experimental Staphylococcosis in Sheep — Observations on Bacteriology and Pathology Following Challenge.

*Australian Veterinary Journal*, 1979, 55 (10): 475 — 480.

**AUTHORS' SUMMARY:** Sheep were vaccinated with a killed *Staphylococcus aureus* vaccine (2 doses) which had been cultured *in vitro* (Group 1), a killed *S. aureus* vaccine (2 doses) cultured *in vivo* (Group 2) or a single dose of a live vaccine (Group 3). Other sheep were used as non-vaccinated controls. All sheep were challenged by intravenous injection of  $2.6 \times 10^{11}$  washed, viable *S. aureus* organisms, the vaccinated animals being given the challenge inoculum at various intervals after vaccinations. The control sheep survived for 29th (mean) after challenge. Animals given killed vaccines survived longer, (particularly Group 2) if challenged less than 40 days post-vaccination. Animals in Group 3 survived longer if challenged after 40 days post vaccination than those in Group 1 or 2. There were no significant differences between the treatment groups for numbers of *S. aureus* recovered from blood in the 3h period following challenge. Histological and bacteriological evidence showed that the kidneys were more severely affected by the challenge inoculum than heart, spleen, liver or lungs. The kidneys showed both toxigenic and lymphoreticular reactions and large numbers of staphylococci were recovered more reliably from kidneys than other organs.

IBAR/1980 POHL, P., ANTOINE, O.,  
85 GHYSELS, G., CHASSEUR, M.L. CHARLIER, G. and THOMAS, J.

Multiple Resistant *Slamonnella dublin* in Belgium.

*Ann. Med. Vet.*, 1979, 123 (7): 485 — 492

**AUTHORS' SUMMARY:** In Belgium, since 1971, the number of *S. dublin* infections in cattle and man has increased continuously.

The strains are nearly always resistant to antibiotics. In spite of their different origins (either human or animal) they are of the same biotype and seem to possess the same plasmids.

The proportion of *S. dublin* isolated yearly from man represents approximately 10% of the proportion of the same serotype isolated from cattle in the course of the preceding year.

Considering these facts, contamination of human beings by these strains and these plasmids appears to be derived from cattle.

IBAR/1980 JONES, J.E.T.

86 Bacterial endocarditis in the pig with special reference to streptococcal endocarditis.

*J. comp. Path.*, 1980, 90 (1): 11 — 28

**AUTHOR'S SUMMARY:** Thirty cases of bacterial endocarditis in the pig are reported; 23 (77%) were associated with streptococci, principally of Lancefield's groups C and L. The other 7 cases were associated with *E. rhusiopathiae* (6) or *A. equuli* (1). Heart valves on the left side were affected more than twice as often as those on the right. Histologically acute and chronic lesions were observed; a striking feature of the acute lesions was the predominance of the bacterial component of the vegetations. There were no morphological differences between the vegetations caused by streptococci and those caused by the other bacteria. Focal or diffuse myocarditis and myocardial fibrosis were commonly seen in association with endocarditis. Renal infarction was the most common extracardiac lesion.

IBAR/1980 RAMISSE, J., BREMENT,  
87 A.M., VIAUD, M.A. and RABREAUD, C.

List and Classification of Streptococci Isolated over Winter Season 1978-1979.

*Revue Med. Vet.*, 1980, 131 (2): 141 — 151

**AUTHORS' SUMMARY:** During several animal pathology syndromes, 299 streptococci strains were isolated in Laboratory, which is about 25% of all the microbial strains isolated over the winter season 1978 — 1979. These strains' biochemical and serological properties were

studied. Could not be all classified. Their classification was worked out according to pathological syndromes. Strains serological dispersion is observed, however D group is prevalent. Even in mammitis, streptococci (B and C group), that are the usual cause, are not very frequent. Antibigrams show a rather great sensitivity to penicillin, cephaloridin and rifamycin.

IBAR/1980 DUFRENE, M., AUGIER, 88 J., DAYOT, G. and AUGIER-GIBORY, S.  
Tuberculin Titration and Heterogeneity of Antigen Involved in the Tuberculin Reaction.

*Rec. Med. Vet.*, 1980, 156 (2): 133 - 136

**AUTHORS' SUMMARY:** In sensitized guinea-pigs, the comparison of the diameters of the papules obtained by intradermic injections of the same bovine tuberculin and of the same standard (constituted by human tuberculins) depends on the strain with which the guinea-pigs have been sensitized.

When the latter have been sensitized with a human strain, the standard is privileged and the tuberculin the strength of which is to be determined is underestimated.

On the contrary, when the guinea-pigs have been sensitized with a bovine strain, tuberculin to be titrated is overestimated.

This happens because there are antigenic differences between the human and bovine strains. Indeed, these differences are not negligible since the strength ratio of the tuberculin in the two sensitization systems is not very different from 5.

It is clear, in view of these results, that a tuberculin titration should be carried out under homogeneous antigenic conditions: it is essential that the tuberculin of which the strength is to be determined and the standard tuberculin had been prepared with strains of the same species and that the animals had been sensitized by strain also belonging to the same species.

IBAR/1980 RAJENDRAN, M.P., 89 KUMAR, R. and NARAYAN RAO, C.V.  
Studies on Clinico-Pathology of Swine Brucellosis

*Indian Vet. J.*, 1980, 57 (2): 102 - 104

**AUTHORS' SUMMARY:** The clinical and pathological findings in cases of swine brucellosis in a herd of York shire pigs are described. The predominant clinical symptoms were still-births, abortion, weak births and abscesses involving joints and udder. There was no correlation between seropositivity and clinical symptoms. Microscopically, focal collection of macrophages, proliferation of reticular tissue and hyperplasia of the follicles were noted in the lymph nodes. Among the genital organs, histological changes were limited to seminal vesicle and epididymis, with scanty infiltration of lymphocytes and macrophages in the interstitial tissue.

IBAR/1980 ISHINO SEISHI, OKA 90 MOTOI, TERUI SHINICHI and IKEDA SUMIO  
Pathological and Microbiological Studies on Calf Pneumonia Occurring in Mass Rearing Facilities.

*Nat. Inst. Anim. Hlth Quart.*, 1979, 19 (3): 91 - 103

**AUTHORS' SUMMARY:** Pathological and microbiological studies were conducted on lesions in the lungs of 194 calves from mass rearing facilities. Macroscopically, the lesions were classified into six forms: non-lesion, atelectasis, mild pneumonia, moderate pneumonia, advanced pneumonia, and advanced pneumonia accompanied with abscess. Histopathological examination revealed bronchopneumonia in most of the calves. Lesions more advanced than moderate pneumonia were complicated with desquamation, severe exudation, and necrosis. Bacteriologically, *Pasteurella* sp. was isolated often in combination with *Staphylococcus* sp. from about a half of the atelectatic cases. With the development of pneumonic lesions, *Pasteurella* sp. was isolated at a high frequency in combination with *Haemophilus* sp., *Streptococcus* sp., and *Corynebacterium* sp. Prominent necrosis was more often seen in cases with *Pasteurella haemolytica* isolated than in cases with only *Pasteurella multocida* isolated. *Mycoplasma* sp. and *Ureaplasma* sp. were isolated from distinctly pneumonic lesions. Advanced pneumonic lesions were observed in many calves over 30 days of age. The importance of environmental and managerial improvement was also emphasized, since calf pneumonia tended to break out in facilities under unsatisfactory conditions in the present work.

IBAR/1980 HONMA TOSHIO, ONUMA  
91 MISAO, MIKAMI TAKE-  
SHI and IZAWA HISAO  
Evaluation of Syncytium  
Assay for Bovine Leukemia  
Virus

*Jpn. J. Vet. Sci.*, 1980, 42  
(1): 1-4

**AUTHORS' SUMMARY:** As a new method for the detection of bovine leukemia virus (BLV) infection, syncytium assay was evaluated with lymphocytes from infected cattle and sheep. Only when lymphocytes from cattle with enzootic bovine leukosis and from sheep experimentally infected with BLV were used, syncytium formation was induced among indicator cells. A direct proportional dose response was presented by the number of inoculated lymphocytes from BLV-infected cattle to the number of syncytia formed. Treatment of lymphocytes from cattle with an adult form of lymphosarcoma with anti-BLV serum could inhibit the syncytium formation, but neither antiserum against bovine syncytial virus nor the sera from cattle with sporadic form of lymphosarcoma could inhibit. These results indicate the specificity and the usefulness of this assay for the detection of BLV infection.

IBAR/1980 RATNAMOHAN, N., GRI-  
92 MES, T.M. BAGUST, T.J.  
and SPRADBROW, P.B.

A Transmissible Tumour  
Associated with Reticuloen-  
dotheliosis Virus Infect-  
ion.

*Australian Veterinary Journal*,  
1980, 56 (1): 34 - 38

**AUTHORS' SUMMARY:** Histiocytic lympho-  
sarcomas of the intestine, liver, spleen and  
sciatic nerve were found at necropsy in a  
36-week-old laying hen that was culled from  
a flock of 1800 birds because of emaciation.  
Type C particles were observed in ultrathin  
sections of liver and spleen. The serum of the  
hen contained reticuloendotheliosis virus  
(REV) antigen, and antibody against REV,  
but lacked antibodies reactive with Marek's  
disease virus or subgroups A and B of Rous  
sarcoma virus.

The tumour was transmitted to chickens  
using a suspension of the initial tumours.  
These experimental tumours were then trans-  
mitted to further chickens, using cultured

spleen cells, viable spleen cells that had been  
stored frozen, and disrupted spleen cells. The  
tumours, which developed after incubation  
periods as short as 2 weeks, were histologically  
similar to those in the original hen. A few  
chickens also developed feather abnormalities.  
The chickens with experimentally transmitted  
tumours developed antibody against REV and  
REV antigen was demonstrated in cultured  
cells from these chickens. The chickens failed  
to develop antibody against Rous sarcoma  
virus and only 1 of 29 developed antibody  
against Marek's disease virus.

IBAR/1980 AGUILAR-SETIEN, A.,  
93 PASTORET, P.P., TOMA,  
B., JOUBERT, L.,  
MICHAUX, C. and  
SCHOENAERS, F.

Anamnestic Immune Res-  
ponse Against Infectious  
Bovine Rhinotracheitis  
Virus (*Bovine Herpesvirus*  
1), in Cattle, After Expo-  
sure to Pseudorabies Virus  
(*Sus Herpesvirus* 1) Ant-  
igens.

*Ann. Med. Vet.*, 1979, 123  
(6): 429 - 434

**AUTHORS' SUMMARY:** Neutralizing antibody  
titres against infectious bovine rhinotracheitis  
virus were measured in the sera of cattle belong-  
ing to two farms where Aujeszky's disease had  
been diagnosed in this species.

All the animals possessing neutralizing  
antibodies against pseudorabies virus possessed  
also antibodies neutralizing infectious bovine  
rhinotracheitis virus.

Experimental injection of pseudorabies virus  
antigens in cattle immunized against infectious  
bovine rhinotracheitis virus, has provoked a  
steady increase in the level of seroneutralizing  
antibodies against both pseudorabies and in-  
fectious bovine rhinotracheitis viruses.

IBAR/1980 PRASAD, L.B.M. and  
94 SPRADBROW, P.B.

Ultrastructure and Infecti-  
vity of Tissue from Normal  
and Immunodepressed  
Chickens Inoculated with  
Turkey Herpesvirus.

*J. Comp. Path.*, 1980, 90 (1): 47  
— 56

**AUTHORS' SUMMARY:** A study was made of the ultrastructural changes and infectivity in tissues of normal and cyclophosphamide (Cy)-treated chickens inoculated with turkey herpesvirus (HVT).

In untreated infected chickens, the ultrastructural in lung and lymphoid tissues were characterized mainly by invaginations in the nuclear and plasma membranes and an increased number of lysosomes in the cytoplasm, and they were observed in only a small proportion of cells. A few naked herpes virions were seen in the nucleus and cytoplasm and the virus particles in the cytoplasm often appeared degenerated. Enveloped virus was not detected in any tissue. In Cy-treated chickens, the virion degradation was rarely seen. Increased lysosomal activity in the phagocytic cells of untreated chickens was believed to account for the cytoplasmic degradation of the naked virions.

In untreated chickens, the cell-associated infectivity was first detected in buffy coat, lung, spleen and thymus 2 days after inoculation. No virus was detected, either by cell culture or by electron microscopy, in skin or feather quill-tips of infected chickens up to 3 weeks after inoculation. The virus titres in tissues of Cy-treated chickens were considerably higher than those in the corresponding control on the 10th day post-inoculation.

IBAR/1980 HORNER, G. W. and  
95 HUNTER, R.  
Experimental Infection in  
Pigs with Encephalomyocarditis.

*New Zealand Veterinary Journal* 1979, 27 (10): 202 — 203

**AUTHORS' SUMMARY:** Non-suppurative myocarditis in pigs that were experimentally infected with encephalomyocarditis (EMC) virus is reported. Two of the four pigs that were infected with the virus died suddenly from myocardial failure, and all four had gross and microscopic changes consistent with EMC infection. This confirms the pathogenic potential of some strains of EMC virus for young pigs.

IBAR/1980 BLANCOU, J., ANDRAL,  
96 L., SAMUDIO, A. and CRISPIM SILVA, L.

Red Foxes (*Vulpes vulpes*)  
Experimental Rabies. II.  
Excretion of the Rabic  
Virus after Infection.

*Revue Med. Vet.*, 1979, 130  
(11): 1473 — 1482

**AUTHORS' SUMMARY:** In a study on 50 red foxes (*Vulpes vulpes*) infected by the oral or intramuscular method with various wild rabic virus doses, the virus has been searched in the saliva on 15 animals before they died and in the salivary glands and saliva on 42 animals dead of rabies.

On the 42 animals, the rabic virus has been found in 42 cases (100%) in the salivary gland and in 23 cases (54%) in the saliva. On alive animals the longest delay for the virus to appear is 6 days before death, 5 days before the first symptoms. It is the longest delay observed till now, concerning carnivorous experimental rabies.

IBAR/1980 DEWAN, M.L. and UDDIN  
97 MOSLEH  
Experimental Transmission  
of Marek's Disease in In-  
digenous (deshi) and Exotic  
Poultry Birds.

*Bang. Vet. Jour.*, 1979, 13  
(2-4): 1 — 3

**AUTHORS' SUMMARY:** Marek's disease (MD) could not be transmitted to the indigenous chicken through experimental inoculation and feeding of infective materials. Intramuscular inoculation and feeding of infective material produced the disease in the White Leghorn chicken. Macroscopic Lesions detected were characterised by nodular swellings in the ovary and presence of numerous minute whitish spot on the surface of the kidney. Microscopic examination of the tissue section revealed infiltration of small, medium and large lymphocytes associated with few plasma cells and round cells. The heterogenous infiltration of lymphocytes and the less pyroninophilic cytoplasm of these cells (when strained with Methyl green Pyronin strain) was found to be characteristic of the Marek's disease. Apparent lack of susceptibility of the indigenous chickens may be attributed to an inherent genetic factor.

IBAR/1980 ANOSA, V.O. and ISOUN,  
98 T.T.

Haematological Studies on  
*Trypanosoma vivax* Infect-  
ed of Goats and Intact  
and Splenectomized Sheep.

*J. Comp. Path.*, 1980, 90 (1):  
155 - 168

**AUTHORS' SUMMARY:** *Trypanosoma vivax* infection of sheep and goats, although manifesting varying severity in individual animals, was characterized by an initial phase of "crisis" lasting several weeks which was associated with high fluctuating parasitaemia, a marked drop in red cell values, leucopaenia and a transient macrocytosis which was later replaced by microcytosis. Many animals died during this phase. Animals which survived the crisis passed into a recovery phase characterized by low infrequent parasitaemia, a recovery of the red cell values, sometimes attaining pre-infection values, and leucocytosis. Mortality was generally higher in goats than in sheep and with "wild strains" isolated from cattle and inoculated directly into experimental animals than with laboratory-adapted strain 36/15.

The anaemia associated with crisis was haemolytic, with a viable decrease in <sup>51</sup>Cr-red cell survival, the degree of shortening being related to the degree of anaemia at the time of measurement. The spleen was the major site of red cell destruction in animals with mild anaemia while the liver was the major site in severely anaemic animals. The bone marrow showed erythroid hyperplasia early in crisis but terminally the percentage of erythrocytic elements drifted towards normal. There was a gross increase in the red marrow mass of the long bones, but regenerative forms of red cells were consistently absent from the blood of severely anaemic animals, suggesting some interference with marrow function.

Splenectomy reduced the incubation period of *T. vivax* in sheep but did not affect the duration and mortality of the disease, nor did it ameliorate the anaemia or effect the intensity and frequency of parasitaemia.

IBAR/1980 DOLAN, T.T., RADLEY,  
99 D.E., BROWN, C.G.D.,  
CUNNINGHAM, M.P.  
MORZARIA, S.O. and  
YOUNG, A.S.

East Coast Fever: 4 Further  
Studies on the Protection

of Cattle Immunized with a  
Combination of Theilerial  
Strains.

*Veterinary Parasitology*, 1980,  
6 (4): 325 - 332

**AUTHORS' SUMMARY:** Four experiments were carried out in which cattle immunized with three cryopreserved stabilates, *Theileria parva* (Muguga), *T. parva* (Kiambu 5) and *T. lawrencei* (Serengeti transformed) and simultaneously treated with a single dose of long acting tetracycline were challenged with field isolates of *T. parva*. Three of the challenge isolates were from Tanzania and one from Kenya. The immunized cattle showed either mild or inapparent disease reactions while susceptible control cattle suffered severe disease and 72% died. These experiments further demonstrated the efficacy of the immunization method against *T. parva* isolates from a wide distribution within East Africa. The value of such laboratory screening of field isolates prior to field exposures is discussed.

IBAR/1980 DUFFUS, W.P.H. and  
100 WAGNER, G.G.

Comparison Between Cer-  
tain Serological Tests for  
Diagnosis of East Coast  
Fever.

*Veterinary Parasitology*, 1980,  
6 (4): 313 - 324

**AUTHORS' SUMMARY:** The indirect fluorescent antibody (IFA), indirect haemagglutination assay (IHA), complement fixation (CF), capillary agglutination (CA) and immunodiffusion (ID) serological tests using *Theileria parva* piroplasm antigen were compared over a wide range of immune serum samples from cattle exposed to *T. parva* experimentally or naturally.

The ID test showed a lack of sensitivity with most sera, whilst difficulties in interpretation were found with the CA test when using the necessarily high serum concentrations. The CF, IFA, and IHA tests on experimentally infected cattle sera showed good correlation to 50 days post infection whereafter the CF titres dropped below base-line values.

With field serum samples the IHA test was the most sensitive of all the serological assays used. It is suggested that the difference in the sensitivity may be related to varying levels of specific IgG<sub>2</sub>.

The CF, IHA and CA tests utilize antigens contaminated with soluble bovine erythrocyte components, which could give false positives in sera containing iso-antibodies to bovine erythrocytes, and absorption of test sera before use with normal bovine erythrocytes is therefore recommended. In conclusion, for experimental work the CF, IFA and IHA tests are recommended: for field work both the IFA and IHA tests should be used.

IBAR/1980 JENKINS, G.C., MCC-  
101 RORIE, P., FORSBERG,  
C.M. and BROWN, J.L.  
Studies on the Anaemia in  
Rabbits Infected with *Trypanosoma brucei brucei*.

*J. Comp. Path.*, 1980, 90 (1):  
107 — 120

**AUTHORS' SUMMARY:** Measurements of red cell counts, haemoglobin concentrations, packed cell volumes, reticulocyte percentages and pyruvate kinase levels were carried out daily in rabbits infected with either *Trypanosoma brucei brucei* S42 or *T. b. brucei* 427.

With both stocks, rabbits became anaemic within 7 days, the anaemia being initially more severe in those animals infected with the S42 stock. Mean cell volumes, percentage of reticulocytes and pyruvate kinase levels increased during the infection, whereas mean cell haemoglobins remained relatively constant and mean cell haemoglobin concentrations decreased.

Red cell T<sub>50</sub> values were reduced in both infections, especially when the infecting organisms was *T. b. brucei* S42. Bone marrow aspirates showed a general hyperplasia.

The evidence is indicative of an anaemia which is haemolytic in origin, in spite of normal serum bilirubin concentrations. Possible mechanisms of haemolysis are discussed and it is concluded that the major cause is red cell damage due to the attachment to the erythrocytes of immune-complex factors, together with sequestration and destruction of red cells in the spleen and other organs of the reticuloendothelial system.

IBAR/1980 CORREA, W.M., CORREA,  
102 C.N.M. and GOTTSALK,  
A.F.  
Bovine Abortion Associated  
with *Anaplasma Marginale*

*Can. J. Comp. Med.*, 1978,  
42 (2): 227 — 228

**AUTHORS' SUMMARY:** During the period from June 1974 to June 1975, five bovine foetuses between seven to nine months old were received for necropsy from four different countries of the S. Paulo State, Brazil. All of them were from brucellosis-free herds.

Necropsy revealed slight liver enlargement generally accompanied by capsular petechial hemorrhages. Enlargement and congestion of the spleen, epicardial and endocardial petechiae were present in three foetuses and one of them had lungs with some hemorrhagic lobules.

Cardiac blood films of all the foetuses stained by the Pappenheim's panoptic method showed *Anaplasma marginale* in two to 20% of red corpuscles. When stained with acridine orange and immunofluorescent methods blood films of the first foetus specifically showed *A. marginale*.

IBAR/1980 OLSSON GERT and HOL-  
103 TENIUS PAUL

Studies on the Epidemiology of *Ostertagia ostertagi* in Calves

*Nord. Vet. Med.*, 1980, 32  
(1): 28 — 37

**AUTHORS' SUMMARY:** The number of parasites in the abomasum of calves that have been grazing during different periods of the seasons 1976 and 1977 has been examined. After infection from overwintering L3 — larvae a new generation of parasites develops. How big this will be depends primarily on the amount of rainfall.

IBAR/1980 LUDRI, R.S. and RAZ-  
104 DAN, M.N.

Efficiency of Nitrogen Utilization by Zebu Cows and Buffaloes. I. Nutrient Utilization and Nitrogen Balances on Prefomed Protein Diets.

*Trop. Agric.*, 1980, 57 (1):  
83 — 90

**AUTHORS' SUMMARY:** In an experiment on eight adult non-pregnant females, four each of Tharparkar Zebu and Murrah buffalo breeds, maintained under identical management and fed on four levels of dietary protein and constant energy, it was found that the buffaloes consumed more dry matter per unit metabolic body size than the cows. A dietary protein intake 40% less than the recommended amount of digestible crude protein (DCP) had no adverse effect on appetite or the digestibilities of the food consumed. Buffaloes digested less crude protein in comparison to cows, but maintained positive nitrogen balances even on 40% of the recommended DCP intakes. The experiment confirmed that the mechanism of nitrogen utilization is better developed in buffaloes than in cows.

IBAR/1980 BANTING A. de L. and  
105 BERTRAND, J.F.

Adaptation of the Rumen Microflora when Changes are Introduced in the Diet of Fattening Young Bulls.

*Rec. Med. Vet.*, 1979 155.  
(7-8): 621 - 623

**AUTHORS' SUMMARY:** The authors report on the results of an experiment carried out in France: a preparation, based on lyophilized rumen juice was administered to young bulls starting their fattening diet. During that period, the rate of growth of the animals treated proved superior by 204 g per day to that of the controls.

IBAR/1980 MICHALK, D.L. and  
106 SAVILLE, D.G.  
Supplementary Feeding of Range Sheep.

*Journal of Range Management*,  
1979, 32 (6): 422 - 429

**AUTHORS' SUMMARY:** Droughts significantly reduce the production from range sheep and in severe cases affect survival. In order to avoid losses in times of feed shortage, pastoralists must either supplement sheep at pasture or implement total hand-feeding strategies. To employ either practice necessitates familiarity with the principles of supplementary feeding, many of which are summarized in this paper. In addition to discussing the nutrient

requirements of grazing sheep, the expected responses to supplementary feeding for different classes of sheep and production activities are reviewed. The question of when to commence feeding is discussed and information is provided on the formulation of diets with respect to the physical form and the nutritive value of the ingredients. Finally, research highlighting the importance of feeding frequency is reviewed.

IBAR/1980 LAUENROTH W.K. and  
107 DODD, J.L.

Response of Native Grassland Legumes to Water and Nitrogen Treatments.

*Journal of Range Management*,  
1979, 32 (4): 292 - 294

**AUTHORS' SUMMARY:** The response of native shortgrass prairie legumes to water and nitrogen additions was evaluated utilizing a replicated factorial design of two water and two nitrogen treatments. Responses measured were densities and above ground biomass by species. Water treatment greatly increased both density and biomass of legumes, presumably because of more favourable conditions for nitrogen fixation and increased competitive advantage under nitrogen deficient conditions.

IBAR/1980 KRISHNAMURTHY, D.,  
108 GERA, K.L., SINGH, JIT  
and NIGAM, J.M.

Experimental strangulated intestinal obstruction in buffalo calves: peritoneal fluid and blood serum alterations.

*Indian Vet. J.*, 1980, 57 (2):  
155 - 159

**AUTHORS' SUMMARY:** Strangulated intestinal obstruction was created in eight buffalo calves to study the haematology and chemistry of blood and peritoneal fluid. The results were compared with four animals operated for exploratory laparotomy only. The animals subjected to bowel obstruction died between 48 and 84 hours. Haematological examination revealed neutrophilia and leukocytosis followed by leukopenia. Hypoglycaemia and high values of urea nitrogen content of serum and perito-

neal fluid were recorded as significant changes in animals subjected to bowel strangulation.

**IBAR/1980 SOLI, NILS E.**

109 Chronic copper poisoning in sheep.

*Nord. Vet. Med.*, 1980, 32  
(2): 75 - 89

**AUTHOR'S SUMMARY:** A review is given of chronic copper poisoning in sheep. The sensitivity of sheep to excess of copper is described and compared to that of other ruminant species. Possible reasons for the accumulation of copper in the liver are mentioned. The absorption, distribution and excretion of copper in sheep are briefly described. Possible modes of action of copper on target cells are reviewed. The interaction of copper with the most important interfering substances is described. An account is given of the features of the disease, clinical findings, clinical pathology and necropsy findings. Possible methods of treatment and control are discussed.

**IBAR/1980 ROGERS, R.J., CONNOLE,**

110 M.D., NORTON, J.,  
THOMAS, A. LADDS, P.W.  
and DICKSON, J.

Lymphadenitis of Cattle  
due to Infection with Green  
Algae.

*J. Comp. Path.*, 1980, 90 (1):  
1 - 9

**AUTHORS' SUMMARY:** Lymph nodes that were diffusely or focally green in colour were detected in cattle at slaughter in northern Australia on eight occasions over the past 8 years. These lesions were caused by infection with green algae of the order *Chlorococcales* but specific identification of the 4 strains isolated was not possible in the light of present knowledge. The infection was restricted to retropharyngeal and mandibular lymph nodes in all but one instance, when a mediastinal node was also involved. The infection evoked a largely proliferative inflammatory response. Non-progressive or slowly progressive infections were established in the peritoneal cavity of rats inoculated i.p. with cultures of the organism. The presence of numerous strongly PAS and GMS positive granules, well developed chloroplasts and the green colour of the organisms, both of individuals and colonies, serve to

differentiate it from the morphologically similar *Prototheca* species which are probably achloric algae.

**IBAR/1980 ROSS, A.D.**

111 Small Intestinal Carcinoma  
in Sheep

*Australian Veterinary Journal*,  
1980, 56 (1): 25 - 28

**AUTHOR'S SUMMARY:** This communication reports a high prevalence of small intestinal carcinoma. Seventeen cases were detected during a survey of 6,248 sheep (0.272%) from 3 export abattoirs in southern New South Wales.

The neoplasm appeared on the serosal surface of the jejunum and ileum as a ring of dense white fibrous tissue which caused stenosis and cranial dilation of the intestine. The mucosa and muscularis were thickened and folded but otherwise grossly normal except for 3 cases where small polyps were present. Metastatic lesions occurred regularly in mesenteric lymph nodes and sclerotic deposits were often present on the surface of abdominal viscera. In one case metastatic nodules were found throughout the liver and lungs. Histologically, the glandular architecture of the mucosa and submucosa was disturbed and thickened with fibrous tissue. The muscularis was intact; however an intense desmoplastic reaction under the serosa contained scattered islands of PAS-positive epithelial cells; distinct acini were uncommon and mitotic figures rare.

**IBAR/1980 DELAHAUT, Ph., BECK-**

112 ERS, J.F. and ECTORS, F.

Effect of Sodium Azide on  
Progesterone Degradation in  
Bovine Blood Samples.

*Ann. Med. Vet.*, 1979, 123 (8):  
567 - 572

**AUTHORS' SUMMARY:** Progesterone degradation was measured in bovine blood samples left on the bench in air at room temperature for a few days. Progesterone level is reduced by 50% after 4 to 6 h and this degradation seems to be due to the presence of red blood cells. The addition to each sample of 5 mg/ml sodium azide give a 90% protection of progesterone after 4 days at room temperature.

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