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References to books should include name and initials of author(s), year of publication (in parentheses), the exact title (underlined), town of publication, page number (if page number specifically cited).

References to annual reports should state the country, year of reference, followed by the name of the department or organisation, e.g. Kenya (1955) An. Rep. Dept. Vet. Services, p. 50 (if specific page cited).

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PRODUCTION TRAITS OF KENYA SAHIWAL CATTLE

D. KIMENYE,
Department of Animal Production University of Nairobi P.O. Box 29053, Kabete, Kenya.

SUMMARY

Three thousand nine hundred and ninety-five (3995) lactation records of 1183 Sahiwal cows milked at the National Sahiwal Stud, Naivasha, Kenya were analysed. The mean 305 day milk yield, butterfat percentage, lactation length, dry period and calving interval were 1455 ± 10 kg, 5.00 ± 0.02 percent, 274 ± 0.8 days, 149 ± 1.8 days and 412 ± 1.4 days respectively. These production and reproduction data suggest that Sahiwals under grazing conditions in Kenya perform well when compared to Sahiwals in India where they are mostly stall-fed.

INTRODUCTION

Sahiwal cattle, also known as Montgomery cattle, are largely bred in the district of Montgomery, the Punjab, Pakistan (Joshi and Phillips, 1953). They were imported into Kenya in 1939 and again in the period between 1945 and 1963 in recognition of their superior milk yields when compared to other zebu breeds. By 1963, 60 bulls and 12 females had been brought from four herds in India and Pakistan (Meyn and Wilkins, 1974). The earlier groups were used in the up-grading of the East African Zebu, the results of which were reported by Mahadevan, Galukande and Black (1962). Following the recommendations of Mason (1965), all the good Sahiwal grade cattle were transferred to Naivasha to form the present National Sahiwal Stud. The objective of this paper is to describe the mean production levels and to compare them to those of other Sahiwal herds.

MATERIALS AND METHODS

The data used in this investigation were obtained from the National Sahiwal Stud, Naivasha, Kenya. They consisted of a variable number of records on 305-day milk yields, butterfat percentages, butterfat yields, lactation lengths, preceding dry periods and calving intervals of 1183 cows. The study was restricted to records of cows which were not sick for more than 20 consecutive days in a lactation and those which were not complicated by death or abortion. Only the milk yields attained up to 305 days were included in the study. No lactation records were excluded from the analyses for being short as had been done by Galukande, Mahadevan and Black (1962) and Osman (1970). Milk samples for butterfat determination were only taken from the cows yielding over three kilograms of milk per day. Monthly butterfat percentage records of each cow were averaged to obtain the lactation butterfat percentage records.

Naivasha which lies between 1°S and 37°E at an altitude of 2200 metres above sea level, has a modified tropical climate. The rainfall (average 487 mm per year) is low, unreliable and bimodally distributed. There is usually a long rainy season March to May and a short one in November and December. The temperature humidity indices are low (average 62.5) and as such cattle are not expected to experience stress due to them alone.

Cows were grazed on natural pasture (mainly Cynodon spp. and Pennisetum clandestinum) and milked twice daily
without calves at foot. Cows producing more than 5 kg of milk/day were supplemented at a rate of one kilogram of concentrate containing about 16 percent crude protein for every three kilograms of milk produced above 5 kg. Other routine management practices were also observed (Meyn and Wilkins, 1974).

The means discussed here were calculated using least squares procedures (Harvey, 1966) after allowing for the effects of lactation number, year of calving, season of calving and year by season interactions.

RESULTS AND DISCUSSION

A summary of the production levels attained at Naivasha, is presented in Table 1. The mean milk yields achieved at Naivasha (Table 1) were lower than those reported in India for the same breed by Kavitkar, Saxena and Chowdhary (1968) and Nagpal and Acharya (1971). Sahiwals at Naivasha grazed on unimproved pastures with hardly any supplementation while those in India were mainly stall-fed. Large phenotypic variations in milk yield, which are commonly observed in *Bos indicus* studies were also found in the Naivasha herd. These variations indicate possibilities of increasing yields if proper selection if done since the heritability of the trait in this herd is moderate (Kimenye, 1980).

Average butterfat content was equal to earlier estimate reported by Gaba and Jain, (1972) on Sahiwals. Larger phenotypic variations than those reported on Mpwapwa cattle by Kiwuwa and Kyomo (1971) and Nganda cattle by Marples (1965) were observed. These variations were indicative of errors in the sampling and the testing milk for butterfat content in this herd. Marples (1965) attributed the low coefficient of variation to the close supervision of the sampling of milk and the testing of butterfat at Entebbe. Mahadevan, Galukande and Black (1962) observed inconsistencies in butterfat records of Sahiwal grades in Kenya and accordingly concentrated their studies on milk yields leaving aside butterfat records. From this study and other studies indicated above, it appears that more supervision of the butterfat testing team at Naivasha is warranted.

Means for lactation lengths and dry periods were similar to those reported by Singh and Choudhury (1961) and Malik and Sindhu (1968). Although lactation lengths were long, they showed large variation which were also reflected

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>LSQ Mean ± S.E.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>305 milk yield, kg.</td>
<td>3995</td>
<td>1455 ± 10.40</td>
<td>41</td>
</tr>
<tr>
<td>Butterfat, percent</td>
<td>3995</td>
<td>5.00 ± 0.02</td>
<td>25</td>
</tr>
<tr>
<td>Butterfat yield, kg.</td>
<td>3995</td>
<td>72.26 ± 0.57</td>
<td>45</td>
</tr>
<tr>
<td>Lactation length, days</td>
<td>3995</td>
<td>274 ± 0.8</td>
<td>19</td>
</tr>
<tr>
<td>Dry period, days</td>
<td>2858</td>
<td>148 ± 1.8</td>
<td>64</td>
</tr>
<tr>
<td>Calving interval, days</td>
<td>2858</td>
<td>412 ± 1.4</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 1: Production traits of the Kenya National Sahiwal Stud.
Table 2: Production traits of some Indian Sahiwal herds.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>Lactation milk yield kg.</th>
<th>Lactation length days</th>
<th>Calving Interval days</th>
<th>Dry Period days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh and Choudhury (1961)</td>
<td>1489 46</td>
<td>265 19</td>
<td>484 23</td>
<td>-</td>
</tr>
<tr>
<td>Gehlon and Malik (1967)</td>
<td>-</td>
<td>-</td>
<td>439 20</td>
<td>183 43</td>
</tr>
<tr>
<td>Malik and Sindhu (1968)</td>
<td>972 30</td>
<td>274 22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kushwana and Misra (1969)</td>
<td>1400 42</td>
<td>297 26</td>
<td>498 25</td>
<td>196 50</td>
</tr>
<tr>
<td>Naggal and Acharya (1971)</td>
<td>1856 22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chopra et al., (1973)</td>
<td>-</td>
<td>270 25</td>
<td>439 20</td>
<td>183 43</td>
</tr>
</tbody>
</table>

in milk yields. The correlation between lactation length and milk yield was 0.60 ± 0.03 while the regression coefficient of milk yield on lactation length was 6.01 ± 0.02 kg. Lactation length accounted for up to 35 percent of total variations in milk yield and was, as such an important cause of variation in milk yield.

Although all the cows in the herd were artificially inseminated, and in general reproductive efficiency of the tropical cattle tends to decline with the use of artificial insemination because heat observation problems, the calving intervals were short in comparison with the report of Kushwana and Misra (1969). This indicated a high level of efficiency of the AI inseminators and the herdsmen who observed the heat signs.

From the study of the production traits, it does appear that Sahiwal in Kenya are as productive as the Sahiwal in India.

ACKNOWLEDGEMENTS

I am grateful to Mr. H. Were formerly Senior Animal Husbandry Research Officer Ministry of Agriculture for encoura-gement and assistance in data collection. A generous research grant from NORAD is gratefully acknowledged.

REFERENCES


Received for publication on 4th December, 1979
CHRONIC COPPER POISONING IN BALAMI SHEEP: A CASE REPORT

C.N. CHINHEME,
Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria,
Nigeria.

SUMMARY
A suspected case of copper poisoning in a farm flock of eleven Balami sheep is described. Gross and histopathological findings together with liver copper values are recorded. The source of copper was not apparent. The influence of stress as a predisposing factor in hepatic copper release and the resulting haemolytic crisis is discussed.

INTRODUCTION
The problem of copper (Cu) intolerance in sheep was recognized over ninety years ago (Ellenberger and Hofmeister, 1883). Since then Cu poisoning in sheep has continually been a big problem, especially in the sheep-producing areas of the world. The first outbreak of chronic Cu poisoning in sheep was diagnosed in Germany (Schaper and Luetje, 1931). Veterinarians have used clinical and biochemical findings to diagnose chronic Cu poisoning in the United States (Boughton and Hardy, 1934), Britain (Eden, 1940), Australia (Albiston et al., 1940) and New Zealand (Cunningham, 1944). Muth (1952) reported a suspected case of Cu poisoning in a farm flock following pasturing on Ladino clover grown in an orchard sprayed with Bordeaux mixture. The feeding of a copper-supplemented diet caused copper poisoning (Pearson, 1956) while an outbreak of chronic copper poisoning was believed to be due to sheep grazing pastures top-dressed with copper compounds (Pryor, 1959).

Postmortem findings in sheep dying from Cu poisoning have been previously described (Boughton and Hardy, 1934; Grayson, 1949; Muth, 1952).

Reports of Cu poisoning in sheep have come from widely separated geographical areas of the world. The present report is to document a suspected case of chronic Cu poisoning involving two Balami sheep in a farm flock in Zaria, Nigeria.

MATERIALS AND METHODS
Two adult Balami sheep (a ram and a ewe) were submitted to the Department of Veterinary Pathology and Microbiology of Ahmadu Bello University, Zaria, for postmortem examination. The animals were among eleven Balami sheep which were bought and transported in a truck from Marguba in Borno State to Zaria — a distance of about 880 kilometers — just less than a week before death. They were reported to have shown symptoms of anorexia, weakness and lagging behind. Blood smears made from ear veins were stained with Giemsa and examined microscopically for blood parasites (Babesia spp). Thorough post-mortem examinations were made of the two sheep. Tissue samples obtained from the liver, kidney, brain, spleen, lymph nodes, and intestines were fixed in 10 per cent phosphate buffered formalin and processed by routine methods for paraffin sectioning. Sections were cut at 5μ and stained with haematoxylin and eosin (H & E). Special stains used on the liver sections were Van Gieson’s and Masson’s trichrome while silver impregnation technique was used on some kidney sections for the purpose of
detecting leptospiral organism. A sample of urine obtained was examined microscopically for presence of erythrocytes. Portions of the liver were frozen and later analyzed for Cu using a Perkin-Elmer Model 290 B atomic absorption spectrophotometer at the Soil Science Laboratory, Ahmadu Bello University, Zaria.

RESULTS

External examination of the carcasses showed yellow skin and visible mucous membranes. Both animals were well nourished and showed similar gross and histological changes in their organs and tissues.

On gross examination the liver was enlarged, yellow and friable. The gall bladder was distended with approximately 55ml of dark yellow and viscous bile but the bile duct was patent. There was extreme jaundice of all body fat and tissues. The spleen was enlarged and dark. The lymph nodes were enlarged, congested and oedematous. The enlarged kidneys were dark and the urinary bladder contained approximately 315ml of dark-red urine.

The pericardium and epicardium showed multifocal areas of petechial and ecchymotic haemorrhage. Similar lesions were seen on the serosae of the stomach and intestines. There was about 350ml straw-coloured fluid in the thoracic cavity of each of the carcasses. Frothy fluid seen in the trachea extended into the respiratory bronchi and bronchioles.

Liver tissues from the two sheep assayed for Cu showed values of 1790 and 1860 ppm on a dry weight basis. Blood smears were negative for Babesia species and no leptospiral organisms or erythrocytes were seen in the urine sample.

Histologically most lobules of the liver showed severe centrilobular degeneration and necrosis and these were associated with marked congestion in the necrotic areas. The affected hepatocytes were swollen and many had pyknotic nuclei. Several phagocytic cells containing haemosiderin pigments were seen in the sinusoids. Many renal cortical tubules were dilated and contained pinkish-to-purplish casts in the lumen. The cytoplasm of the tubular epithelial cells were granular and stained eosinophilic. Several glomeruli were distended while some were shrunken and associated with the presence of pink-stained deposits between the capsule and the glomerular tufts. Sections of the kidney specially treated by silver impregnation technique were negative for Leptospira species.

Diffuse congestion and haemosiderosis were the only significant findings in the spleen. The lymph nodes showed diffuse oedema and haemosiderosis mainly in the sinuses while in some sections, congestion and haemorrhage were marked. The colonial villi had dilated lacteals and there were submucosal congestion, oedema and thrombosis.

DISCUSSION

Copper poisoning in sheep does not seem at the moment to pose a major threat to the development of this industry in Nigeria. The present report is to reiterate and highlight the fact that this disease can occur in any region of the world where sheep farming is practised. The source of Cu in this case was not apparent. Since losses may continue for as long as five months after the ingestion of copper (Boughton and Hardy, 1934) it may be difficult or impossible in some instances to locate the source of poisoning. In some past disease outbreaks, investigators have reported such
Various sources of Cu as contaminated feed (Schaper and Luetje, 1931; Bischoff and Haum, 1939; Muth, 1952; Pearson, 1956; Pryor, 1959) and medications containing copper sulphate for prevention of helminthiasis (Boughton and Hardy, 1934). Another cause of hepatic Cu accumulation in the haemolytic disease was found to be pyrrolizidine alkaloids in hepatotoxic plants — *Heliotropium europaeum* and *Echium plantagineum* which damage the liver and cause the organ to collect and continuously accumulate Cu from normal diets (Anon, 1949/50; St. George-Grambauer and Rac, 1962; Bull, 1963/64).

Various types of stress factors including malnutrition, transportation and lactation have been suggested as precipitating the liberation of Cu from the liver. Few investigators have referred to stress as a predisposing factor in hepatic Cu release. Grayson (1949) suggested that stress could lead to derangement in the normal function of the liver and indirectly was responsible for the Cu release. The strain of travelling as an important factor in promoting the haemolytic crisis of Cu poisoning has been stressed (Anon, 1947-52). It has further been considered that forms of stress such as starvation after good feeding, exposure to cold and sudden unaccustomed exercise are important predisposing factors leading to liver Cu release (Allcroft, 1955). The predisposing factor that might have caused liver Cu release in the present reported case could be stress arising from the long distance transportation of the animals from Marguba to Zaria a few days prior to their developing clinical symptoms and death.

The carcasses were in good flesh, suggesting that the animals were well fed. It has been suggested that sheep are less liable to develop the disease if they are in good health (Fincham, 1945).

Descriptions of gross and histopathological lesions in the parenchymatous organs — liver, kidney and spleen agree with those of others (Boughton and Hardy, 1934; Grayson, 1949; Muth, 1952; Pearson, 1956; Jensen, 1974); however spongy degeneration of the midbrain, pons and cerebellum as described in sheep with induced Cu poisoning (Doherty *et al*., 1969) was not seen in either of the sheep examined here.

Although there is great variation, the Cu content of the livers of normal sheep rarely exceeds a concentration 500 p.p.m. D.W. (Albiston *et al*., 1940). The figures obtained from liver Cu determinations in the two sheep here were much higher than normal and this finding when considered together with the gross and histopathological findings was consistent with chronic Cu poisoning in sheep. Negative findings of evidence of babesiosis and leptospirosis — disease conditions that may show similar clinical symptoms and lesions in sheep also lent support to the diagnosis of chronic Cu poisoning.

ACKNOWLEDGEMENT

The author is indebted to the staff of Soil Science Laboratory, Ahmadu Bello University, Zaria, for carrying out the liver copper estimation.

REFERENCES


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HAEMATOLOGICAL PARAMETERS OF LARGE WHITE PIGS IN NIGERIA

D.I. SAROR and F. SANTIAGO,
Faculty of Veterinary Medicine and School of Agriculture, Samaru, Ahmadu Bello University,
Zaria, Nigeria

SUMMARY

Erythrocyte and leukocyte values were recorded from 5 litters of Large White pigs raised under improved husbandry practices ranging in age from 16 days to 146 days of age. The erythrocytes, haemoglobin and packed cell volume increased with age and became stabilized at 30 days of age. These parameters attained values reported for adult pigs 45 days after birth. Total leukocytes also increased with age and attained adult levels at 45 days of age. Lymphocytes were the predominant leukocytes observed. A comparison of blood parameters of the same breed of pigs raised by private farmers under local husbandry practices showed lower haemoglobin, packed cell volume, erythrocytes and leukocyte counts but higher eosinophil values. It is suggested that these values may serve as baseline haematological parameters of Large White pigs in this area.

INTRODUCTION

The blood picture of pigs is reported to be influenced by factors such as age, nutrition and management (Miller et al., 1961; McTaggart and Rowntree, 1969; Upcott et al., 1973; Schalm et al., 1975). In Nigeria, pigs are raised under varying husbandry and management practices. In view of recent government encouragement in the livestock sector, commercial pig production is getting a big push. Consequently, the indigenous black pig is being replaced by the exotic Landrace or Large White breeds because of their superior meat and growth qualities. Thus efforts are needed to obtain necessary information that could be used to evaluate herd health status and diagnosis of disease conditions. There is, however, a paucity of information on the blood picture of this category of pigs in Nigeria. In a preliminary investigation to determine normal haematological parameters of Large White pigs on private and commercial farms in this area, it became apparent that the presence of diseases including dermatitis, ectoparasites, gastrointestinal parasites, baby pig anaemia and the prevailing substandard management practices would invalidate any data collected from such pigs as baseline values for assessment of haematological derangements in Large White pigs in Nigeria.

The study reported here is based on pigs raised under improved management conditions and were free from overt clinical diseases.

MATERIALS AND METHODS

Pigs used in this investigation were the Large White breed maintained at the School of Agriculture Pig Farm, Ahmadu Bello University. The naturally born piglets were suckled by their respective dams which were fed a commercial pig ration. Water was provided ad lib. The litters were raised on concrete floor where each sow had its pen. These pens had separate concrete feeding and water troughs which were cleaned daily. Piglets were routinely treated with
iron-dextran injection 5 to 7 days after birth to prevent iron-deficiency anaemia. The pigs were weaned 8 weeks after birth. None of the male pigs was castrated. The pigs comprised males and females in equal proportions. No overt clinical disease was detected in any of the pigs in the course of the investigation.

Blood samples were taken from the anterior vena cava. In one litter of 8 piglets, sampling was done beginning at 16 days after birth and continued at about two week intervals until the pigs were 62 days old when they were sold. Four other litters were sampled beginning 2 to 3 weeks after birth. A few older pigs under the same management, whose history was known were also included. Blood samples were placed into tubes containing EDTA as anticoagulant and analysed using routine laboratory procedures (Saror and Schilhorn van Veen, 1979). All sampling was done between 08.00 hours and 09.00 hours. Blood parameters determined were packed cell volume (PCV), erythrocytes (RBC), total leukocytes (WBC) and haemoglobin (Hb). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Differential leukocyte counts were based on 100 cells. A qualitative evaluation of erythrocyte morphologic features was done by noting the presence and numbers of nucleated erythrocytes per two high power fields and the extent of polychromasia of erythrocytes. Statistical analysis was done by computer.

RESULTS

The changes with age in the haematological parameters of 8 Large White pigs from 16 days of age to weaning are presented in Table 1. The changes observed in all the pigs investigated are presented in Table II. It can be seen in Table I that there was an increase in PCV, Hb and RBC from 16 days of age to 30 days of age when these parameters became stabilized. No significant variations were encountered thereafter until the pigs were weaned. Morpholo-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>16</th>
<th>30</th>
<th>47</th>
<th>62</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV 1/1</td>
<td>34.0 ± 2.5</td>
<td>36.9 ± 2.4</td>
<td>37.5 ± 1.5</td>
<td>36.5 ± 6.0</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>11.0 ± 0.9</td>
<td>12.6 ± 0.7</td>
<td>12.5 ± 0.4</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>RBC 10^12/1</td>
<td>6.4 ± 0.7</td>
<td>7.4 ± 0.6</td>
<td>6.8 ± 0.3</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>WBC 10^9/1</td>
<td>8.5 ± 1.9</td>
<td>17.3 ± 3.9</td>
<td>16.7 ± 2.1</td>
<td>20.9 ± 3.2</td>
</tr>
<tr>
<td>Band Neutrophils</td>
<td>0.6 ± 0.5</td>
<td>0.1 ± 0.5</td>
<td>0.1 ± 0.5</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>36.3 ± 6.4</td>
<td>32.8 ± 8.3</td>
<td>30.0 ± 7.1</td>
<td>31.9 ± 6.4</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>60.2 ± 6.9</td>
<td>65.3 ± 7.2</td>
<td>66.9 ± 5.6</td>
<td>64.1 ± 6.7</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>1.7 ± 0.9</td>
<td>1.4 ± 1.8</td>
<td>2.0 ± 1.4</td>
<td>3.0 ± 1.9</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>1.2 ± 0.9</td>
<td>1.4 ± 1.8</td>
<td>2.0 ± 1.4</td>
<td>3.0 ± 1.9</td>
</tr>
<tr>
<td>Baophils %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>MCV Pg</td>
<td>53.1 ± 4.2</td>
<td>50.3 ± 5.2</td>
<td>55.6 ± 4.0</td>
<td>50.5 ± 2.9</td>
</tr>
<tr>
<td>MCH f1</td>
<td>17.2 ± 1.5</td>
<td>17.2 ± 1.8</td>
<td>18.2 ± 1.1</td>
<td>16.8 ± 0.9</td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>32.4 ± 0.5</td>
<td>34.0 ± 1.1</td>
<td>33.5 ± 0.7</td>
<td>33.2 ± 0.9</td>
</tr>
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<td>Parameter</td>
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<td>-----------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>No of Animals</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age in Days</td>
<td>146</td>
<td>129</td>
<td>60</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 2: Means and Standard Deviations of Haematological parameters of Growing Lake White Pigs.
gical features of peripheral erythrocytes observed in 16 day old pigs included the presence of few nucleated erythrocytes and polychromasia which was a routine feature of the pigs up to 45 days of age, however, with declining intensity, and was insignificant in 62 day old pigs.

The proportion of neutrophils to lymphocytes in peripheral blood remained fairly stable throughout the observation period, with lymphocytes as the predominant leukocytes. Neutrophil band cells were observed with decreasing frequency as the pigs advanced in age. Eosinophils were stable in pigs up to 30 days of age. Thereafter, a significant increase (P > 0.05) in eosinophil count was observed. Monocytes showed little variation while basophils were only occasionally seen. There were minor variations observed in the MCV of pigs up to 62 days of age while the MCH and MCHC values remained stable during the observation period.

The age-related haematological changes observed in all the pigs investigated are presented in Table II. The PCV, Hb and RBC values increased with age and attained adult levels by the time the pigs were 45 days old. Total WBC increased with age and became stabilized at adult levels at 45 days of age. Thereafter, no significant differences were observed in WBC of pigs up to 146 days of age. The proportion of neutrophils to lymphocytes was stable throughout the observation period with lymphocytes as the predominant leukocytes. Pigs of 129 days and older had significantly higher (P > 0.05) lymphocyte counts than younger pigs. Small but nonsignificant variations were observed in eosinophils, monocytes and basophils. Significant differences (P > 0.05) were observed in MCV of pigs up to 28 days of age. Thereafter, the values remained stable. The MCH showed moderate but significant variations (P > 0.05) up to 28 days of age but became stable by the time the pigs were 45 days of age. No significant (P < 0.05) age-related changes were observed in the MCHC of pigs investigated.

Table 3: Means and standard deviations of haematological parameters of Large White pigs raised on private farms*.

<table>
<thead>
<tr>
<th>Age (Estimated)</th>
<th>0–8 weeks</th>
<th>3–4 months</th>
<th>5.7 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Animals</td>
<td>34</td>
<td>63</td>
<td>22</td>
</tr>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV I/1</td>
<td>31.2 ± 6.2</td>
<td>34.1 ± 3.5</td>
<td>33.5 ± 4.2</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>9.3 ± 2.5</td>
<td>10.9 ± 1.1</td>
<td>10.9 ± 1.4</td>
</tr>
<tr>
<td>RBC x 10^12/l</td>
<td>6.2 ± 1.3</td>
<td>6.6 ± 0.7</td>
<td>7.1 ± 0.9</td>
</tr>
<tr>
<td>Total WBC x 10^9/l</td>
<td>7.1 ± 2.7</td>
<td>12.7 ± 4.6</td>
<td>11.4 ± 4.1</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>34.6 ± 11.9</td>
<td>35.6 ± 13.5</td>
<td>35.6 ± 8.2</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>61.4 ± 11.0</td>
<td>54.3 ± 13.5</td>
<td>60.4 ± 8.1</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>2.6 ± 2.7</td>
<td>8.9 ± 9.0</td>
<td>3.5 ± 2.7</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>1.3 ± 0.9</td>
<td>1.1 ± 0.9</td>
<td>0.6 ± 1.0</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0.05 ± 0.24</td>
<td>0.06 ± 0.25</td>
<td>0.0</td>
</tr>
<tr>
<td>MCV fl</td>
<td>50.3 ± 7.0</td>
<td>51.9 ± 6.1</td>
<td>47.7 ± 4.6</td>
</tr>
<tr>
<td>MCH pg</td>
<td>14.8 ± 2.8</td>
<td>16.5 ± 1.9</td>
<td>15.4 ± 1.4</td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>29.3 ± 3.1</td>
<td>31.1 ± 5.0</td>
<td>32.4 ± 1.7</td>
</tr>
</tbody>
</table>

* From Saror and Gyang (1979).
DISCUSSION
The mean haematological values of Large White pigs observed in this study are in close agreement with values reported for similar pigs in temperate climates (Miller et al., 1961, Upcott et al., 1973; Schalm et al., 1975; Hlousek, 1978). The erythrocytic parameters are, however, lower than those reported by McTaggart and Rowntree (1969) for Large White pigs raised under "minimal disease" conditions perhaps due to their nutritional status and other environmental factors under tropical conditions. The age related changes observed in these pigs are also similar to those reported by others (Imlah and McTaggart, 1977). The reported crossover of neutrophil and lymphocyte ratios in peripheral blood of young pigs was not observed in this study. It is probable that the switch was missed because of the two-weekly sampling intervals. The results of this study indicate that the blood parameters of Large White pigs in this area become stabilized to near adult levels by the time the pigs are weaned.

Previous field observations of exotic breeds of pigs raised under traditional husbandry practices showed that haematological values were lower than those obtained in this investigation. Table III presents the blood picture of similar pigs in this area raised by private farmers and fed largely on waste food from student hostel kitchens comprising of rice, yams, fruit peelings, yam peelings and cooked maize or guinea corn flour (Saror and Gyang, 1979). It can be seen that the pigs raised by private far-
mers under native husbandry practices had lower PCV, Hb and RBC values. Their erythrocyte indices were also lower than those from pigs in this study. These differences are probably due to differences in nutritional status and standards of husbandry. The higher eosinophil values and lower total leucocyte counts recorded from pigs on private farms may be due to the presence of parasitic conditions and the stress of undernourishment.

ACKNOWLEDGEMENTS
The authors express their gratitude to the Principal, School of Agriculture, Samaru, for permission to use the pigs. Our thanks also go to Mr. Felix Akuse for technical assistance and to the Ahmadu Bello University Research Board for funding the project.

REFERENCES

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SUMMARY

Using Meat Inspection data from the Veterinary Department, Kabete, Kenya, prevalence of cysticercosis in different abattoirs, in each province and at the KMC plants were determined for the period 1975-79. A great variation in the prevalence of C. bovis was observed in the local abattoirs, ranging from 0.74% in Vipingo in the Coast Province to over 18% in Kisii district. Prevalence at the KMC plants were, on the average, higher than in the local abattoirs (a mean of 19.3, 15.1 and 6.22% for Athi River, Nakuru and Mombasa respectively). It was suggested that the differences in prevalence from abattoir to abattoir were determined by local conditions which may include differences in rainfall, temperature, soil moisture and differences in the customs of the different ethnic groups of people and to some degree, differences in the thoroughness of infection. It is recommended that more studies be made to clarify the causes of these variation.

INTRODUCTION

The anthropozoonotic nature of tae-niasis and cysticercosis was first described and experimentally confirmed by Kuchemeister and Oliver respectively in 1853 and 1869 (Kean, Mott and Russel, 1978). More than a century later, infection of man and cattle, the hosts of T. saginata and Cysticercus bovis respectively, is known to be worldwide, with the highest prevalence in developing countries (Soulsby, 1965; Froyd, 1965; Pawloski and Schultz, 1972). In Kenya, there is generally a lack of detailed information concerning the prevalence and distribution of C. bovis. Previous investigators (Grindle, 1978; Ginsberg, Cameron, Coddard and Grieve, 1956; MacOwan, 1958; Froyd, 1960; 1965; Ginsberg 1954; 1955; Mann and Mann, 1947) used data from one slaughterhouse, the Kenya Meat Commission (KMC) at Athi River to study the prevalence of cysticercosis. The assumption was that cattle slaughtered at KMC came from all the ecological zones of Kenya. It is, however, evident that the same KMC now processes less than 10% of all the beef in the country.

Although Froyd (1965) described distribution of C. bovis in Kenya, his sample size was relatively small (47,000 cattle). Mango (1971) did not give details of how or where he collected his data and hence his results are not directly comparable with the present or other studies. Other investigators (Ginsberg et al., 1956; MacOwan, 1958; Froyd, 1960; Ginsberg, 1955) in their study of the distribution of C. bovis in Kenya classified infected cattle at KMC into European or African-derived without any further breakdown into their zones of origin.

The present study was carried out in an attempt to assess more accurately the extent and trend of C. bovis infection in different parts of Kenya using data from local abattoirs. Cattle slaugh-
tered in these abattoirs are assumed to have been drawn from the vicinity of the abattoirs. It is, however, regretted that at the time of this study, about 1/3 of the abattoirs were inspected by the staff of the Veterinary Department while the other 2/3 were inspected by Public Health Department. The annual returns prepared by the latter were, unfortunately, not sufficiently detailed or comprehensive to be used in this study.

**MATERIALS AND METHODS**

The annual and monthly returns of all cattle inspected by the Meat Inspectors of Veterinary Department were studied for the period 1975-1979 and the number of cattle killed and found infected to any degree with *C. bovis* was recorded. The returns from 31 abattoirs in the country and from the three KMC slaughterhouses at Athi River, Nakuru and Mombasa were examined. Using the least significant difference, Duncan’s new multiple range test and variance statistics, the prevalence of *C. bovis* in each abattoir was made and compared with the data from other abattoirs in the Province. The mean monthly prevalence was used as source of data. The status of cysticercosis in each province was worked out using the pooled data from all abattoirs in that province while the pooled data from all provinces provided data for the whole country.

**RESULTS**

The prevalence (% of infected cattle) of cysticercosis in different local abattoirs in Kenya between 1975-1979 are summarized in Table 1. There were significant differences in prevalences between many abattoirs. Vipingo in the Coast and Embu in the Eastern Provinces had the lowest prevalence of under 1% while Kisii in Nyanza province and Ngong in the Rift Valley provinces had relatively high prevalences of 18.8 and 10.3% respectively. The overall prevalence of *C. bovis* in each province is given in Table II. Significant differences were again evident between the provinces over a period of time. The Coast province had the least, under 2% and Nyanza province had the highest, over 9%. The prevalence of *C. bovis* in the three KMC plants, however, showed little differences between them (Table III). The overall prevalence in the entire country is given in Table IV and graphically presented in figure 1. There is a downward trend in prevalence between 1975 and 1979.

Prevalence of bovine cysticercosis in a given abattoir has not been consistently uniform. In Vipingo, for example, the 1975 data indicated that there were eight monthly reports in which prevalence was zero and four months in which it was about 1.5%. In 1978, there were nine months in which prevalence was zero and three in which it was about 1.3%. Likewise in Kisii, the 1977 monthly reports showed a range in prevalence of 11 — 19%, and of 14 — 30% in 1978, and 12 — 26% in 1979. Other abattoirs had varying monthly prevalence of *C. bovis*.

The differences in prevalence of cysticercosis at KMC, Athi River and Nakuru were not significant but were much higher than in the smaller abattoirs. The 1979 figures for Nakuru were exceptionally low (2.1%). At Mombasa KMC, the range of prevalence was 2.3 — 12.4% between 1975-1979.

Not all the abattoirs examined had been in operation during the period of study. In 1975, for example, there were only 10 abattoirs that were inspected
Table 1: Prevalence of cysticercosis in Kenya's abattoirs over a 5-year period (1975–1979).

<table>
<thead>
<tr>
<th>Test of significance</th>
<th>Given number</th>
<th>Abattoirs</th>
<th>X</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB: Judged by Duncan's New Multiple Range test; those underlined are not significantly different at the 5% level.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vipingo</td>
<td>0.74 ± 0.68</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Embu</td>
<td>0.91 ± 0.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Nyeri</td>
<td>1.10 ± 0.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Machakos</td>
<td>1.11 ± 0.77</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Tala</td>
<td>1.30 ± 1.63</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Nanyuki</td>
<td>1.35 ± 1.16</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Malindi</td>
<td>1.40 ± 0.48</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Mariakani</td>
<td>1.90 ± 1.05</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Nyahururu</td>
<td>1.97 ± 1.16</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Naivasha</td>
<td>2.07 ± 2.57</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>*Athi River</td>
<td>2.11 ± 1.86</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Karatina</td>
<td>2.13 ± 1.19</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Kericho</td>
<td>2.87 ± 1.12</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Kitale</td>
<td>2.92 ± 1.32</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Bungoma</td>
<td>3.60 ± 1.61</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Nyongoro</td>
<td>3.64 ± 2.57</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Waithaka</td>
<td>4.33 ± 4.22</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Homa Bay</td>
<td>4.37 ± 2.65</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Dandora</td>
<td>4.63 ± 2.45</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Kakamega</td>
<td>5.12 ± 2.74</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Eldoret</td>
<td>5.30 ± 1.25</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Muranga</td>
<td>5.80 ± 2.96</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>Thika</td>
<td>5.85 ± 7.43</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Kiambu</td>
<td>6.07 ± 1.77</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Kisumu</td>
<td>6.41 ± 4.39</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>Webuye</td>
<td>7.7 ± 2.88</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>Ongata Rongai</td>
<td>8.32 ± 4.47</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>Meru</td>
<td>9.06 ± 1.95</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>Gilgil</td>
<td>10.13 ± 3.60</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>Ngong</td>
<td>10.26 ± 5.70</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Kisii</td>
<td>18.83 ± 2.57</td>
<td>35</td>
</tr>
</tbody>
</table>
Fig. 1: Trend in the prevalence of cysticercosis in Kenya between 1975 – 1979.

Table 2: Prevalence of Cysticercosis in Provinces of Kenya between 1975—79.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast</td>
<td>2.5 ± 2.69</td>
<td>2.3 ± 0.71</td>
<td>0.7 ± 0.26</td>
<td>0.7 ± 0.46</td>
<td>1.3 ± 1.24</td>
<td>1.5</td>
</tr>
<tr>
<td>Central</td>
<td>*</td>
<td>*</td>
<td>4.1 ± 2.37</td>
<td>2.1 ± 1.23</td>
<td>5.1 ± 4.14</td>
<td>3.77</td>
</tr>
<tr>
<td>Nairobi</td>
<td>≠3.2</td>
<td>6.9 ± 5.30</td>
<td>4.8 ± 3.20</td>
<td>2.3 ± 0.66</td>
<td>3.7 ± 2.92</td>
<td>4.18</td>
</tr>
<tr>
<td>Eastern</td>
<td>5.5 ± 3.3</td>
<td>6.6 ± 8.79</td>
<td>3.0 ± 6.14</td>
<td>3.2 ± 0.16</td>
<td>3.7 ± 2.85</td>
<td>4.4</td>
</tr>
<tr>
<td>Rift Valley</td>
<td>8.8 ± 7.46</td>
<td>5.0 ± 3.66</td>
<td>5.4 ± 4.75</td>
<td>6.6 ± 4.93</td>
<td>4.5 ± 3.65</td>
<td>6.06</td>
</tr>
<tr>
<td>Western</td>
<td>≠7.6</td>
<td>8.6 ± 1.91</td>
<td>3.6 ± 1.23</td>
<td>5.8 ± 1.70</td>
<td>5.1 ± 4.35</td>
<td>6.14</td>
</tr>
<tr>
<td>Nyanza</td>
<td>≠10.8</td>
<td>≠11.6</td>
<td>5.8 ± 6.12</td>
<td>10.8 ± 11.98</td>
<td>9.4 ± 7.74</td>
<td>9.68</td>
</tr>
</tbody>
</table>

≠ Only one source of data on record
* No data available
Table 3: Prevalence of *C. bovis* in the three KMC plants between 1975—1979.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Athi River</td>
<td>20.5 ± 10.67</td>
<td>17.3 ± 1.9</td>
<td>17.7 ± 4.9</td>
<td>21.7 ± 10.0</td>
<td>19.3 ± 4.5</td>
<td>19.3</td>
</tr>
<tr>
<td>Nakuru</td>
<td>18.9 ± 7.4</td>
<td>12.4 ± 10.00</td>
<td>21.9 ± 10.4</td>
<td>19.9 ± 9.4</td>
<td>2.1 ± 1.4</td>
<td>15.1</td>
</tr>
<tr>
<td>Mombasa</td>
<td>12.4 ± 5.04</td>
<td>11.6 ± 8.9</td>
<td>2.3 ± 1.9</td>
<td>4.8 ± 2.2</td>
<td>8.6 ± 3.8</td>
<td>6.22</td>
</tr>
</tbody>
</table>

Table 4: Prevalence of Bovine Cysticercosis in Kenya 1975-1976 — Total cases.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cattle killed</th>
<th>Number Infected with <em>C. bovis</em></th>
<th>Prevalence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>202731</td>
<td>29531</td>
<td>14.57</td>
</tr>
<tr>
<td>1976</td>
<td>305814</td>
<td>42620</td>
<td>13.94</td>
</tr>
<tr>
<td>1977</td>
<td>271617</td>
<td>28367</td>
<td>10.44</td>
</tr>
<tr>
<td>1978</td>
<td>211159</td>
<td>20505</td>
<td>9.71</td>
</tr>
<tr>
<td>1979</td>
<td>253865</td>
<td>20846</td>
<td>8.21</td>
</tr>
</tbody>
</table>

N.B. Data do not take into account beef inspected by the Public Health meat Inspectors.

by the Veterinary Department, Kabete, and for which the monthly and annual returns were available. The number of these abattoirs rose to 12, 28, and 31 in the years 1976, 1977 and 1978 respectively. There was no change in the numbers in 1979.

**DISCUSSION AND CONCLUSION**

The importance of cysticercosis to cattle industry cannot be overemphasized. The serious economic loss it causes in Kenya has been recognized since the turn of this country (Kenya Veterinary Annual Report, 1908-9). At this stage of Kenya’s economic development, there is a shift in emphasis from pastoral to a cash or export economy in animal production. Cysticercosis is bound to play a very negative role in the success of this development. As a strategy to control or reduce the effects of cysticercosis, a thorough study of its epidemiology, distribution across the country and the trend of incidence over several years is indispensable.

The prevalence of cysticercosis in this country varies a great deal between abattoirs and also within the same abattoir over a period of time. At KMC, Athi River alone, this variation has ranged from 8% (Mann and Mann, 1947) to a peak of 44.2% (MacOwan, 1958). Ginsberg (1954) recorded an average of 56.5% infection in calves. During this study, monthly prevalence of cysticercosis at the KMC plants: Athi River, Mombasa and Nakuru has ranged from 6-38%, 0.6-25% and 0.37% respectively. At other abattoirs, the range has also been very large in some but relatively small in others. Vipingo on the Coast, for example, has monthly prevalence over 58 months ranging from 0-4% while Ngong has a prevalence rate ranging from 0.17% in 50 months. apart from this range of variation within one abattoir, it is interesting to note
some variation between two or more abattoirs that are geographically close together. Gilgil and Naivasha (which are approximately 15 miles apart) had an overall prevalence of 10.1% and 2.1% respectively taken from about 36 monthly data. Similarly, Kisii and Homa Bay, (about 20 miles apart) both in Nyanza province, had a prevalence of 18.8% and 4.4% respectively. It would be difficult to think that factors influencing prevalence rates of *C. bovis* at such close centres are different yet the ethnic groups and geographical factors at the same centres are similar.

Differences in prevalence rates between abattoirs that are distant apart can be associated with natural differences between them such as mean rainfall, temperature, soil type, soil moisture and even vegetation which are known to influence the life span of ova of *T. saginata* (Chapman, 1945; Duthy, Van Someren, 1948; Luckner, Douvres, 1960; Luckner, 1960; Round, 1961). Soil cover (grass), temperature and relative humidity influence in turn, rate of evaporation. In Kenya, there are different rainfall zones ranging from under 500mm per annum to about 2,000mm. (E.A. Meteorological Department, 1975). Similarly, mean temperature ranges from about 26°C at the Coast, 27°C in Lodwar and Isiolo to under 17°C in most parts of the Rift Valley.

Other possible explanations for the variation is the degree of thoroughness in inspection and differences in the customs of people in Kenya. The KMC plants which process meat for export is a good example of this. It has provided consistently higher prevalence rates of cysticercosis than have those abattoirs located in regions from where KMC draw their animals. Under ordinary conditions, it is known that cysts of *C. bovis* can easily escape the inspector’s eye (Griffith, 1950; Ginsberg, 1954) and it would seem that much more effort in inspection is required in these abattoirs if a true prevalence picture is to be expected. Kenya has many ethnic groups including pastoral and settled farmers. The degree of man/cattle association is, therefore, also different. Differences in ethnological groups is known to influence the prevalence of taeniasis and hence cysticercosis (Pawloski and Schultz, 1972), and in Kenya, Froyd (1965) described results supporting this phenomena. The customs of each of the Kenya’s ethnic groups perhaps account for the greatest part in epidemiology of taeniasis/cysticercosis in this country.

In conclusion, the recorded prevalence of cysticercosis which is found to be lower in local abattoirs than in the better established KMC slaughter-house is probably influenced greatly by thoroughness of inspection and accuracy of recording data. The geographical differences between the abattoirs and the subsequent differences in the precipitation, evaporation or temperatures are possible cause of variation. The customs of the Kenya’s ethnic groups probably play a role in the epidemiology of this tapeworm. The chief factors known for maintaining the transmission of taeniasis and cysticercosis are the insanitary disposal of human excreta and eating of raw or insufficiently cooked meats (Froyd, 1965; Eisa, Mustafa and Soliman, 1962). It would, therefore, seem that Health Education must rank high in the priorities for determining control measures against this parasite. Studies must, however, continue to determine precise information about factor(s) influencing the epidemiology of this tapeworm. Such studies must
be supported by Public Health planners and researchers, Social scientists, Veterinary Research Scientists and Politicians. However, it is gratifying to note some downward trend in the prevalence of cysticercosis in the period under study.

ACKNOWLEDGEMENTS

The author appreciates, the kind support given him by the Director of Veterinary Services, Kabete, and his team, for giving him access to the monthly and annual returns. The author also would like to thank the Director of Veterinary Research Department, Muguga, for supporting the study and for permission to publish the results.

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TRICHOPHYTOSIS IN FARM ANIMALS AND TRIALS FOR TREATMENT

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SUMMARY
Sixty hair and scale samples from 30 cows, 30 buffaloes and 2 camels showing skin lesions suggestive of ringworm infection were examined. The results showed that:

1. *Tr. verrucosum* was the cause of trichophytosis in the cows and buffaloes.
2. Double infection with *Tr. verrucosum* and *Tr. gypseum* was the cause of ringworm in the two camels.
3. The efficacy of local treatment in camels with one of the following fungicides: 6% salicylic acid, 6% benzoic acid, 5% tincture of iodine and 2% precipitated sulphur was compared with a mixture of Salicylic acid 6.0 Benzoic acid 8.0 Glycerine 10.0 ml. Alcohol 100.0 ml.

INTRODUCTION
The reported frequency of infection with different types of pathogenic fungi in cows and buffaloes in Egypt varies considerably. Abdallah et al., (1971) found in a cattle farm in Assiut (Upper Egypt) that *Trichophyton verrucosum* was the etiological agent of trichophytosis in 52.5% of the cases examined, whereas *Trichophyton gypseum* caused only 47.5% of the cases. On the other hand, Refai (1976) stated that *Trichophyton verrucosum* was the only cause of cattle and buffalo-ringworm in Egypt.

The literature on ringworm in camels is scanty. Nasser (1969) found that *Tr. verrucosum* was the main causative agent of camel ringworm. The present investigation is a trial to link between the clinical picture of ringworm and the laboratory diagnosis in 62 cases of cows, buffaloes and camels as seen in different parts of North Egypt.

Clinical Data

Cows and Buffaloes
Single or multiple lesions were noticed on the head, neck, chest, eyelids and sometimes on other parts of the body of cows and buffaloes in herds in different provinces (El Gharbia, El-Beihara, Alexandria, El-Monoufia) in North Egypt. They varied from oval or rounded, small lesions (about 1 cm. in diameter) to large (4–5 cm) lesions devoid of hair and covered with whitish, cigar ash-coloured scales.

Camels
Multiple lesions were observed in a young camel (5–6 months old), whereas single lesions were seen in an adult female-camel. Some of the lesions were 5–8 cm. in diameter. They were similar in appearance to those in cattle and occurred on the neck and chest. The other lesions were multiple, small (2–3 cm. in diameter), rounded or oval, greyish-white or asbestos coloured and situated on the nasal bridge, neck, chest, abdomen, and other parts of the body. (Fig. 1).

*Animal Health Institute, Dokki, Cairo, Egypt
The two camels had recently been purchased by the Faculty of Veterinary Medicine, Alexandria University, from El-Monoufia and El-Behaira Provinces.

**MATERIAL AND METHODS**

Hair and scales were scraped from the periphery of affected areas of the skin of 30 cows and 30 buffaloes and 2 camels. Samples were subjected to routine mycological examination as described by Emmons *et al.*, (1977) and the results of the simple direct smear method and primary cultures were recorded. Each sample was cultured on 5 tubes of Sabouraud’s agar with cyclo-heximide (Actidione) and observed for 1–4 weeks. Final diagnosis was made in 1–4 weeks after the primary cultures had been grown on Sabouraud’s dextrose agar in accordance with the morphological features of isolants as described by Hazen *et al.*, (1960).

The Pathogenicity of each isolate was checked by duplicate guinea pig inoculation. This was carried out after the method of Bonk *et al.*, (1962) but with some modifications.

In our method the hair was clipped from two areas, one of which was about 4 cm. wide, 5 cm. long on the back and the other on the thigh. The skin was then scraped gently with a blunt scalpel to produce slight abrasions. Two to 4 week-old mycelial mats were scraped from the surface of Sabouraud’s dextrose agar and mixed in a mortar with some of the aforementioned media and subsequently was spread over the skin.

The development of infection was recorded in terms of erythema, scaling and degree of crusting and its extent was graded from + to ++++. Microscopic examination of hair and skin scales was performed weekly so as to isolate the causative agent.

Small biopsy specimens were examined histologically using Periodic acid Shiff stain.

Treatment trials were only carried out on camels where the lesions on
<table>
<thead>
<tr>
<th>Organism</th>
<th>Mycological Examinations</th>
<th>Microscopic Morphology</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton verrucosum</td>
<td>Absent, no mycelium</td>
<td>Absent, no mycelium</td>
<td>-</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>Absent, no mycelium</td>
<td>Absent, no mycelium</td>
<td>-</td>
</tr>
<tr>
<td>Tinea capitis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Results of mycological and histopathological examinations and guinea pig inoculation

<table>
<thead>
<tr>
<th>Mycological Examinations</th>
<th>Microscopic Morphology</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Notes:**
- Absent, no mycelium
- Cultures were negative for mycelium.
each part of the body were divided into 5 separate zones each of which received one of the following treatments: 6% salicylic acid, 6% benzoic acid, 5% tincture of iodine, 2% precipitated sulphur, or a mixture of Salicylic acid 6.0. Benzoic acid 8.0 Glycerine 10.0 ml. and Alcohol 100.0 ml. respectively.

The treatments were topically applied by smearing daily for 5-8 weeks, after which the results were recorded.

RESULTS

The results of the mycological examination of the hair and scales and the morphological appearance of the isolates are summarized in Table 1. Only *Tr. verrucosum* was isolated from the samples obtained from 30 cows and 30 buffaloes; both *Tr. verrucosum* and *Tr. gypseum* were isolated from the 2 camels as concurrent infections. *Tr. verrucosum* was isolated from the largest lesions (5-8 cm. in diameters) of the neck and chest while *Tr. gypseum* was demonstrated from the small lesions (2-3 cm. in diameter) on other sites of infection.

Culture of *Tr. verrucosum* isolated from the buffaloes and cows showed polymorphism even in single cell cultures. But not those from the camels. Differences in the cultural characters were not associated with differences in the virulence as shown in Table 1.

The efficacy of different preparations applied locally to the two camels is shown in Table 2. It is seen that the local application of benzoic acid alone or in combination with other ingredients gave better results as judged by the time needed to cure the lesions. Iodine comes next to the aforementioned applications. *Tr. verrucosum* infection responded to benzoic acid treatment more rapidly than *Tr. gypseum* infection.

Table 2: The effect of local application of various drugs on trichophytopsis in two camels.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time needed for cure (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
</tr>
<tr>
<td>Salicylic acid 6%</td>
<td>8</td>
</tr>
<tr>
<td>Benzoic acid 6%</td>
<td>5-6</td>
</tr>
<tr>
<td>Iodine tincture 5%</td>
<td>6</td>
</tr>
<tr>
<td>Sulphur ointment 2%</td>
<td></td>
</tr>
<tr>
<td>Mixture*</td>
<td>5</td>
</tr>
</tbody>
</table>

* Consisted of: Salicylic acid 6.0 gm, benzoic acid 8.0 gm., glycerine 10.0 ml., and alcohol 100.0 ml. Not effective.

DISCUSSION

In the present investigation *Tr. verrucosum* was isolated from 30 cows and 30 buffaloes from the Northern regions of Egypt. Refai (1976) reported the same Trichophyton species in cows and buffaloes from the southern parts of Egypt. This finding agrees with reports from other countries (Gentles and O.Sullivan, 1957; and Oka and Asuma 1968).

Double mycotic infection with *Tr. verrucosum* and *Tr. gypseum* was found in the two camels. It is the first time this is reported in camels in Egypt. The finding of *Tr. verrucosum* in ringworm infection of camels partly confirms the suggestion of Nasser (1969) that this agent may be the main cause of camel ringworm. It is interesting to note that Rieth and Schirman (1958),
and Kachnic (1967) also had reported double mycotic infection in man.

ACKNOWLEDGEMENT

We are indebted to Prof. Dr. A.A. Sami, Dean of the Faculty for his criticism and to Prof. Dr. I.M. Abou El-Azm for his help and guidance.

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Received for publication on 15th January, 1980
A NOTE ON HYALOMMA M. RUFIPES KOCH, 1844 OFF DOMESTIC STOCK IN THE SUDAN

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Central Veterinary Research Laboratories, Soba-Sudan.

SUMMARY

Hyalomma m. rufipes behaviour off domestic stock was investigated in Tulus area during April-October 1973.

The cycle of development apparently starts shortly after the advent of infested herds into the area. Female Oviposite in stem cracks of Acacia nilotica, Larvae hatch and feed on birds and rodents and drop as fed nymphs. Nymphal moulting takes place between April and June and young adults attack to domestic animals.

INTRODUCTION

Several species of Ixodid ticks undergo their biological development in sites associated with animal habitat, (Barnett, 1961 Hoogstraal, 1956 and Grey 1961). The author thus considered that useful data on their ecology and seasonal cycle would be gathered if these habitats were investigated.

On these bases Hyalomma m. rufipes Koch, 1844 which occurs extensively in Southern Darfur was investigated during the period April-October 1973.

MATERIAL AND METHODS

The investigation was carried out in Tulus area (11° N Lat. 24° 33°E Long.) This area is in the Baggara Repeating Pattern of the low rainfall savanna (Harrison, 1958). The rainfall average is 600 mm mainly during June-October but rain showers start earlier (April or May).

Ticks were searched for under debris, in tree cracks and holes, along cattle routes and in stables and a single collection from a hare -- Lepus saxitiles. This survey covered an area of nine miles transect comprising the land systems described by Harrison, 1958. Ticks thus collected were preserved in wide mouthed bottles in 70% alcohol. Adult ticks were identified to species according to keys provided by Hoogstraal, 1956.

In Tulus area large numbers of nomadic cattle, sheep and camels are watered from shallow water-wells at Wadi Bulbol bed during the dry season (December-June).

RESULTS AND DISCUSSION

The data obtained in this survey was expected to throw light on some aspects of the seasonal activity of H. m. rufipes.

Shortly after the advent of nomadic herds (in December), which are infested with this tick engorged females drop and seek refuge in stem cracks of Acacia nilotica. Oviposition and hatching take place presumably during January-period. Larvae probably feed during April and a part of May and drop as fed nymphs. Larvae of this tick have a strong preference for birds on which they feed and drop as fed nymphs. (Keiser, personal communication). However, in Tulus area engorged nymphae were collected from a hare, Lepus saxitiles. Nymphal moulting takes place in April-June.

The obtained data also indicated that the unattatched ticks of this species occurred on all land divisions but they were more common on alluvial land systems, specially under fig and mango trees. Nymphae undergoing moulting
Table 1: *Hyalomma m. rufipes* monthly collection from ground sources in Tulus area (1973)

<table>
<thead>
<tr>
<th>Month</th>
<th>M</th>
<th>F</th>
<th>MN</th>
<th>Site of collection</th>
<th>Type of soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>128</td>
<td>90</td>
<td>130</td>
<td>Under debris of Fig tree</td>
<td>Alluvial</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>20</td>
<td>132</td>
<td>Under debris of Mango tree</td>
<td>Alluvial</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>—</td>
<td>Under debris Balanites</td>
<td>N*</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>9</td>
<td>—</td>
<td>Acacia nilotica stem cracks</td>
<td>Cracking Clay</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>—</td>
<td>9</td>
<td><em>Hares Lepus saxitiles</em></td>
<td>—</td>
</tr>
<tr>
<td>May</td>
<td>58</td>
<td>36</td>
<td>75</td>
<td>Under debris — Fig trees</td>
<td>Alluvial</td>
</tr>
<tr>
<td></td>
<td>132</td>
<td>102</td>
<td>29</td>
<td>Mango tree under debris</td>
<td>Alluvial</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>35</td>
<td>—</td>
<td>Balanites tree under debris</td>
<td>Naga</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>100</td>
<td>20</td>
<td>Stable wall cracks</td>
<td>Sand</td>
</tr>
<tr>
<td>June</td>
<td>69</td>
<td>125</td>
<td>20</td>
<td>Fig trees under debris</td>
<td>Alluvial</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>104</td>
<td>14</td>
<td>Mango trees under debris</td>
<td>Alluvial</td>
</tr>
<tr>
<td>July</td>
<td>29</td>
<td>22</td>
<td>—</td>
<td>Along cattle routes</td>
<td>Alluvial</td>
</tr>
<tr>
<td>August</td>
<td>7</td>
<td>6</td>
<td>—</td>
<td>Along cattle routes</td>
<td>Alluvial</td>
</tr>
<tr>
<td>Sept.</td>
<td>5</td>
<td>2</td>
<td>—</td>
<td>Along cattle routes</td>
<td>Alluvial</td>
</tr>
<tr>
<td>October</td>
<td>7</td>
<td>2</td>
<td>—</td>
<td>Along cattle routes</td>
<td>Alluvial</td>
</tr>
</tbody>
</table>

* Cemented clay soil.  
MN = Nymphs

were however only restricted to alluvial soils with special preference for debris under fig and mango trees. A lesser degree of dissipation rate is expected in the moist alluvial bed than on sand and Naga.

*Hyalomma m. rufipes* occurs extensively on cattle, camels and horses in Southern Darfur, especially in the Baggara Repeating Pattern where localities resembling Tulus area are very frequent. Preliminary data on the ecology and seasonal activity of this tick were obtained. Adequate data may be obtained by more investigation. However, at this stage it could be concluded that the utilization of pasture and water outside the alluvial areas may minimize animal infestation with this tick.

ACKNOWLEDGEMENT

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MYCOPLASMAL INFECTION OF GOATS AND SHEEP IN THE SUDAN

HARBI, M.S.M.A., ABDULLA, A.E.D., EL TAHIR, M.S. SHALLALI, A.A., ABDEL GABAR, K.M.A. and MANSOUR, E.A.,
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SUMMARY

The importance of goat pleuropneumonia and relevant infections in sheep due to Mycoplasmas are discussed. A Mycoplasma resembling M. mycoides presumed as the causative agent of CCPP in the Sudan. This was confirmed serologically by the growth inhibition test and artificial reproduction of disease in goats even with the pure culture. The final isolation of M. mycoides was hampered by the overgrowth of M. arginini which happened to occur in the same culture. M. arginini was isolated for the first time from goats in the Sudan. In sheep it was isolated in association with other pathogens. M. gallinarum and a virus were isolated from a case of sheep pneumonia. M. arginini was recorded for the first time from eye infection in sheep in the Sudan. The report of Agalactia in goats in the Sudan is not confirmed. The authors advise that CCPP in this country should not be described as Abu Nini. The latter could be a syndrome due to various pathogens, alone or occurring in association with mycoplasmas.

INTRODUCTION

The importance of goat pleuropneumonia in the Sudan provided stimulus for investigation into this disease over the last two decades (Karib 1958, El Nasri 1964, 1967, Abdulla and Lindley 1967, 1969, Abdulla, Harbi, El Tahir, Salim, Salih, and Muktar 1979). In some of these investigations it was shown that the disease could be produced artificially. This was obtained by spraying lung lymph into the nostrils of healthy goats (Karib 1958) and by the intratracheal endobronchial inoculation of the lung suspension or pleural exudate from sick goats (Abdulla and Lindley 1967, Abdulla et al. 1979). The last authors also showed that cultures in inanimate media or just in normal goat serum were capable of setting up the disease when inoculated endobronchially. Treatment of the disease also received some attention and successful trials were reported (Otte 1960, El Nasri 1964). Identification of agents causing this disease was first reported by Pillai in the early sixties (El Nasri 1964). These agents were identified serologically as M. mycoides subsp. mycoides* by the CF test (Lemcke 1964). El Nasri (1967) reported isolation of two strains from goats which were found to be identical and serologically not related to Pillai strains and M. mycoides. Both strains showed antigenic relationship with the Nigerian strain OSB42. Ten years later, Harbi (1977) isolated M. ovipneumoniae from an outbreak of CCPP.

The first mycoplasma isolated from sheep in this country was reported by Khan (1960). It was from a case of sheep pneumonia. The organism was identified by the author as a PPLO from cultural characteristics only. After a lapse of time Muna El Mahi (1974) isolated M. arginini from pneumatic lungs of sheep.
In this paper we report the results of surveys of outbreaks of pneumonia or pleuropneumonia in sheep. A small scale study was also carried out to detect the role of Mycoplasma in eye infection.

Agalactia of sheep and goats has not been confirmed in the Sudan. In surveying the aetiology of mastitis in goats, Ibrahim (1969) was able to isolate Mycoplasma from goats suffering from acute form of mastitis. From cultural characteristics and biochemical findings only, he ascribed the strain to *M. agalactiae*. This result stimulated the present authors to further survey mastitis in goats with the purpose of elucidating the exact agent of the disease.

**MATERIAL AND METHODS**

**Specimens**

Pleural fluid was collected from goats suffering with pleuropneumonia by the method of Abdulla (1966). Lung samples from goats or sheep were taken under aseptic conditions. Milk was collected in sterile containers from goats with clinical and subclinical mastitis. Swabs from inflamed eyes were brought from sheep in Kadaru export quarantine near Khartoum. Material obtained from the field was cultured in Brucella Broth and Agar (Albimi), Mycoplasma Broth Base and Mycoplasma Agar Base (Oxoid). These media were prepared according to the Manufacturers' instruction. However, they were supplemented with 20% horse serum, three grams of yeast extract per litre, 0.6 grams per litre of DNA, 200,000 units of penicillin per ml and one ml of 10% solution thallium acetate. Pure goat serum prepared according to Abdulla and Lindley (1967) was also used as a medium. Incubation at $37^\circ C$ in humid chamber was carried out under aseptic conditions.

**Animal Inoculation**

Nine Nubian goats 1–2 years old and five sudanese desert sheep 1–2 years old and three Butana calves 6 months to one year old were used in this experiment. Animals were free from bacterial, protozoal infection and internal parasites. They were kept in separate enclosures; one of each species was housed apart as control. Body temperature was recorded one week before and after incubation.

Two goats among the group were inoculated endobronchially with the pleural exudate from diseased goat brought to Khartoum Veterinary Clinic for treatment. Both goats exhibited acute pneumonic symptoms, high temperature and died 6 and 7 days respectively after inoculation.

Ten folds serial dilution of pleural exudate collected from these goats were made in the above mentioned media. One tenth ml was streaked onto the corresponding solid media.

Cultures obtained in pure goat serum were used in inoculation of goats and for maintenance of the growth of the organisms in the other media. Two sheep were inoculated with the pleural fluid from an experimentally infected goat and so were two Butana calves. The respective doses were 5.0 ml and 20.0 ml. One sheep received 0.5 ml of culture of *M. arginini* at the log phase into one of the eyes.

**Cultural, Biochemical and Serological Procedures**

These were carried out for identification of the strain as described by Ernø and Stipkovits (1973). However only
those tests mentioned in this paper were used. Reference strains and sera were generously provided by Prof. Freundt, E.A. Director FAO/WHO Collaborating Centre for Animal Mycoplasma, Aarhus, Denmark; and by Dr. P. Perreau of the I.E.M.V.T., France.

RESULTS

No growth was obtained when material from the living sick goats or aspirated from their lung homogenate were cultured in Brucella and Mycoplasma media (see material and methods) growth however was obtained in whole goat serum. After three subcultures into goat serum it was possible to shift to the other described media. Mycoplasma Broth and Agar media (Oxoid) were found to be satisfactory for further sub-cultures. The organism belongs to the family Mycoplasmataceae showing 7 mm zone of inhibition with digitonin. The urease test was negative and further identification was carried out on the grounds that the strain belongs to the genus Mycoplama. The growth characteristics and biochemical findings of our isolate KVC1 (from Khartoum Veterinary Clinic), and Gladysdale are shown in Table 1. The result of the growth inhibition test of the last two strains were shown in table II. Glucose fermentation and arginine hydrolysis were inconsistent in the first subculture. However the fifth subculture became constant and positive for arginine. Except for arginine hydrolysis the strain exhibited characteristics typical of gladysdale. PG3 exhibited distinct digestion of inspissated serum but was poorly inhibited with a hyperimmune serum to gladysdale. It was also noted that KVC1 killed goats only after the fourth subculture but the fifth did not. When the fifth subculture was checked for growth inhibition it was only positive with anti-serum to strain G230 of M. arginini. This indicated the possible co-existence of M. mycoides from the start. This suspicion was supported by the inconsistent biochemical tests in the early subcultures. Trials to inhibit arginini in the early cultures to keep M. mycoides alone was not successful.

Table 1: The Growth characteristics and biochemical reactions of three strains of mycoplasmas

<table>
<thead>
<tr>
<th>CHARACTERS</th>
<th>STRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KVC 1</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>typical Fried egg appearance 7 mm</td>
</tr>
<tr>
<td>Sensitivity to digitonin</td>
<td>-</td>
</tr>
<tr>
<td>Urease activity</td>
<td>-</td>
</tr>
<tr>
<td>Phosphatase activity</td>
<td>-</td>
</tr>
<tr>
<td>Glucose Fermentation</td>
<td>+</td>
</tr>
<tr>
<td>Catabolism of arginine</td>
<td>+</td>
</tr>
<tr>
<td>Reduction of tetrazolium</td>
<td>+</td>
</tr>
<tr>
<td>Digestion of coagulated serum</td>
<td>-</td>
</tr>
<tr>
<td>Hemolysis of sheep RBCS</td>
<td>(αβ)</td>
</tr>
</tbody>
</table>
Table II: Growth inhibition test — Antiserum & Zone of inhibitions

<table>
<thead>
<tr>
<th>test</th>
<th>Strain examined</th>
<th>PG 3</th>
<th>Gladysdale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc Method</td>
<td>KVC1</td>
<td>0</td>
<td>5mm</td>
</tr>
<tr>
<td></td>
<td>Gladysdale</td>
<td>0</td>
<td>5mm</td>
</tr>
<tr>
<td></td>
<td>PG 3</td>
<td>4mm</td>
<td>3mm</td>
</tr>
<tr>
<td>Well Method</td>
<td>KVC1</td>
<td>0</td>
<td>5mm</td>
</tr>
<tr>
<td></td>
<td>Gladysdale</td>
<td>0</td>
<td>6mm</td>
</tr>
<tr>
<td></td>
<td>PG 3</td>
<td>4.8</td>
<td>3mm</td>
</tr>
</tbody>
</table>

Table III: Mycoplasma isolated from goats and sheep in the Sudan

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>Host &amp; Recovery Site</th>
<th>No isolates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. mycoides</em> subsp mycoides</td>
<td>Goat-Pleurropnuemonia-lung</td>
<td>2</td>
<td>Pillai 1961 cited by El Nasri 1964</td>
</tr>
<tr>
<td><em>M. mycoides</em> subsp. capri</td>
<td>Goat pleurropnuemonia-lung</td>
<td>2</td>
<td>El Nasri 1967</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Goat Pleurropnuemonia-lung</td>
<td>&gt;1</td>
<td>Abdulla &amp; Lindley 1967, 69</td>
</tr>
<tr>
<td><em>M. agalactiae</em></td>
<td>Goat mastitis udder</td>
<td>1</td>
<td>Ibrahim 1969</td>
</tr>
<tr>
<td><em>M. ovipneumoniae</em></td>
<td>Goat Pleurropnuemonia-lung</td>
<td>1</td>
<td>Harbi 1977</td>
</tr>
<tr>
<td><em>M. mycoides</em> subsp mycoides</td>
<td>Goat Pleurropnuemonia-lung</td>
<td>1</td>
<td>Harbi <em>et al</em> 1979</td>
</tr>
<tr>
<td><em>M. arginini</em></td>
<td>Goat Pleurropneumina</td>
<td>1</td>
<td>Harbi <em>et al</em> 1979</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Sheep-pneumonia lung</td>
<td>1</td>
<td>Khan 1960</td>
</tr>
<tr>
<td><em>M. arginini</em></td>
<td>Sheep-pneumonia lung</td>
<td>&gt;1</td>
<td>El Mahi 1974</td>
</tr>
<tr>
<td><em>M. gallinarum</em></td>
<td>Sheep-pneumonia lung</td>
<td>&gt;1</td>
<td>Harbi <em>et al</em> 1979</td>
</tr>
<tr>
<td><em>M. arginini</em></td>
<td>Sheep conjunctivitis eye</td>
<td>2</td>
<td>Harbi <em>et al</em> 1979</td>
</tr>
</tbody>
</table>

*M. arginini* was the constant isolate from sheep with eye infection, pneumonia or pleurropneumonia (table III). In one case however, *Pasteurella multocida*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were isolated. From sheep with pneumonia *M. gallinarum* and a virus were isolated. We failed to isolate *M. ovipneumoniae* from sheep and goats in the above mentioned media. Milk from goats with mastitis was free from Mycoplasma.

**DISCUSSION**

Isolation of PPLO from a goat suffering from acute contagious pleuropneumonia was reported since the early fifties (Longley 1951). Two organisms have so far been accepted as the cause of the disease viz *M. mycoides subsp. capri* (Cottew, Watson, Erdag and Arisoy 1969) and *M. mycoides* (Ojo 1976). Extensive studies by the above authors were conducted on the cultural bioche-
mical and serological characteristics of these two species. Strains among the species of *Mycoplasma mycoides* and *M. mycoides* subsp. *capri* and their homologous hyperimmune sera showed strong cross-reactivity and antigenic overlap. In spite of this there appears to be great difference as to the experimental cross infection in animals.

The subcutaneous or endobronchial inoculation of calves and sheep with a virulent young culture (3rd subculture) of the strain in question or with pleural fluid or lung tissue of experimentally infected goats failed to induce lesions or cause disease in animals. However goats inoculated with the above material or kept in contact with the diseased ones rapidly contracted pleuropneumonia (Abduilla et al. 1979). These trials were worthwhile especially because we noticed that the strain was more towards *M. mycoides* than *M. mycoides* subsp. *capri*. It was interesting to know that cattle and sheep usually kept in contact with the diseased goats did not contract infection while goats did.

From these findings and the fact that the present isolate from CCPP was similar to *M. mycoides*, it appears that the question already raised, that goats may play a role in the dissemination and perpetuation of CBPP (Perreau 1971) is not valid. This interpretation should however be taken with much prudence since in a recent finding (Cottew and Yeats 1978) it was found that the small colonies (SC) of *M. mycoides* harboured by goats could possibly be pathogenic to cattle.

Harbi (1977) identified *Mycoplasma* from goats as *M. ovipneumoniae* which was isolated from a typical case of CCPP according to the clinical and necropsy findings described by the D.V.O. (Gedarif, Kassala Province). The fact that the *M. ovipneumoniae* could possibly be the cause of CCPP (Masiga and Rurangirwa 1979) encourages authentic pathogenicity studies to give a clear-cut conclusion about this malady. The present authors are in the opinion that more than one type of *Mycoplasma* could cause this disease. Other agents which may exist in such infection of goats together with Mycoplasmas encourage search for these pathogens, especially when Mycoplasma other than those accepted as cause of pleuropneumonia viz: *capri* and *mycoides* are isolated. We concluded that Abu Nini of goats in the Sudan could possibly be a syndrome due to synergistic action of various pathogens among which *Mycoplasma* does exist. In this case CCPP should not be referred to as Abu Nini or vice versa. It is important to know that *M. mycoides* which was identified at the outset in this survey had been overgrown by *M. arginini*, a known ubiquitous organism which frequently harbours the respiratory tract (Leach 1970).

The *mycoplasmas* isolated from sheep with pneumonia or pleuropneumonia do not seem to have been the main cause of the disease. The inoculation of early subcultures of *Mycoplasma* alone in sheep failed to induce infection. However the effect of synergism was remarkable when the bacteria isolated from the diseased animals viz: *P. multocida*, *Pseudomonas aerginosa* and *Proteus vulgaris* were isolated with the *Mycoplasma*. Chickens are the natural host for *M. gallinarum* whose presence in sheep infection has not been previously reported. It is highly probable that the sheep merely acted as a passive host for this *Mycoplasma*. *M. arginini* failed to induce infection when injected
into the eye. This is in conformity with the recent findings (Trotter et al. 1977). Agalactia in goats supposedly present in the Sudan was not substantiated in this study.

ACKNOWLEDGEMENT

The authors are grateful to the Director Vet. Res. Administration. We thank Prof. E.A. Freundt and his collaborators for the generous provision of materials and confirmation of our isolate. We equally thank Dr. P. Perreau of the I.E.M.V.T., France. The assistance of the Technical Staff of the Department of Mycoplasma is highly appreciated. We are thankful to the P.U.S. Animal resources (MAFNR) Khartoum for permission to publish this work.

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CAPRINE BRUCELLOSIS: SEROLOGICAL STUDIES IN NIGERIA

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SUMMARY

Brucellosis in Nigerian goats was investigated by the use of the Rose Bengal plate, serum (tube) agglutination, 2-mercapto ethanol, Rivanol, Coombs antiglobulin and complement fixation tests. The Coombs (AGT) proved the most effective at a reaction rate of 25%. The RBPT and tube agglutination tests showed a very high correlation when both serum and their samples were negative. The disease exists in both active and chronic forms among goats. It is suggested that appropriate measures be taken for its eradication and control.

INTRODUCTION

According to Banerjee and Bhatt (1970), the earliest reference to the occurrence of brucellosis in Nigeria could be found in the Annual Report of the Veterinary Department (1927) when ten cases of bovine contagious abortion were reported. By 1931, the Nigerian Medical Authorities had also recorded two cases of undulant fever diagnosed clinically. Since then, sporadic cases of bovine brucellosis have emanated from various parts of the country. Nuru and Dennis (1975) examined 1142 samples from 266 breeding herds in various parts of Northern States and recorded a higher infection rate amongst local Fulani herds (6 to 7.1%) than in government-owned farms (3.6%). Esuruoso (1965) recorded the first case of the disease in Western State in one of two Friesian cows that had aborted on the same day. Serological as well as cultural evidence of bovine brucellosis were produced in later years. (Esuruoso and Hill, 1970, and Esuruoso and Van Blake 1972). Br. abortus biotypes 1 and 2, the predominant bovine strains in Nigeria were readily isolated from hygroma fluids and milk samples, (Eze, 1978).

In the Eastern State, an abortion storm was reported in a government herd of Zebu cattle by Adam and Mackay (1966). Most of the investigations on brucellosis in Nigeria have therefore been confined to cattle. However, Collard (1962) found Br. abortus agglutinating antibodies in man and showed a close correlation between the degree of human infection and the density of cattle. Alausa and Osoba (1975) and Alausa and Awoseyi (1976) found a seropositivity rate of 53.5% to 55% to Br. abortus on sera collected from people aged between 18 and 55 years. In both instances, higher antibody titres than in the general population were found among slaughtermen, butchers, veterinary workers and other people whose occupation constantly brought them in close contact with cattle. The possible occurrence of Br. canis was recently indicated by Okoh, Alexiev and Aghonlahor (1978) who isolated the organism from a batch of exotic dogs and suggested this species as a potential source of infection. The first comprehensive report on the disease in Nigerian breeds of goats was by Falade, Ojo, Sellers (1975) who found 4.27% of 2550 goats examined had antibodies in excess of 50 international units. Within
the last eighteen months, a survey was carried out in selected places by the use of the conventional and additional serological techniques. The findings of this investigation are hereby reported.

MATERIALS AND METHODS

A total of seven hundred and five (705) goat serum samples was collected from abattoirs, slaughter houses and goat markets (Kano, Bodija, Oja-Oba) and stored at -20°C until ready for use. From each of one hundred and thirty-eight (138) goats was also collected about 5-8 ml of milk in sterile universal bottles. These were refrigerated within one hour of collection and tested later. All serum and milk samples were then examined by the following methods.

Rose-Bengal plate test (RBPT). Acidified Rose Bengal stained Br. abortus S99 standardized antigen was used. The technique employed was that described in an earlier paper, Falade (1978).

Seurm (Tube) Agglutination Test—SAT.

The method used was also reported in an earlier paper Falade loc cit. Results were however converted into International units (I.U.) of antibody. All serum samples positive by both tests (RBPT and SAT) and two hundred randomly selected negative samples were further examined by the following additional tests.

Rivanol test
2 gm of Rivanol/Acrinol* or 2-Ethoxy-6-9- diamino acridine lactate was dissolved in 500 ml of normal saline to give a 0.4% solution. 0.9 ml of this solution was added to 0.3 ml of each test serum, the mixture kept at room temperature for 15 minutes and later centrifuged at 2000 rpm for 10 minutes. The supernatate (0.2 ml) was diluted (1/5) with 0.8 ml of normal saline containing 0.5% phenol. Further dilutions and the techniques of the test were done exactly as for the tube agglutination method.

2-Mercaptoethanol Test
0.2M (1.56%) solution of 2-Mercaptoethanol** was prepared by dissolving 7.8gm in 500ml of normal saline. To 0.5 ml of this solution was added 0.3 ml of normal saline and 0.2ml of each test serum. The mixture was incubated at 37°C for 1 hr and later treated in a five tube test as for the tube agglutination method.

Coombs antiglobulin test
0.5 ml of haemoglobin-free serum from each of ten brucella negative goats was used for the preparation of the goat antiglobulin by the method of Proom (1943). The titrations of the antiglobulin and procedure for the test were as described by Unel, Williams and Stableforth (1969).

Complement Fixation test

Fresh complement was obtained from guinea pigs and stored shortly at -4°C before use. The hemolytic serum (HIB) was also prepared in rabbits but was inactivated at 58°C for 50 minutes to destroy the native complement. The 3% sheep red blood cells used was estimated with the sicca hemometer.*** The titrations of the various components and the techniques used for the test were those described by Morgan et al. (1978).

Milk Tests

The milk ring test was performed as previously described, Falade (1978).

*Dauchi Seiyaku Co. Ltd., Tokyo 103, Japan
**Koch-Light Laboratories, Buck, England.
***Testa Laboratorium, Capenhagen, Denmark
Also 5ml of each sample was centrifuged at 2500 rpm for 30 minutes and to the supernatant was added a few drops of rennet. After 1 hr at room temperature, this was centrifuged again to obtain the whey suspension. The whey tube and Rose Bengal agglutination tests were performed.

**RESULTS**

Brucella antibodies were detected in 44 (4.38%) of the 705 samples tested by the SAT. However 29 of these (4.01%) had antibodies in excess of 50 I.U. The RBPT, although it showed a high correlation with the SAT when both tests are negative, could only detect two of the positive samples (Table 1). Infection rates are in fact higher since by the use of additional tests many more positive samples were detected (Table 2a). Between two and seventeen of the SAT negative sera were positive although at low titres by all the additional tests used.

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>*Extent of clearing</th>
<th>No. of sera</th>
<th>International Units (I.U.)</th>
<th>RBPT Positive</th>
<th>Totals</th>
<th>Percent</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>661</td>
<td>0</td>
<td></td>
<td></td>
<td>661</td>
<td>93.76</td>
</tr>
<tr>
<td>1/10</td>
<td>1</td>
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<td>17</td>
<td>-</td>
<td>15</td>
<td>2.01</td>
</tr>
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<td></td>
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<td>3</td>
<td>21</td>
<td>-</td>
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<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>23</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>27</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/20</td>
<td>1</td>
<td>1</td>
<td>30</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>34</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>47</td>
<td>-</td>
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**Above 50 international units**

<table>
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<tr>
<th>Serum dilution</th>
<th>*Extent of clearing</th>
<th>No. of sera</th>
<th>International Units (I.U.)</th>
<th>RBPT Positive</th>
<th>Totals</th>
<th>Percent</th>
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<td>4</td>
<td>80</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>93</td>
<td>-</td>
<td></td>
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<td>4</td>
<td>3</td>
<td>106</td>
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<tr>
<td>1/80</td>
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</tr>
<tr>
<td></td>
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<td>-</td>
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<td>372</td>
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<td>4</td>
<td>0</td>
<td>424</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>Totals</td>
<td>705</td>
<td>2</td>
<td></td>
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<td>705</td>
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</tbody>
</table>

*Extent of Clearing
1. 25%
2. 50%
3. 75%
4. 100%
Table 2(a): Comparison of serological tests on 244 goat serum samples

<table>
<thead>
<tr>
<th>Serological Tests</th>
<th>Serum Dilutions</th>
<th>Totals</th>
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<tbody>
<tr>
<td></td>
<td>1/10</td>
<td>1/20</td>
</tr>
<tr>
<td>Sat</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>RBPT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ME – Test</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Rivanol test</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Coombs AGT</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

CFT Majority of samples were anticomplementary but 53 samples showed positive reactions at \( \frac{1}{2} (9) \) and \( \frac{1}{4} (44) \) dilutions of antiserum.

Table 2 (b): Results of additional serological tests on sat RBPT negative sera

<table>
<thead>
<tr>
<th>Serological TESTS</th>
<th>No. Negative</th>
<th>No. Positive at Serum dilutions</th>
<th>Totals</th>
</tr>
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<td></td>
<td></td>
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<td>1/20</td>
</tr>
<tr>
<td>ME-test</td>
<td>198</td>
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<td>1</td>
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<tr>
<td>Rivanol test</td>
<td>192</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Coombs AGT</td>
<td>183</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

CFT 9 Positive at \( \frac{1}{2} \) dilution.

(Table 2b). The Coombs antiglobulin test proved most effective; but since its use on a large number of samples is time-consuming, the equally effective Rivanol modification of the serum agglutination test is recommended. A total of 46 (31.15%) milk samples was positive by the MRT. Both the whey agglutination and Rose Bengal, plate tests were however negative.

**DISCUSSION AND RECOMMENDATIONS**

The reaction rate of 4.01% by the SAT in this investigation is similar to that previously detected by Falade et al. (1975). There is no record of brucellosis vaccination in small ruminants in Nigeria. Therefore, using the diagnostic criterion of 1/10 titre to the AGT in unvaccinated sheep as suggested by Unel
et al. (1969), sixty-one (25%) of 244 goats examined could be classed as reactors. The RBPT detected only 2 (4.5%) of the 44 SAT-positive samples. The ineffectiveness of the RBPT on goat and human sera had been previously described (Falade, 1974, 1975, 1978). It seems therefore that it gives a positive result only when the SAT titre is at least 1/40. However, the correlation between these tests is highest when both are negative, for example, all the 661 serum and 138 whey samples were negative to both tests. Whereas the SAT detects predominantly IgM or 19S antibodies, both mercaptethanol and rivanol destroy the activity of the IgM or 7S antibodies, while the activity of the IgG or 7S antibodies is unaffected (Morgan, 1967). These tests are useful in differentiating antibodies resulting from infection and vaccination, but also as the CFT and AGT, in detecting the chronic carrier animals.

Both active and chronic brucella infections exist in Nigerian goats. With the total goat population estimated at approximately 22 million (FAO Report 1965), uncontrolled incidences of brucellosis are therefore of paramount economic and public health significance. Hence urgent and appropriate measures must be taken for its eradication and control.

REFERENCES


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ANTIBACTERIAL ACTIVITY OF SOME ALKALOIDAL SALTS

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SUMMARY

Caffeine citrate, quinine hydrochloride and quinine sulphate, among 12 alkaloidal salts tested proved to possess a marked antibacterial activity against *staph. aureus*, *strept. Pyogenes Coryne. ovis*, *E. Coli*, *Salmonella typhi* and *Pseud. aeruginosa*. The minimum inhibitory concentrations on these microorganisms were determined.

Using the sensitivity test, the antibacterial activity of these alkaloidal salts was compared with terramycin, chloramphenicol, ampicillin, nalidixic acid, neomycin, colistin sulphate, gentamycin, erythromycin, streptomycin, penicillin G, cephalothin and thiophenicol and proved that the antibacterial effect of caffeine citrate against *Strept. Pyogenes* was greater than that of terramycin, nalidixic acid, neomycin, colistin sulphate, streptomycin, penicillin, cephalothin and thiophenicol. Caffeine citrate surpassed all the studied antimicrobial against *Staphylococcus aureus* with exception of ampicillin, penicillin and cephalothin. In case of *Corynebacterium ovis*, the caffeine citrate exceeded all the tested antimicrobial agents except chloramphenicol.

Concerning *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, caffeine citrate surpassed all the antimicrobial agents with the exception of chloramphenicol, gentamycin and cephalothin.

Caffeine citrate was more potent than quinine salts on the tested bacteria. Growth of *Leptospira icterohaemorrhagiae* and *Leptospira canicola* were inhibited by these alkaloidal salts.

Topical application of caffeine citrate preparations enhanced the healing of infected wounds in buffaloes whereas quinine salts delayed it.

INTRODUCTION

The great need for the availability of new, effective and inexpensive antimicrobial agents has encouraged many investigators to search for such materials in the active constituents of plants (Osborn 1943, Ayad, Shihata and Atef 1971, Fujita, Yamada, Azuma and Hirozawa 1978 and Hilal, Zinat, Haggag and Soliman 1978).

The antimicrobial activity of plant alkaloids and their salts need more investigations, and so the present study was undertaken to investigate their activity against a variety of bacterial species.

MATERIAL AND METHODS

Alkaloidal salts

Atropine sulphate, caffeine citrate, emetine hydrochloride, ephedrine hydrochloride, nicotine sulphate, physostigmine sulphate, pilocarpine nitrate, quinine hydrochloride, quinine sulphate, strychnine sulphate, tubocurarine chloride and yohimbine hydrochloride were used.

Microorganisms

The antibacterial activity of these alkaloidal salts was investigated against
Streptococcus pyogenes, Staphylococcus aureus, Corynebacterium ovis, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Leptospira icterohaemorrhagiae and Leptospira canicola.

Media

The antibacterial activity of alkaloidal salts against each microorganism was tested using a specific medium. Therefore, nutrient agar (Oxoid), blood agar, Sabouroud's dextrose agar with antibiotics, nutrient broth, modified liquid sabouroud's EMJH medium (Ellinghausen and McCullough, 1965) modified by Johnson and Harris, (1967) and EMJH enrichment media were prepared and employed.

METHODS

A. In vitro

Determination of sensitivity of the tested microorganism to the effect of the chosen alkaloidal salts were carried out using the gutter technique (Cooper and Woodman 1946).

The minimal inhibitory concentration of these alkaloidal salts against the tested bacteria was determined using plate dilution test (Bail and Scott, 1966).

Furthermore, their activities were compared with the activity of some antibiotics using the disc inhibition zone method (Grove and Radall, 1955).

The sensitivity of Leptospira to graded concentrations of alkaloidal salts was determined using the fluid dilution method (Grove and Radall 1955) on EMJH medium.

Experiments

B. In vivo.

Fifteen Egyptian buffaloes were used to investigate the in vivo antibacterial effect of the alkaloidal salts which proved to have antimicrobial effect in vitro. The buffaloes were divided into five equal groups. Experimental wound (7 x 5 cm) was created on both sides of the neck of each animal and infected with a mixed culture of Staphylococcus aureus and Streptococcus pyogenes. Forty-eight hours following the infection, the wounds of the first three groups were firstly dressed with a 5% solution, then painted with a 5% ointment of caffeine citrate, quinine hydrochloride or quinine sulphate, respectively. The fourth group were dressed with 1% acriflavin solution and then painted daily with terramycin ointment (each gram contains 30 mg oxytetracycline and 10,000 Units polymyxine sulphate). Wounds of the fifth group were left without treatment.

The healing time of wounds treated with the alkaloidal salts was compared with those treated with terramycin ointment.

RESULTS

Screening of the antibacterial activity of different alkaloidal salts against Staphylococcus aureus, e.g. Streptococcus pyogenes, Corynebacterium ovis, Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa using the gutter technique revealed that caffeine citrate, quinine hydrochloride and quinine sulphate inhibited the growth of all studied bacteria, whereas the other alkaloidal salts were ineffective.

The antibacterial activity of graded concentrations of caffeine citrate, quinine hydrochloride and quinine sulphate, using the bore method, are shown in Fig. 1. Their mean values of the inhibition zones are recorded in Table 1.
Fig. 1: The antibacterial effect of graded concentrations of caffeine citrate (A), quinine hydrochloride (B) and quinine sulphate (C) on *Corynebacterium ovis*. 
Table 1: Effect of graded concentrations of alkaloidal salts on the growth of some pathogenic bacteria (Using the bore method).

<table>
<thead>
<tr>
<th>Concentration of alkaloidal salt</th>
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<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staph. aureus</td>
<td>Strept. pyogenes</td>
<td>Coryne. ovis</td>
<td>E. coli</td>
<td>Salmonella typhimurium</td>
<td>Pseud. aeruginosa</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Caffeine citrate</td>
<td>10.0%</td>
<td>31+2.2</td>
<td>32+2.4</td>
<td>38+2.9</td>
<td>30+2.8</td>
<td>31+3.1</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>24+2.0</td>
<td>26+2.1</td>
<td>33+2.4</td>
<td>25+2.6</td>
<td>27+2.4</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>20+1.8</td>
<td>21+1.9</td>
<td>29+2.0</td>
<td>17+1.6</td>
<td>18+1.6</td>
</tr>
<tr>
<td></td>
<td>1.0%</td>
<td>18+1.6</td>
<td>20+1.6</td>
<td>23+1.8</td>
<td>16+1.4</td>
<td>17+1.6</td>
</tr>
<tr>
<td>Quinine HCl</td>
<td>10.0%</td>
<td>31+2.1</td>
<td>31+2.0</td>
<td>32+2.8</td>
<td>27+3.1</td>
<td>25+2.7</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>22+1.8</td>
<td>21+1.9</td>
<td>23+2.5</td>
<td>16+1.5</td>
<td>15+1.4</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>18+1.6</td>
<td>17+1.5</td>
<td>18+1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0%</td>
<td>10+0.8</td>
<td>12+1.1</td>
<td>16+1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinine Sulphate</td>
<td>10.0%</td>
<td>21+3.1</td>
<td>33+2.8</td>
<td>34+3.2</td>
<td>28+2.6</td>
<td>25+2.1</td>
</tr>
<tr>
<td></td>
<td>5.0%</td>
<td>24+2.6</td>
<td>22+2.3</td>
<td>25+2.1</td>
<td>17+1.6</td>
<td>15+1.4</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>20+1.6</td>
<td>18+1.6</td>
<td>21+1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0%</td>
<td>12+1.0</td>
<td>13+1.1</td>
<td>18+1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each figure represents 6 replicates.

The minimal inhibitory concentration of caffeine citrate, quinine hydrochloride and quinine sulphate on the tested bacteria were also determined and are recorded in Table 2. The antibacterial activity of caffeine citrate against the tested micro-organisms was more potent than those of quinine salts. The minimal inhibitory concentrations in caffeine citrate ranged from 1.8 — 2.1 mg/ml media corresponding to 1.0 — 1.0 mg/ml media for quinine salts.

The sensitivity of the tested micro-organism to the alkaloidal salts was compared with that of various types of antibiotics and the inhibitory zones

Table 2: Minimal inhibitory concentration of caffeine citrate, quinine hydrochloride and quinine sulphate (mg/ml media) on the tested bacteria.

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Caffeine citrate</th>
<th>Quinine hydrochloride</th>
<th>Quinine sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>2.0</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Strept. pyogenes</td>
<td>1.8</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Coryn. Ovis</td>
<td>1.9</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>2.1</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>2.0</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Pseud. aeruginosa</td>
<td>2.0</td>
<td>1.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>
are measured and recorded in Table 3. The observed antibacterial effect of caffeine citrate against *Streptococcus pyogenes* was greater than that of terramycin, nalidixic acid, neomycin, colistin sulphate, streptomycin, penicillin, cephalothin and thiophenicol. Caffeine citrate surpassed all the studied antimicrobial agents against *Staphylococcus aureus* with the exception of ampicillin, penicillin and cephalothin. In the case of *Corynebacterium ovis*, the antibacterial effect of caffeine citrate exceeded all the tested antimicrobial agents except chloramphenicol.

Caffeine citrate surpassed all the antimicrobial agents against *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, with the exception of chloramphenicol, gentamycin and cephalothin.

It is also clear that caffeine citrate was more potent than quinine hydrochloride and quinine sulphate on the tested bacteria.

The effect of caffeine citrate, quinine hydrochloride and quinine sulphate against *Leptospira icterohaemorrhagiae* and *Leptospira canicola* was studied using the serial dilution technique. The minimum inhibitory concentration of these alkaloidal salts were found to be 1.0, 0.25 and 0.25 mg/ml media respectively. Thus *Leptospira icterohaemorrhagiae* and *Leptospira canicola*

Table 3: Diameter of inhibitory zones (mm) caused by some antibiotics, caffeine citrate and quinine salts on some micro-organisms (using the sensitivity disc method).

<table>
<thead>
<tr>
<th>Substances</th>
<th>Conc.</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Strept. pyogenes</em></td>
</tr>
<tr>
<td>Terramycin</td>
<td>30 Ug</td>
<td>23*</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 Ug</td>
<td>30</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 Units</td>
<td>30</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 Ug</td>
<td>16</td>
</tr>
<tr>
<td>Neomycin</td>
<td>10 Ug</td>
<td>23</td>
</tr>
<tr>
<td>Colistin</td>
<td>10 Ug</td>
<td>10</td>
</tr>
<tr>
<td>Sulphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>30 Ug</td>
<td>23</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10 Ug</td>
<td>30</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10 Ug</td>
<td>25</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 Units</td>
<td>25</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30 Ug</td>
<td>18</td>
</tr>
<tr>
<td>Thiophenicol</td>
<td>30 Ug</td>
<td>22</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Saturated</td>
<td>27</td>
</tr>
<tr>
<td>Citrate hydrochloride</td>
<td>Solution</td>
<td>20</td>
</tr>
<tr>
<td>Quinine</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Quinine sulphate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Diameter of inhibitory zones (mm).

— No inhibition zones noticed.
were found more sensitive to quinine salts than caffeine citrate.

The experimentally infected wounds in buffaloes showed purulent discharge 2 days after infection. Wounds treated with terramycin were reduced to 0.2 x 0.2 cm within 8 days, whereas those treated with 5% caffeine solution and ointment reached 0.5 x 0.5 cm after 8 days. But quinine salts seemed to retard healing of wounds as they remained unchanged in size. Untreated wounds were reduced in their dimensions from 7 x 5 cm to 3.0 x 2.7 cm.

**DISCUSSION**

In the present work, preliminary screening of the antimicrobial activity of 12 alkaloidal salts revealed that caffeine citrate, quinine hydrochloride and quinine sulphate had an antimicrobial effect against the tested microorganisms. These results were to some extent in agreement with those obtained by Raj and Dhala (1965), Ayad et al. (1971) and Ghoneim, Atef and Shihata (1971). Atropine sulphate, emetine hydrochloride, ephedrine hydrochloride, nicotine sulphate, physostigmine sulphate, pilocarpine nitrate, strychnine sulphate, tubocurarine chloride and yohimbine hydrochloride had no effect against these microorganisms. These findings are in agreement with those of Osborn (1943), Sanders, Wax and McClung (1945), Spencer, Koniuszzy, Rogers, Shavel, Eston, Kaczka, Fredrick, Phillips, Wallti, Folkers, Malanga and Seefer (1947), and Johnson, Johnson and Poe (1949).

Using the plate dilution technique, the minimal inhibitory concentration of caffeine citrate against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ovis*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas* were found similar to those reported by Raj and Khala (1965) and Ayad et al. (1971).

The minimal inhibitory concentration of quinine hydrochloride and quinine sulphate ranged from 1-2 mg/ml media. These results were in agreement with those obtained by Ayad et al. (1971).

It was also observed that the antibacterial activity of quinine salts were more potent on Gram positive than Gram-negative bacteria. This is in accordance with Ayad et al. (1971) and Ghoneim et al. (1971).

The antibacterial effect of quinine salts could be attributed to their direct toxic effect on the protoplasm of the cell (Laurence, 1973 and Sharaf, 1974) or to the interference of quinine with the normal metabolism of bacterial cells preventing them from the utilization of riboflavin (Goodman and Gilman, 1968).

Moreover, the antibacterial activity of caffeine citrate, quinine hydrochloride and quinine sulphate was compared with that of some antibiotics and their potency varied according to the type of organism. Generally, the alkaloidal salts, surpassed some of these antibiotics against certain microorganisms.

Caffeine citrate and quinine salts in concentrations of 1 and 0.25 mg/ml media respectively inhibited the growth and motility of *Leptospira icterohaemorrhagiae* and *Leptospira canicola*.

*In vivo* studies caffeine citrate preparations were effective in healing experimentally infected wounds in buffaloes but less than terramycin.

The observed antimicrobial activity of caffeine citrate was attributed to its interference with normal cellular function of infecting bacteria (Pridham, 1961).
Quinine salt preparations markedly decreased pus formation in the wounds but delayed their healing, which may be attributed to the direct toxic effect of quinine salts on the protoplasm of the cell (Laurence, 1973 and Sharaf, 1974) preventing regeneration of the edges of these wounds or to an inhibitory action on an enzyme responsible for wound healing.

REFERENCES
Johnson, R.C. and Harris, V.G. (1967): J. Bact. 94/ 23—31

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PRELIMINARY STUDIES ON THE ANTHelmINTIC PROPERTIES OF THE AQUEOUS EXTRACT OF CALLIANDRA PORtORICENSIS (JACQ) BENTH.

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Department of Pharmacy, University of Nigeria, Nsukka, Nigeria

SUMMARY

Experiments were conducted to determine the anthelmintic properties of Calliandra portoricensis. Two dogs infected with Toxocara canis were successfully treated with an aqueous extract of the plant, an indication that some of the locally used recipes should not be disregarded. The extract however had no effect on Ancylostoma caninum and Hymenolepis diminuta of the dog and rat respectively.

INTRODUCTION

The Alcoholic extract of the root of Calliandra portoricensis (Jacq) Benth (Mimosaceae) is known to be used as an anthelmintic in parts of Ondo and Bendel States of Nigeria (Awomewe, 1976). The root is usually cut into small pieces and macerated in locally distilled alcohol (locally known as “ogogoro”) for about 24 hours, filtered and the extract is taken as such. About half a glass (250ml) of such extract is taken by an adult for worms.

Calliandra portoricensis is a shrub which grows wildly in Nigeria. It is also grown for ornamental purposes (Dalziel, 1955). The aim of this communication is to confirm or refute the vermifugal properties of the plant.

MATERIALS AND METHODS

Plant collection and extraction.

The plant was collected late in August, 1976, identified and deposited at the Forestry Research Institute Herbarium, Ibadan, Nigeria. The roots were thoroughly washed and cut into small pieces and then ground with a "Waring blender". The ground root was then soaked in ethanol (50%) for 72 hours. The extract was then suction-filtered and the filtrate was concentrated to dryness in vacuo.

Tests for anthelmintic activity

Anthelmintic studies were carried out in 7 – 9 months old local dogs (weight range 8 – 10 kg) and rats (weight range 200 – 240 gm).

Experiment I

Two local dogs naturally infected with Toxocara canis were isolated and allowed one week of rest in cages. Egg count was carried out once a day for 3 days before and once after the extract was administered orally at 15 mg/kg body weight (in divided doses, three times daily for 3 days). The dogs were sacrificed 24 hours after the end of treatment. The abdomen was opened, the small intestine was ligated at the pyloric end before the whole intestine was opened. The lumen of the intestine was then examined for the presence of worms.

Experiment II.

The larval culture of Ancylostoma caninum was prepared as described by

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Oyerinde (1976). The cultures, packed in petri-dishes were kept within a moist chamber at room temperature (29\(+2^\circ\) C) for seven days. Developed third stage larvae were harvested with a modified Baerman’s apparatus. Twelve local dogs (weight range, 8 — 10 kg) were then infected percutaneously on shaven parts of the abdomen with 500 larvae each. The dogs were then divided into three groups of four each and then isolated in metabolic cages. The dogs were examined for hookworm egg excretion on the 30th day precisely as in experiment I before and after the administration of drugs. Those in group I were left untreated, group II dogs were given the drug at the rate of 20 mg/kg body weight orally (in divided doses three times daily for three days), while those in group III were treated with Disophenol at 7 mg/kg body weight subcutaneously.

**Experiment III**

The maximum tolerated dosage (MTD) of *Calliandra portoricensis* for Norway rats (weight range 200 — 240 gm) was found by the method of Basil (1963) in which 10 rats in five groups of two each were utilized. The first group was treated with a dosage of 10 mg/kg body weight orally which was assumed to be non-toxic. Subsequent groups were then treated with dosages five times that of the previous group until a dosage level was reached at which all rats receiving it died. The highest level at which all rats in a group survived was then taken as the M.T.D. for this experiment.

Twelve rats which were naturally infected with *Hymenolepis diminuta* were isolated for one week. The rats were divided into three groups as in experiment II. Group I was left untreated, group II was given 250 mg (MTD) of the *Calliandra portoricensis* extract while those in group III were treated with Niclosamide (N-2\(^{1}\)-chloro-4\(^{1}\)-nitrophenyl-5 chlorosalicyamide) at 30 mg/kg body weight orally. The drugs were given in three cycles of 5 days each with 10 days interval to prevent the phenomenon of auto-infection.

Faecal egg counts were taken daily for three days before and after the administration of the drugs. Activity was regarded as being present if the average egg count showed a 50% decrease after the administration of drugs.

**Phytochemical tests**

1. **Test for saponins**

(a) Four hundred mg of the plant extract was mixed with water in a test tube. The presence or absence of frothing was noted.

(b) Four hundred mg (in one ml) of the extract was added to 10 ml of standardised blood suspension prepared according to the method of Wall et al. (1952). After five minutes the absence or presence of haemolysis was noted.

2. **Test for alkaloids**

Four hundred mg of the extract was stirred with 4 ml of 10% HCl on a steam bath. One ml of the filtrate was treated with a few drops of Mayer’s reagent and another ml with Dragendorff’s reagent in the same way. Presence or absence of precipitation or turbidity was recorded.

3. **Test for Cardial glycosides**

Three tenths of a ml of the reagent which consists of a few crystals of naphthoresorcinol and 3 mls of concentrated HCL was added to 500mg of the extract in a test tube. This was heated in a water bath at 50\(^{\circ}\)C for 10 minutes.
Colour change to pink, green, yellow, final red and or precipitate was regarded as evidence for the presence or absence of cardiac glycoside.

4. General test for Terpene derivatives

Plates of dimensions 20 x 5 cm were coated with 0.5 mm layer of silical gel and activated for 1 hour at 105°C. The extract mixed with silical gel was placed on top of a silical gel column and eluted with n-hexane-ethyl acetate (1:1). Separations were carried out in solvent-saturated camag tanks at room temperature (24-26°C) with a development of 1 hour. The plates were then sprayed with anisaldehyde reagent which consisted of 0.5 ml of anisaldehyde. 50 ml of acetic acid and 1 ml of concentrated sulphuric acid. After spraying the plates were heated at 100°C for 15 minutes until the appearance of coloured spots (Stahl, 1969).

5. Test for Tannins

Five hundred mg of extract was stirred with 10 ml distilled water and filtered. The addition of ferric chloride (FeCl₃) reagent to the filtrate resulting in the absence or presence of tannin was recorded.

RESULT

Worm as well as egg counts for Toxocara canis were negative for the two dogs after treatment with the extract of Calliandra portoricensis in Experiment I.

In Experiment II the average egg counts per day for the dogs infected with Anyclostoma caninum for the control (group I) the test (group II) and the monitor (group III) before treatment were 2.34, 3.33 and 32.11 million eggs per day respectively while those after treatment were 2.82, 3.35 and 0.01 million eggs per day respectively (Table I). Similarly the figures for the rats infected with Hymenolepis diminuta were 1.34, 1.32 and 0.73 million eggs per day before treatment and 1.82, 1.02 and 0.04 million eggs per day after treatment (Table I).

The preliminary chemical tests showed that the extract contained no alkaloids, saponins, tannins, cardiac glycosides but the appearance of yellow spots showed that the extract apparently contained some terpene derivatives.

DISCUSSION

Two dogs naturally infected with Toxocara canis were cleared of the infection. This is an indication that we should not disregard all the information passed to us by herbalists.

The results of experimentally infected dogs with Anyclostoma caninum and rats naturally infected with Hymenolepis diminuta show that the extract had no effect on A. caninum and H. diminuta (Table I). This when compared with the monitor (control) groups showed that the method of evaluation was good enough since the average egg count gave dramatic reduction in the number of eggs passed per day. It is interesting to note that no intestinal worm is specifically implicated as the ultimate species against which action is sought in the prescription of this vermicide in those parts of Nigeria where it is used for the treatment of helminthiasis. The only desirable approach therefore is not to rely on the herbalist but to utilize laboratory animals harbouring different species of parasites against which the vermicide is to be used. This will unfold anthelmintic spectrum of the herb and at the same time form the basis on which the herbs can be recommended for inclusion in the African Medicinal plant pharmacopoeia.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Treatment</td>
<td>1.32</td>
<td>0.95</td>
<td>3.82</td>
</tr>
<tr>
<td>After Treatment</td>
<td>2.11</td>
<td>2.24</td>
<td>0.34</td>
</tr>
<tr>
<td>Average BES count per 24 hours in millions</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| II Acrylamide camphurum (d08) (g) | 12 |
| III Hypeamolips camphurum (rals) | 12 |
| IV Tosoacana pars (d08) | 2 |

Table I: Results obtained from analytical studies with extracts of Calluna borealis.

Note Grouped
More work on the plant is in progress (chemistry, biological and pharmacological) before the active constituents can be isolated and before further recommendations can be made on its possible use in veterinary or human medicine. The possibility of it becoming a commercial ascaricide will also depend on its availability and its ease of cultivation.

ACKNOWLEDGEMENTS

We thank the Director of Forestry Research Institute, Ibadan, Nigeria for the identification of the plant and we are grateful for the technical assistance of Mr. Umoh of the Chemistry Department, University of Nigeria, Nsukka.

REFERENCES


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THE STABILITY OF VIABILITY AND IMMUNIZING POTENCY OF LYOPHILIZED, MODIFIED EQUINE ARTERITIS LIVE VIRUS AND VACCINE

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SUMMARY

The Bucyrus strain of equine arteritis virus, previously modified by 131 serial passages in primary cell cultures of horse kidney followed by 111 passages in primary cell cultures of rabbit kidney, was further passaged in E. Derm. (NBL-6) cells, a continuous diploid cell line of equine origin. Pools of the 16th and 25th passages in the latter cell line were lyophilized and stored in lots at 37°C, 23 – 28°C, 4°C and –20°C. The vaccine virus was unstable at 37°C and at 23 – 28°C, but stable at 4°C and 20°C for at least one year.

The stable vaccine protected two horses from arteritis on subsequent inoculation with virulent virus, whereas two unvaccinated control horses inoculated along with the two vaccinated horses developed severe signs of disease and died from acute arteritis.

INTRODUCTION

Equine viral arteritis is a disease of the equidae that occurs in epizootics, with the most consistent feature being the necrosis of the media of small arteries with well developed muscular coat (Bryans et al, 1957; Doll et al, 1957a; Doll et al, 1957b; Jones et al 1957; Jones, 1969; McCollum et al, 1962). It also causes instant abortion in pregnant mares. Previous reports have described effective vaccines developed by attenuating the virus by serial passaging in primary cell cultures of horse, hamster and rabbit kidneys (McCollum et al, 1961; McCollum et al, 1962a; McCollum et al 1962b; McCollum, 1969; Doll et al, 1968; Wilson et al, 1962).

A diploid cell culture of equine origin, E. Derm. (NBL-6), has been developed (Kniazzeff et al, 1965) which readily supports the growth of arteritis virus. The studies recorded in this report were designed to ascertain the stability of viability and immunizing potency of arteritis vaccine produced in this continuous cell line.

MATERIALS AND METHODS

Vaccines

One of us (W.H.M.) modified the Bucyrus strain of equine arteritis virus (EAV) by passaging it 131 times in primary horse kidney cell cultures, and then 111 times in primary cell cultures of rabbit kidney. The products were additionally passaged 16 or 25 times in E. Derm. cell cultures for use as the vaccines in this study. The vaccines were divided into 4 lots and the lots placed respectively in medium containing 2% or 5% horse serum, or 2% or 5% fetal calf serum, and 95% or 98% Eagle’s minimal essential medium containing 0.06% NaHCO₃ (w/v). The final preparations were dispensed in 2ml aliquots and freeze-dried. Each of the lots of vaccine was, after freeze-drying, divided into four groups for storage at 37°C, room temperatures (23 – 28°C), 4°C and –20°C respectively.

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Viability of Vaccine Virus

The viability of the vaccine virus was determined simultaneously by tube titration and plaque counting. In the tube method dilutions of the virus were inoculated onto pre-formed monolayers of LLC-MK₂ line of monkey kidney cells, and the development of cytopathic effects followed daily for 7 days. The end-point was determined by the method of Reed and Muench (1938). In the plaque method bottle monolayers of RK-13 line of rabbit kidney cells were used. The cells were on the flat surface, and four days after virus inoculation cells were fixed and stained in a buffered formalin-crystal violet solution, and plaques counted.

Vaccination of horses

Two healthy horses with no known previous experience with arteritis virus were tested and found to be seronegative. They were then inoculated intramuscularly (cervical muscle) with vaccines stored at 4°C for 12 months. One horse received the 16th passage and the other the 25th passage of the virus in the E. Derm. cell line.

Blood was collected from each horse (through the jugular vein) daily for 2 weeks, and subsequently for 6 weeks at weekly intervals. Rectal temperatures were taken twice daily (a.m. and p.m.) from just prior to vaccination for 14 days.

Challenge of Immunity

Challenge took place 8 weeks after vaccination. The challenge virus was obtained from the infected spleen of a horse that had died 5 days after nasopharyngeal inoculation with virulent EAV. Each horse received by nasopharyngeal inoculation 4.8 x 10⁷ p.f.u. in 10 ml spleen suspension. Two vaccinat-ed and two unvaccinated seronegative horses were challenged with equal amounts of the virulent virus.

Rectal temperatures were recorded twice daily, and the following samples were taken from the horses just before challenge and daily after challenge for up to 18 days:

(a) Blood for leukocyte counts;
(b) Nasopharyngeal swabs;
(c) Blood for sera;
(d) Blood foruffy coats;

Sera, buffy coats and nasopharyngeal swab extracts were inoculated onto preformed monolayers and passed 3 times to find out if virus was present. Total white blood cell counts were performed in suspension in a Technicon Model I Autoanalyser. For differential counts blood smears on microscope slides were stained with Wright's blood stain, and 100 leukocytes counted in each sample.

Titration of Sera

This was done by the plaque reduction test for virus neutralizing antibody. The titre of each serum sample was expressed as the highest dilution of serum reducing the plaque count by at least 50% relative to the number of plaques formed by an equal volume and titre of virus suspension not mixed with serum.

RESULTS

Stability of Viability of Vaccine

(a) Tube Culture Method

The infectivity titres ranged between 6.0 and 7.5 log₁₀ TCID₅₀/ml before and immediately after lyophilization. The titres of vaccine aliquots stored at 37°C diminished rapidly, and by the end of 4 weeks activity could no
longer be detected. The decrease in titre of vaccine lots stored at room temperature (23-28°C) was slower. Even at the end of 5 months virus could still be detected (0.67 to 1.0 log_{10} TCID₅₀/ml, but after 6 months the samples became uninfected. The vaccine was found to be considerably stable at 4°C. After 12 months titres of 4.5 to 5.5 log_{10} TCID₅₀/ml were obtained. At -20°C the vaccine titred 5.3 to 6.5 log_{10} TCID₅₀/ml, at the end of 12 months. These results are presented graphically in Fig. 1.

(b) Plaque Assay Method

The results from this method are recorded graphically in Fig. 2. The vaccine titred 5.4 x 10⁷ to 1.6 x 10⁸ pfu/ml immediately before and after lyophilization. In those aliquots stored at 37°C virus was last detected at the end of 3 weeks (3.3 x 10² pfu/ml). With regard to room temperature samples, virus was detected up to the end of 6 months. Infectivity titres of vaccine samples stored at 4°C decreased slowly, and were still high after 12 months (1.9 x 10⁶ to 1.9 x 10⁷ pfu/ml). The

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Fig. 1: Viability of lyophilized equine arteritis vaccines after storage at 37°C, ambient room temperature (23 – 28°C), 4°C and -20°C as ascertained by the tube titration method using cell culture of the LLC-MK₂ line of monkey kidney. The vaccines used were Buc. Art. V. HK-131 RK-111 ECID-16 and HK-131 RK-111 ECID-25. The numeral following the cell culture indicates the number of serial passages in that cell line. The drying medium was Eagle’s minimum essential medium with 2% or 5% foetal calf serum (FCS) or horse serum (HS) added.
Fig. 2: Viability of lyophilized equine arteritis vaccines after storage at 37\(^0\)C, ambient room temperature (23-28\(^0\)C), 4\(^0\)C and -20\(^0\)C as ascertained by plaque formation in cell culture of the RK-13 line of rabbit kidney. The vaccines used were Buc. Art. V. KH-131 RK-111 ECID-16 and HK-131 RK-111 ECID-25. The numeral following the cell culture indicates the number of serial passages in that cell line. The drying medium was Eagle's minimum essential medium with 2% or 5% foetal calf serum (FCS) or horse serum (HS) added.
viability of aliquots stored at \(-20^\circ C\) decreased only slightly over the 12-month test period, titrating between 1.2 \(\times 10^7\) and 1.2 \(\times 10^8\) pfu/ml at the end of 12 months.

The tube culture method was found to be more laborious yet less sensitive than the plaque assay method.

Response of Horses to Vaccination and Subsequent Challenge with Virulent Virus

(a) Febrile Reactions

The normal body temperatures of horses range from 37.4\(^\circ\)C to 38.5\(^\circ\)C. Temperatures above 38.5\(^\circ\)C were considered significant. Graphic representations of temperature fluctuations after vaccination, and after challenge are given in Figure 3. One of the two vaccinated horses showed only a slight febrile reaction on the 5th day (38.7\(^\circ\)C) and on the 11th day (38.8\(^\circ\)C) after vaccination. The second vaccinated horse showed significant reaction on day 12 (39.5\(^\circ\)C), after which the temperature returned to normal. Following challenge, one of the vaccinates developed temperatures ranging from 39.0 to 38.7\(^\circ\)C from days 9 to 11. The second vaccinate reacted from days 2 to 4 with temperatures of 40.3 to 39.0\(^\circ\)C. The unvaccinated controls reacted typically to infection with virulent arteritis virus.

(b) Total and Differential White Blood Cell Counts

White blood cell counts performed on blood samples taken before inoculation with the virulent virus were regarded as 100%. Figure 4 shows the percentage change in leukocyte counts from pre-challenge levels. All 4 horses experienced some leukopenia during the period of observation. However, whereas the counts from the vaccinated animals returned to normal, the unvaccinated ones experienced a terminal increase in the leukocyte counts.

The neutrophil counts fluctuated in the vaccinated horses. Counts from the unvaccinated animals dropped on day 1, but increased steadily thereafter until each horse died.

Lymphopenia was more severe and persistent in the unvaccinated animals than either leukopenia or neutropenia. In both unvaccinated animals after an initial rise in lymphocyte counts there was a steady drop until both horses died. Lymphocyte counts fluctuated around normal in the vaccinated animals. The lymphocytes were clearly the type of white cells most severely affected.

(c) Recovery of Virus from Horses after Challenge Inoculation

As shown in Table 1 virus was recovered from the nasal swabs of all 4 horses from day 1. Virus recovery from buffy coats was earlier in the unvaccinated animals, and continuous until death. Buffy coats from the vaccinates yielded virus later, and only intermittently until day 7 or 8, whereas sampling was continued until day 18. Sera from the vaccinates yielded no virus, but virus was recovered from the sera of the unvaccinated animals from day 1 or 2 until death.

(d) Serum Virus Neutralizing Antibody

The results are shown in Table 2. After vaccination each horse showed significant specific antibody levels (1:8 to 1:128 and 1:16 to 1:128 respectively). The antibody levels increased further on challenge inoculation (1:512 to 1:2,048 and 1:16,384 to 1:8,192 respectively).

There was no opportunity to measure the antibody levels in the unvaccinated
Fig. 3: (A) Average daily temperatures of horses vaccinated intramuscularly with modified arteritis virus which had been lyophilized and stored at 4°C for 12 months before use. Horse 68-844 was vaccinated with vaccine virus passed HK-131 RK-111 ECID-16 times, and 68-1254 with vaccine virus passed HK-131 RK-111 ECID-25 times.

(B) Average daily temperatures of vaccinated and unvaccinated horses inoculated intranasally with virulent Bucyrus strain of equine arteritis virus. Horses 68-844 and 68-1254 were previously vaccinated, while horses 1442 and 1450 were unvaccinated.
Fig. 4: The percentage of change from preinoculation levels (100%) of the daily counts of total leucocytes, neutrophils and lymphocytes of vaccinated and unvaccinated horses inoculated intranasally with virulent Bucyrus strain of equine arteritis virus. Horses 68-844 and 68-1254 were previously vaccinated intramuscularly with lyophilized, modified equine arteritis virus, while horses 1442 and 1450 were unvaccinated.
Table 1: Recovery of virus from vaccinated and unvaccinated horses after intranasal inoculation with the virulent Becturus strain of equine arteritis virus.

<table>
<thead>
<tr>
<th>Horse Treatment</th>
<th>Days sampled post-Challenge</th>
<th>Days Virus Recovered</th>
<th>Clinical Issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>0+ - 18</td>
<td>1-6 4-6,7 None None</td>
<td>Protected</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>0+ - 18</td>
<td>1-6 3-5,8 None Mild</td>
<td>Protected</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>0+ - 5</td>
<td>1-5 1-5 1-5 Severe</td>
<td>Died 6th day*</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>0+ - 7</td>
<td>1-7 2-7 2-7 Severe</td>
<td>Died 8th day*</td>
</tr>
</tbody>
</table>

Day 0 = on day of challenge but before challenge inoculation
* Day after challenge inoculation.

Table 2: Serologic and clinical response of horses to vaccination with lyophilized, modified equine arteritis virus after storage for 12 months at 4°C and subsequently challenge inoculated with virulent arteritis virus.

<table>
<thead>
<tr>
<th>Horse Treatment</th>
<th>Vaccination Weeks post-Vac</th>
<th>Titre</th>
<th>Challenge Weeks Post-chall</th>
<th>Titre</th>
<th>Clinical Issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>Pre</td>
<td>&lt;1:4</td>
<td>1</td>
<td>1:512</td>
<td>Protected</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1:8</td>
<td>2</td>
<td>1:2048</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:256</td>
<td>4</td>
<td>1:2048</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1:128</td>
<td>8</td>
<td>1:128</td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>Pre</td>
<td>&lt;1:4</td>
<td>1</td>
<td>1:16,384</td>
<td>Protected</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1:16</td>
<td>2</td>
<td>1:16,384</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:128</td>
<td>4</td>
<td>1:8,192</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1:64</td>
<td>8</td>
<td>1:32</td>
<td></td>
</tr>
<tr>
<td>Unvaccinated</td>
<td></td>
<td></td>
<td>pre</td>
<td>&lt;1:4</td>
<td>Died 6th day+</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td></td>
<td></td>
<td>pre</td>
<td>&lt;1:4</td>
<td>Died 8th day+</td>
</tr>
</tbody>
</table>

+ Day after challenge inoculation
animals. This is because one of them died before the day 7 sampling date, and the other, though bled on day 7, had developed so much haemocoagulation that serum was not obtainable from its blood. This one died on day 8.

**DISCUSSION**

All four horses, vaccinated and unvaccinated, developed some fever after inoculation with virulent EAV. However, the occurrence of only mild signs of arteritis (Bryans et al., 1957; Jones, 1969) and the subsequent recovery of the two vaccinates as opposed to the occurrence of severe signs and subsequent death of the two unvaccinated animals show that vaccination saved the vaccinated horses.

The results obtained from this study show that modified, lyophilized equine arteritis live virus vaccine propagated in E. Derm. continuous cell cultures may be stored at 4°C or −20°C for at least 12 months without loss of viability or immunizing potency. Vaccine stored at 4°C for 12 months was shown to confer protection against clinical arteritis in the two vaccinated horses when challenged with virulent EAV. There was, however, evidence of replication of the virulent virus in the vaccinates as shown by the development of transient fever, viraemia, leukopenia, and anamnestic antibody response. There was also observed a direct correlation between the virus neutralizing antibody titre and the degree of immunity obtained.

The work reported in this communication also shows that leukopenia which is a regular feature of equine viral arteritis (Bryans et al. 1957; Jones, 1969) is due mainly to the depression of lymphocytes.

**REFERENCES**


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OBSERVATIONS ON SOME CHARACTERISTICS OF VIBRIO PARAHAE-MOLYTICUS ISOLATED FROM SHELLFISH IN KENYA

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SUMMARY
Studies on two isolates of Vibrio parahaemolyticus selected from among many isolates recovered from shellfish, sea water and sediment and sea fish are reported herein. The two isolates were serotyped 0–11, K–52 and 0–10, K–23 respectively. Micromorphological study showed both lateral and polar flagella of gram negative, slightly curved rods which at times showed pleomorphism. Growth requirement studies indicated a pH optimum of 8.2 with a minimum of 4.5 and a maximum of 8.5. Furthermore, the sodium chloride concentrations supporting maximum growth ranged from 1.5 to 3.5 percent. Both V. parahaemolyticus isolates were sensitive to chloramphenicol, erythromycin and streptomycin, but resistant to penicillin-G. One isolate was sensitive to tetracycline but resistant to sulphaflurozole whereas the other isolate was resistant to tetracycline and sensitive to sulphaflurozole. The reference strain was sensitive to chloramphenicol, erythromycin and tetracycline but resistant to sulphaflurozole, penicillin-G and streptomycin.

INTRODUCTION
In 1950, a severe gastrointestinal food poisoning occurred in Japan following consumption of a semi-dried fish popularly known in Japan as “Shirasu” (Fujino et al. 1953) affecting 272 persons with 20 fatal cases. The out-

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break was associated with presence of a new bacterium, V. parahaemolyticus isolated from the sick and the dying patients. However, the significance of the involvement of V. parahaemolyti-
cus was not realised until Takikawa described another episode later involving 120 persons, among them 47 hospital personnel and 73 in-patients (Takikawa, 1956). It took many years for the rest of the world to recognise and report the occurrence of this organism, among them: India (Chatterjee et al. 1970), Indonesia (Bonang et al. 1974), United States of America (Dadisman et al. 1973), the Republic of Vietnam (Newman et al. 1972) and many others. The organism has been isolated from estuarine sediments (Ward, 1968; Kaneko and Colwell, 1973); coastal waters (Kampelmacher et al. 1970; Ayres and Barrow, 1978; Baross and Liston, 1970); leg gangrene (Roland, 1970) and faeces from the sick patients (Kudoh et al. 1974; Sakazaki et al. 1968).

In Africa, the cholera-like syndromes of diarrhoea have been associated with V. parahaemolyticus (Bockemuhl and Triemer, 1974). The organisms have been recovered from seafoods, water and sediment obtained from waters off the West African coast (Bockemuhl et al. 1972; Bockemuhl and Triemer, 1974). However, there is no mention of this organism in East African medical literature. Thus the aim of this investiga-
tion was to characterise some of the isolates among those recovered in a preceeding prevalence survey involving
shellfish, seafish, lake fish, sea water and sea sediment. The isolates reported herein were from shellfish.

**MATERIALS AND METHOD**

**Source of shellfish**

Whole shellfish were obtained from fishing centres and fish markets along the Kenya coast and in up-country markets. Samples were transported to the laboratory in cooled iceboxes from the nearby markets. Those obtained from the coastal fishing areas were collected and stored at 4°C for a maximum of 2 days after which they were transported to the laboratory for immediate examination. Homogenates of the shellfish tissue were prepared and processed for bacterial isolation.

**Isolation of bacteria and serotyping**

Fifty grams of shellfish tissue was blended in 450mls of physiological saline in a mechanical homogeniser. Tenfold dilutions of the homogenate were made and 10 ml portions from each dilution were inoculated into glucose salt teepol broth (GSTB) tubes and incubated at 37°C overnight. A loopful of the overnight growth from GSTB tubes showing growth in the highest dilution were streaked onto thiosulphate citrate salt sucrose (TCBS) agar and incubated for 18 to 24 hours at 37°C. Colonies with diameters of 2-4mm with dark green centres, were subcultured and purified. Isolates which on further testing showed alkaline slant and acid butt on triple sugar iron (TSI), were positive for lysine decarboxylase, negative for Voges-Proskauer test and grew in 8% sodium chloride were preserved for further biochemical tests and serotyping. Drs. G.I. Barrow and D.C. Miller of Truro Hospital, London, and Prof. R. Sakazaki of the Public Health Laboratory, Osaka, Japan, kindly serotyped our isolates. The reference strain was kindly provided by Dr. G.I. Barrow. All stock cultures were maintained on nutrient agar slopes containing 3% sodium chloride.

**Morphological studies**

Observations on macroscopic colonial morphology were made on TCBS agar plate cultures. Hanging drop preparations were made from GSTB overnight cultures to study motility. Electron-microscopy was done as follows: Plates of TCBS agar were streaked with various cultures and incubated for 24 hours at 37°C. An area of a bacterial colony measuring 4 x 4 mm was delineated and cut at its edges vertically into the agar medium. This was followed by fixation of the block thus obtained in formaldehyde-glutaraldehyde (Ito and Karnowsky, 1968), dehydration, embedding and sectioning. Various sections were stained with uranyl acetate and lead citrate and carried on 200 mesh grids and examined in a 3M9A Carl Zeiss electron microscope.

**Biochemical tests**

Provisionally, all isolates were inoculated onto TSI, lysine decarboxylase broth, Voges-Proskauer broth and nutrient broth containing 8% sodium chloride. Isolates showing an alkaline slant and an acid butt on TSI, positive for lysine decarboxylase, negative for Voges-Proskauer test and growth in 8% sodium chloride were subjected to tests shown in Tables 1 and 2 using standard methods. However, for the evaluation of sodium chloride concentration ranges that supported growth, values of 0.5%, 1.5%, 3.5%, 8.5%, 9.5%, 10.5%, and 13.5% in peptone water were inoculated with 10⁹ cells per tube and incubated at 37°C. Subsequent bacterial growth was
monitored as turbidity in a spectronic-20 photometer at a wavelength of 400nm. To evaluate optimal pH for growth, peptone water containing 3% sodium chloride was adjusted to pH values of 4.2, 4.5, 5.0, 5.5, 6.0, 7.0, 7.5, 8.2, 8.5, and 9.0 and inoculated with 10⁶ cells per tube. During incubation at 37°C, growth was monitored and scored.

To evaluate antibiotic sensitivity, four Mueller-Hinton agar (Oxoid) plates were inoculated with the reference strain, the two test serotypes and a Vibrio alginolyticus isolate. The plates were inoculated evenly by swabbing an 18 hour culture onto the agar surface so as to obtain a confluent growth. Multidiscs impregnated with chloramphenicol (10μg), erythromycin (10μg), sulphafurazole (100μg), penicillin-G (1.5 units), streptomycin (10μg) and tetracycline (10μg) were placed on the four plates carrying each organism. After an overnight aerobic incubation, zones of inhibition were measured.

RESULTS AND DISCUSSION

Morphological studies

On TCBS agar, the V. parahaemolyticus bacterial colonies had a dark raised green centre, were smooth, moist, circular and opaque. When examined in gram strain preparations, one observed gram negative rods which were slightly curved and occasionally showed pleomorphism. The bacteria were motile and the electron microscopy showed both lateral and polar flagella.

Biochemical studies.

Whereas most of the V. parahaemolyticus isolates did not ferment sucrose, hence the green colour on TCBS, V. alginolyticus readily fermented sucrose producing yellow colonies on the same medium. Table 1. shows results for the remainder of the biochemical tests. It is clear that serotypes of this organism have minor biochemical differences as observed earlier by Colwell (1970). However, Kimura et al. (1979a) and Kimura et al. (1979b) have shown that the medium pH influences both the flagellation and the production of haemolysin by V. parahaemolyticus, whereby in alkaline pH (9.0) the organism grew well but did not form lateral flagella although the M-flagella were unaffected. Anionic detergents have also been shown to inhibit synthesis of L-flagella (Shinoda et al. 1977).

The optimal concentration of sodium chloride tolerated by both serotypes was 3.5%. This fits well with the work of other authors, however, some have used 2% sodium chloride in growth medium when testing for other phenomena (Kimura et al. 1979b), or 2.5% (Boutin et al. 1979) or 5% (Kimura et al. 1979a). The variations in the use of sodium chloride could easily influence

Kanagawa type haemolysis

A modified Wagatsuma blood agar (WBA) medium (Miyamoto et al. 1969) was used. It contained yeast extract (3g), Bactopeptone (10g), sodium chloride (70g), dipotassium phosphate (5g), mannitol (10g), crystal violet (10g) and 5% washed human group “O” erythrocytes. The cultures were inoculated onto the medium and incubated following which absence of clear and complete haemolysis around bacterial colonies indicated a Kanagawa negative strain, whereas presence of clear haemolysis would show a Kanagawa positive strain.
Table 1: Biochemical properties of two serotypes of *Vibrio parahaemolyticus* recovered from sea foods in Kenya.

<table>
<thead>
<tr>
<th>Test done</th>
<th>Serotype tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,10-K23</td>
</tr>
<tr>
<td>a) Presence of:</td>
<td></td>
</tr>
<tr>
<td>oxidase</td>
<td>+</td>
</tr>
<tr>
<td>catalase</td>
<td>+</td>
</tr>
<tr>
<td>Indole formation</td>
<td>+</td>
</tr>
<tr>
<td>Acetyl methyl carbinol</td>
<td>-</td>
</tr>
<tr>
<td>Acid in Methyl red test</td>
<td>-</td>
</tr>
<tr>
<td>Growth in 7% Sodium chloride</td>
<td>+</td>
</tr>
<tr>
<td>Growth in 10% &quot;&quot; &quot;&quot;</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>Citrate (christensen) utilization</td>
<td>+</td>
</tr>
<tr>
<td>Arginine deamination</td>
<td>-</td>
</tr>
<tr>
<td>Malonate utilisation</td>
<td>-</td>
</tr>
<tr>
<td>H₂S formation in peptone agar</td>
<td>-</td>
</tr>
<tr>
<td>Growth at pH 9.0</td>
<td>+</td>
</tr>
<tr>
<td>b) Dissimilation of:</td>
<td></td>
</tr>
<tr>
<td>Glucose (Acid)</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>_d</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>_d</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>-</td>
</tr>
<tr>
<td>Adonitol</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
</tr>
<tr>
<td>d = variable.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Antimicrobial drug sensitivity of several serotypes of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* recovered from sea foods in Kenya (mm growth inhibition).

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>Drug concentration</th>
<th>Vibrio parahaemolyticus isolates</th>
<th>Reference strain Vibrio parahaemolyticus</th>
<th>V. Alginolyticus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>serotype 0-10, K-23</td>
<td>serotypes 0-10, K-52</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>10μg</td>
<td>32(S)*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26(S)</td>
<td>26(S)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10μg</td>
<td>22(S)</td>
<td>20(S)</td>
<td>22(S)</td>
</tr>
<tr>
<td>Sulphafurazole</td>
<td>100μg</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16(S)</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>1.5 units</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10μg</td>
<td>13(S)</td>
<td>13(S) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10μg</td>
<td>17(S)</td>
<td>14(R)</td>
<td>17(S)</td>
</tr>
</tbody>
</table>

<sup>a</sup>, S = Sensitive  
<sup>b</sup>, 0 = highly resistant  
<sup>c</sup>, R = resistant

...the results obtained by different workers and perhaps a standard concentration should be recommended to allow comparison of results among the researchers.

The two strains and also the others not reported herein were Kanagawa negative. It is usual to associate Kanagawa positive strains with pathogenicity whereas the Kanagawa negative ones would be regarded as being non-pathogenic (Miyamoto et al. 1969). However, it is becoming increasingly evident that not all Kanagawa positive strains would consistently produce a positive disease response in test animal species (Brown et al 1977; Sakazaki et al. 1968). Also the findings of Boutin et al. (1979) have shown that there were no marked differences between Kanagawa negative and Kanagawa positive strains in invasiveness of the lamina propria of ligated rabbit loops.

The two strains reported here grew well in alkaline pH (8.2 and above), but there was minimal growth in acid pH values (pH 4.2 and below). This finding agrees with those of Kimura et al. (1979b) who recorded the growth of *V. parahaemolyticus* even at pH 9.0, however, the lowest pH tested by these authors was 6.0.

The antibiotic sensitivity of the two strains, the reference strain and *V. alginolyticus* are shown in Table 2. There were no marked differences between the reference strain and the two serotypes isolated locally except for the resistance to streptomycin and sulphafurazole by the reference strain.

**ACKNOWLEDGEMENTS**

The help given by Drs. G.I. Barrow and D. C. Miller of Truro, Cornwall Hospital, Britain and that of Prof. Sakazaki of the Public Health Laboratory, Osaka, Japan is gratefully appreciated.

**REFERENCES**


Received for publication on 20th May 1980
Short Communication.

BRUCELLA HYGROMA FLUID: POSSIBLE VETERINARY AND HUMAN PUBLIC HEALTH HAZARDS

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SUMMARY
At the Jos slaughter slab, brucellae were constantly cultured from the slab and its surroundings during a period of 19 days within which 8 hygroma cattle were slaughtered and the hygroma fluid drained onto the slab. Human and veterinary public health hazards were discussed.

INTRODUCTION
Swellings in or around one or more joints of brucellosis reactor cattle has been described as one of the major manifestations of bovine brucellosis in certain parts of the world. This condition known as hygroma has been described by many authors: (Camara 1948; Blanchard & Coulibaly (1954) and Earnshaw and O’Brein 1928). It has been associated with abortions and infertility in the affected herds.

Brucella hygromas contain golden-yellow to straw coloured serous fluid with caseous materials, high Brucella antibody titres and Brucella organisms (Thienpont et al. 1958).

In Nigeria, Brucella hygroma, known to the nomadic Fulanis as “Bakale” and thought to be endemic in the big rivers and lake valleys (Eze, unpublished observation) was first described by Earnshaw and O’Brien (1928).

In this paper, the survival of Brucella organisms shed onto Jos slaughter slab from the slaughter of hygroma cattle was assessed and the veterinary and human public health significance discussed.

MATERIALS AND METHODS
Between 12th and 31st December 1976, 8 hygroma cattle were slaughtered and the hygroma fluid spilt on the slab. On each morning, 10 ten ml samples were collected from each of the following locations in and around the slab: a) cracks in the slab (sampled before the day’s slaughter); b) effluent sewage (sampled before and during the day’s slaughter); c) puddles on the slab (sampled during the day’s slaughter and d) areas on the low walls around the slab (sampled during the day’s slaughter).

All samples were plated on serum dextrose antibiotic agar plates within one hour of collection and incubated in candle-jars at 37°C for 4 days.

RESULTS
The results of the cultures are shown in Table 1. Brucella organisms were isolated from cracks in the slab every day throughout the period. Brucella organisms were also isolated from puddles on the slab every day hygroma cattle were slaughtered and some days thereafter. Samples from the edges of the draining sewage yielded brucellae only on the days hygroma cattle were slaughtered. Brucellae were isolated from drops of effluent from the low walls of the slab.
Table 1: Isolation of Brucella organisms from Jos slaughter slab (12th-31st December 1976)

<table>
<thead>
<tr>
<th>Date sampled</th>
<th>Brucellae cultured (No. of samples)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>slab cracks</td>
<td>puddles on slab</td>
</tr>
<tr>
<td>12.12.76**</td>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>13.12.76</td>
<td>A</td>
<td>2</td>
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</tr>
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<tr>
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</tr>
<tr>
<td>15.12.76</td>
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<td>1</td>
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<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>16.12.76</td>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B</td>
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</tr>
<tr>
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<td>A</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>18.12.76**</td>
<td>A</td>
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<td></td>
<td>B</td>
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</tr>
<tr>
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<td>6</td>
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<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
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<td>B</td>
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</tr>
<tr>
<td>21.12.76</td>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>22.12.76</td>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>23.12.76</td>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>24.12.76*</td>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>25 and 26.12.76.</td>
<td>No Sampling</td>
<td></td>
</tr>
<tr>
<td>27.12.76</td>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>28.12.76</td>
<td>A</td>
<td>4</td>
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<td>-</td>
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<tr>
<td>29.12.76</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td></td>
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<td>-</td>
</tr>
<tr>
<td>30.12.76</td>
<td>A</td>
<td>2</td>
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<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>31.12.76**</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes on table 1:
* 1 hygroma animal slaughtered.
** 2 hygroma cattle slaughtered.
no sample taken.
A samples collected before day’s slaughtering,
B samples collected during days slaughtering. 10 samples taken from each location and numbers are shown are those from which Brucella organisms were cultured.
DISCUSSION

Brucella organisms were constantly present in the slab and its surroundings during the experiment. Cattle which fought through the poodles during restraint for slaughter, could be responsible for contaminating both the butchers’ clothes and carcasses being dressed on the slab. Vultures scavenging the slab and its environs for food may also disseminate the infection to grazing grounds.

REFERENCES


Received for publication on 20th November, 1979
BRUCELLOSIS: AN INVESTIGATION IN SELECTED HERDS IN OYO STATE, NIGERIA

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School of Animal Health, Institute of Agricultural Research & Training, Ibadan, Nigeria.

SUMMARY

Brucellosis continues to be the predominant reproductive disease in Nigerian livestock. Serological evidence is produced of the disease in cattle, goats as well as sheep. There is no doubt that the Rose Bengal plate test is ineffective on goats and sheep sera. *Brucella abortus* biotypes 1, 2 and 3 are most commonly encountered. Goats and sheep born either by sero-negative or positive dams do possess antibodies to brucella. Efforts should be geared towards controlling and/or eradicating the disease in the country.

INTRODUCTION

Brucellosis is an endemic disease in Nigeria. It is prevalent in domestic animals such as cattle, goats and dogs and also within the human population. A comprehensive account of the disease in the country as well as the major objectives for its control and eradication had been presented. (Falade, 1979). We report here our recent findings in selected livestock holdings in Ibadan.

MATERIALS AND METHODS

A total of four hundred and eighty-seven sera was collected from 250 sheep, 189 goats and 48 cattle at the livestock stations of the Institute of Agricultural Research and Training, and the National Cereals Research Institute, Moor Plantation. Milk samples were also obtained from 42 goats. Information on the brucellosis vaccination status, age as well as previous history of abortion was also recorded. With the goats and sheep, it was possible in a few cases to identify the offsprings and their respective dams. All serum samples were stored at -20°C until ready for use. The milk samples were refrigerated within one hour of collection and tested immediately after.

Serological tests

The serum samples were examined by the Rose Bengal plate, standard tube agglutination and the Rivanol tests, and the milk samples by the milk ring test. The techniques used for the various tests were those described in earlier reports (Falade, 1978, 1979, Falade and Hussein 1979).

Bacteriological culture

Milk samples positive by the MRT were centrifuged at 300 rpm, the cream and deposits were obtained. These and the synovial fluid from a bovine hygroma were examined for brucella as described by Corbel *et al.* (1978) Biotypes were finally confirmed at the National Veterinary Research Institute, Vom, Nigeria.

RESULTS

Brucella antibodies were detected in 4.8% (12/250) of sheep and 9.0% (17/189) of goat (Table 1). Antibodies were also found in 33 of 48 bovine

*Contact Address: Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.
serum samples and the synovial fluid (Table 2). The MRT was positive for 11 of the 42 milk samples examined. The serological tests were negative for the goats from which milk could be collected. The antibody titres and dates of birth of goats born to their respective dams are as shown in Table 3.

Table 1: *Brucella abortus* antibody titres in goats and sheep

<table>
<thead>
<tr>
<th>No. of Animal</th>
<th>Rose Bengal Plate Test</th>
<th>Serum Agglutination test</th>
<th>Rivanol test</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCG 6 74</td>
<td>Negative</td>
<td>1/40+</td>
<td>1/10+</td>
</tr>
<tr>
<td>132</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>254</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>312</td>
<td>&quot;</td>
<td>1/10++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>330</td>
<td>&quot;</td>
<td>1/40+</td>
<td>&quot;</td>
</tr>
<tr>
<td>331</td>
<td>&quot;</td>
<td>1/10++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>359</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>365</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>380</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>551</td>
<td>&quot;</td>
<td>1/10++</td>
<td>&quot;</td>
</tr>
<tr>
<td>559</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>605</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>616</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>978</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>SFG 59</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>63</td>
<td>&quot;</td>
<td>1/10++</td>
<td>&quot;</td>
</tr>
<tr>
<td>NCS 15</td>
<td>&quot;</td>
<td>1/10++</td>
<td>&quot;</td>
</tr>
<tr>
<td>85</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>140</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>149</td>
<td>&quot;</td>
<td>1/40++</td>
<td>&quot;</td>
</tr>
<tr>
<td>729</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>843</td>
<td>&quot;</td>
<td>1/10++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>853</td>
<td>&quot;</td>
<td>1/20++++</td>
<td>&quot;</td>
</tr>
<tr>
<td>871</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>905</td>
<td>&quot;</td>
<td>1/10++</td>
<td>&quot;</td>
</tr>
<tr>
<td>920</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
<tr>
<td>BS 8</td>
<td>&quot;</td>
<td>1/10+</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Key Note: National Cereals Research Institute Goat (NCG)  
National Cereals Research Institute Sheep (NCS)  
Southern Farm Goat (SFG)  
Bora Sheep (BS)

Table 2: *Brucella abortus* antibody titres in cattle

<table>
<thead>
<tr>
<th>No. of Animal</th>
<th>Rose Bengal Plate test</th>
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<tbody>
<tr>
<td>N 003</td>
<td>++</td>
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<tr>
<td>020</td>
<td>&quot;</td>
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<tr>
<td>021</td>
<td>&quot;</td>
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<td>&quot;</td>
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<td>029</td>
<td>&quot;</td>
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<td>030</td>
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<td>058</td>
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<td>066</td>
<td>&quot;</td>
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<td>068</td>
<td>&quot;</td>
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<td>01116</td>
<td>++</td>
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<tr>
<td>19</td>
<td>++</td>
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<td>168</td>
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<td>739</td>
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<td>++</td>
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<td>474</td>
<td>++</td>
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<tr>
<td>768</td>
<td>++</td>
</tr>
<tr>
<td>SF 708</td>
<td>++</td>
</tr>
<tr>
<td>770</td>
<td>++</td>
</tr>
<tr>
<td>781</td>
<td>++</td>
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<tr>
<td>800</td>
<td>++</td>
</tr>
<tr>
<td>809</td>
<td>++</td>
</tr>
<tr>
<td>WF 048</td>
<td>++</td>
</tr>
<tr>
<td>978</td>
<td>++</td>
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<tr>
<td>358</td>
<td>++</td>
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<tr>
<td>K 049</td>
<td>++</td>
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<td>053</td>
<td>++</td>
</tr>
<tr>
<td>20</td>
<td>++</td>
</tr>
<tr>
<td>685</td>
<td>++</td>
</tr>
<tr>
<td>5575</td>
<td>++</td>
</tr>
</tbody>
</table>

Key Note:  
N: Ndama breed  
WF: White Faced  
K: Keteku  
SF: Synovial
Table 3: *Brucella abortus* antibody titres of goats and sheep offsprings and their respective dams.

<table>
<thead>
<tr>
<th>Parent Stock</th>
<th>First Generation</th>
<th>Second Generation</th>
<th>Date of Birth</th>
<th>Antibody Titres</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RBPT</td>
<td>SAT</td>
</tr>
<tr>
<td>NCG 33</td>
<td>NCG 132</td>
<td>NCG 605</td>
<td>Not known 8/6/74, 14/4/78</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NCG 48</td>
<td>(a) NCG 331</td>
<td>(b) NCG 330</td>
<td>Not known 9/5/77, 9/5/77, 20/12/77, 11/1/79</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>(c) NCG 616</td>
<td>(b) Not known</td>
<td>(a) NCG 330 is a male</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c) None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCS 17</td>
<td>NCS 94</td>
<td>NCS 871</td>
<td>Not known 31/3/75, 3/9/76</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NCS 8</td>
<td>NCS 71</td>
<td>NCS 920</td>
<td>Not known 17/11/74, 4/7/77</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SFG 6</td>
<td>SFG 59</td>
<td></td>
<td>Not known 16/6/79</td>
<td>–</td>
<td>1/10+++</td>
</tr>
<tr>
<td>SFG 2</td>
<td>SFG 63</td>
<td></td>
<td>Not known 18/6/79</td>
<td>–</td>
<td>1/10+++</td>
</tr>
<tr>
<td>NCG 262</td>
<td>NCG 559</td>
<td>NCG 791</td>
<td>Not known 20/8/77, 15/8/79</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NCG 6</td>
<td>NCG 160</td>
<td></td>
<td>Not known</td>
<td>–</td>
<td>1/40+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>18/10/74, 7/7/77</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NCG 9</td>
<td>NCG 365</td>
<td></td>
<td>Not known 15/3/78</td>
<td>–</td>
<td>1/10+</td>
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<tr>
<td></td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>NCS 29</td>
<td>NCS 64</td>
<td>NCS 843</td>
<td>Not known 22/9/74, 10/5/76</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>
DISCUSSION

There is no record of brucellosis vaccination in all the animals examined. Although antibodies were detected in approximately 70% of cattle tested, only 3 (6.25%) could be considered infected using the criteria of the WHO Report of 1971. Similarly, 29 (6.61%) of the total sheep and goats had antibodies although only 4 (less than one per cent) had antibodies in excess of 50 I.U. and could therefore be considered infected. However, according to Unel (personal communication) some unvaccinated sheep and goats with antibody levels of 20 I.U. although classed as "suspicous", not infrequently yield brucelae on post mortem. While the number of culturally positive animals is even much higher in those with at least 40 I.U. (SAT titre of \( \frac{1}{2} + + + + \) and above). Going by the latter criteria, 9 of the 189 goats (4.76%) and 4 of 250 sheep (1.6%) could be regarded as positive.

The single report on ovine brucellosis in the country was by Kramer, Nduaka and Nzoukwu (1967) who found less than one per cent (1/15) of West African Dwarf sheep (ovis jubata) positive for brucella. The present infection rates for goats is similar to previous observations (Falade, Ojo and Sellers 1975, Falade 1978, 1979) and it would appear that brucellosis is more prevalent in goats than sheep. The Rose Bengal plate test proved more useful on bovine sera as all SAT — positive goats and sheep sera gave negative results. The RBPT has previously been considered ineffective on human and goat sera (Falade, loc. cit) and also for the diagnosis of brucellosis in sheep (Kulshreshtha, Kalra and Vasudevan, 1978). The Rivanol test was also less effective on all sera, the titres were lower than SAT titres in cattle, while it was negative for all the goats and sheep sera. The reason for this is not clear as it compared favourably with the SAT in earlier investigations on goat (Falade 1979, Falade and Hussein 1979). Antibodies were detected in serum and synovial fluid of Ndama cow No. 768 at below diagnostic levels. Br. abortus biotype 1 was also isolated from the synovial fluid. The animal is believed to be chronically infected and the CFT would have proved more useful.

The milk samples showed some false MRT positive results probably due to mastitis in more than a third of the samples. The fact that Br. abortus biotype 1 was isolated from four of the MRT positive samples would suggest an udder infection. Br. abortus (untyped) was previously isolated from a goat’s milk (Falade, 1978). Br. abortus biotypes 1, 2 and 3 are more commonly isolated from cattle in Nigeria (Eze, 1978 and Thomas, personal communication). There has been no single report on the isolation of Br. melitensis in Nigerian breeds of goats. This problem is compounded by the fact that aborted materials are usually discarded by the owners. Much public enlightenment is therefore desired in this regard.

From Table 3 it is observed that goats and sheep born either by sero-negative or positive dams do possess brucella antibodies. It is contemplated that such offsprings either acquired the light infection from within the herd or from their respective parent stock although the number of animals involved is too small to make a meaningful conclusion. Finally, from all available evidence, brucellosis is the single most common reproductive disease in Nigerian livestock, the Federal Livestock Department
is currently intensifying its efforts in the control and eradication.

ACKNOWLEDGEMENTS

We are grateful to Dr. P. Omueti of the National Cereals Research Institute, Moor Plantation, for his co-operation and Dr. E.N. Eze of the National Veterinary Research Institute, Vom, for confirming the isolates.

REFERENCES

Received for publication on 19th February 1980
ASPERGILLOSIS IN NEWLY HATCHED TURKEY POULTS

*O.O. FATUNMBI,
Department of Veterinary Medicine, University of Ibadan.

and

O.G. OGUNTOYINBE and SEGUN FALADE,
Institute of Agricultural Research and Training, University of Ife.

Aspergillosis of poultry is generally considered to affect chicks or poults of a week old or older and attributed to mouldy litter, mouldy feed or damp brooding conditions. Although it is more frequently reported in chicks than poults (Witter and Chuter, 1952), Durant and Tucker (1935) concluded that chicks were more resistant to aspergillosis than poults. It appears there is no available literature on egg borne aspergillosis in poults. However, Eggert and Barnhart (1953) reported a case of egg borne aspergillosis in recently hatched chicks. An outbreak of aspergillosis in poults, which may be egg borne or of hatchery origin, is reported.

The outbreak occurred in 500 imported day old poults. Immediately they arrived, adequate brooding and management were provided. Forty-eight hours after being housed, about 100 poults (20%) were showing signs of depression, anorexia and dyspnoea resulting in the death of 92 poults. All were placed on Eura vit\textsuperscript{R} (TAD) after a tentative diagnosis of vitamin deficiency was made, but mortalities did not decline. At 10 days old, about 80% were persistently coughing and emaciated. A few had ophthalmitis. During the ensuing two week interval, 352 poults (70.4%) died.

The dead poults were necropsied. Pustules and excessive mucous exudate were found in the oral cavity and oesophagus of poults that died within the first week of life. Gross examination of the lungs of poults that died at about 10 days old revealed small, spherical hard, whitish-yellow nodules suggestive of aspergillosis (Fig. 1). Lesions were taken from the infected lungs and ophthalmic eyes and sent for microbiology.

Fig. 1: Small, spherical, hard whitish-yellow nodules in lungs. The lung in the centre is normal.

Sections were cut from infected lungs after fixing in 10% formalin and stained with methamine hexamine silver nitrate and haematoxin eosin strains. The litter, feed and drinking water were also cultured. Petri dishes of Sabouraud's agar were placed and exposed in the brooding house. The culture from the infected lungs yielded \textit{Aspergillus fumigatus}. \textit{A. niger} was isolated from the affected eyes. Culture from the brooding house, litter, feed and drinking water did not reveal any fungus. Histopathological examination of the infected lungs showed lesions typical of a pneumo-
mycosis due to *Aspergillus* sp. and the septate branching fungus (Fig. 2). A diagnosis of aspergillosis was therefore established.

A case of egg borne aspergillosis was reported by Eggert and Barnhart (1953). They suggested the fungus had penetrated through the eggshell during incubation and the recently hatched chicks were infected. Clarke and others (1954) diagnosed a case of hatchery borne aspergillosis in chicks where infection could not be traced to the hatcher eggs but was readily found in the incubators, hatcher, incubator rooms and intake ducts. O'Meara and Chute (1959) easily infected chicks in the process of hatching and up to two days of age with *A. fumigatus* spores by contaminating the forced draft incubator with wheat which had been seeded with *A. fumigatus*.

The source of the infection was unknown and because no *A. fumigatus* sp. was isolated from the house, litter, feeding and drinking it is suggested that the outbreak of aspergillosi in these newly hatched poulets might have been hatchery or egg borne.

**REFERENCES**


*Received for publication on 20th May, 1980*
Geographical Distribution of FOOT-AND-MOUTH DISEASE in Africa

OAU/STRC
INTERAFRICAN BUREAU
FOR ANIMAL RESOURCES
MAP No. 438

- Foci reported
- Widespread
- Enzootic/Sporadic but no Foci reported
- No official information available

Geographical Distribution of BRUCELLOSIS in Africa

[Map illustrating the geographical distribution of brucellosis in Africa]

OAU/STRA
INTERAFRICAN BUREAU FOR ANIMAL RESOURCES
MAP No. 439
1980

- Foci reported
- Widespread
- Enzootic/Sporadic but no Foci reported
- No official information available.

Geographical Distribution of RABIES in Africa

OAU/STRC
INTERAFRICAN BUREAU
FOR ANIMAL RESOURCES
MAP No 440

- Foci reported
- Widespread
- Enzootic/Sporadic but no Foci reported
- No official Information available

Bull Anim Hlth Prod Afri (1981)
Geographical Distribution NEWCASTLE DISEASE in Africa

OAU/STRA
INTERAFRICAN BUREAU FOR ANIMAL RESOURCES
MAP No. 441
1980

- Foci reported
- Widespread
- Enzootic/Sporadic but no Foci reported
- No official Information available

Geographical Distribution CONTAGIOUS BOVINE PLEUROPNEUMONIA in Africa

OAU STRC
INTERAFRICAN BUREAU
FOR ANIMAL RESOURCES
MAP No. 442
1980

- Foci reported
- Widespread
- Enzootic/Sporadic but no Foci reported
- No official information available

Bull Anim Hlth Prod Afr (1981)
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57. Discontinuous Natural Phosphate Rock Intakes in Tropical Cattle Feeding.
IBAR/1981 M. GITTER, R. BRADLEY
and P.H. BLAMPIED
*Listeria monocytogenes* infection in bovine mastitis


AUTHORS' SUMMARY: *Listeria monocytogenes* type 4 was isolated from milk of a cow affected with mastitis in the left fore quarter. Histological examination revealed a severe suppurrative mastitis with eosinophil leucocytes predominating among the inflammatory cells. The findings and the public health aspect of the condition are discussed.

IBAR/1981 J.L. SMART, T.A.
ROBERTS, K.G. MCCULLAGH, V. M. LUCKE
and H. PEARSON
An outbreak of type *C. botulism* in captive monkeys

*The Veterinary Record* (1980) 107, 445–446

AUTHORS' SUMMARY: In an outbreak of type *C. botulism* in a group of captive primates, six squirrel monkeys, six white throated capuchin monkeys and two weeper capuchin monkeys succumbed rapidly and died. *Clostridium botulinum* type *C* toxin was detected in the remains of the chopped chicken feed and in nine of 11 blood samples and one of three stomach contents samples from the affected animals.

IBAR/1981 FEINHAKEN, D., SCHNEERSON-PORAT, S. and TAMARIN, R.
Serological Findings and their Relevance in diagnosis of Brucellosis in General Population and in Veterinary Personnel.


AUTHORS' SUMMARY: Forty two selected sera of hospitalized patients clinically diagnosed for brucellosis, on grounds of symptoms, signs and epidemiological data, were investigated by four serological tests. In addition, 42 sera of veterinary personnel exposed to Brucella infection, and 50 control sera of healthy males were also examined.

Antibodies to Brucella were detected in sera of both hospitalized patients and veterinary personnel, but were absent in the control sera.

All sera of acute hospitalized cases positive by serum agglutination. In the symptomless group of veterinary personnel 34/37 were positive by the antiglobulin (Coombs) test. The results of the Rose Bengal correlated well with those of serum agglutination.

The serum agglutination test is recommended principally for serological diagnosis of acute disease. If low titers are detected by serum agglutination, then other tests may be indicated.

The Rose Bengal plate test was positive in all sera of acute patients examined. It is a quick and simple test and may be useful as a screening test for laboratory diagnosis of acute brucellosis.

IBAR/1981 J.H. MARCELIS
35 Interactions Between Bacteria and Iron Binding Proteins

*Veterinary Research Communications*, 4 (1980) 151-164

AUTHOR'S SUMMARY: Growth inhibition due to iron deprivation of 118 strains of staphylococci, enterobacteriaeeae, *Streptococcus faecalis* and *Bacteroides species* was studied in vitro. Apo-transferrin (Tr), apo-lactoferrin (Lf) and a synthetic iron chelator (EDDA) were used as iron binding agents.

*S. epidermides* was more inhibited than *S. aureus*. The majority of the enterobacteriaeae was inhibited by the highest concentrations of EDDA, Tr and Lf. Most bacteroides strains were inhibited by EDDA and all were inhibited by Tr and Lf at physiological concentrations. Production of bacterial iron chelators could be demonstrated in all strains growing at iron deprivation except for *Streptococcus faecalis*. In 8 out of 11 strains of *Bacteroides cytochromes* of type b and c could be demonstrated. In one strain iron deprivation resulted in disappearance of the cytochrome and a reduction of the growth yield. In Bacteroides the production of iron-chelators could not be related to virulence.
IBAR/1981 RENAU'T, L. and BOUR-36 H1S, E. LE


AUTHORS' SUMMARY: The authors describe the isolation of new enteropathogenic strain Escherichia coli 0157: K 88: H 43 of pigs. This strain releases heat-labile (LT) enterotoxin but is deprived of heat-stable (ST). These characteristics confirm the interest of its identification in the mechanisms of colibacillosis after weaning and of its incorporation the vaccination program.

IBAR/1981 RASKOVA and K. 37 RASKA
Enterotoxins from gram-negative bacteria relevant for veterinary medicine


AUTHORS' SUMMARY: The chemistry, mechanism of action, assay methods, pharmacology, and prevention and treatment of diarrhoea due to toxins of gram-negative microbes are discussed. Other virulence factors are mentioned briefly. Special emphasis is placed on non-specific treatment by oral rehydration.

IBAR/1981 OLSSON GERT and HOL-38 TENIUS PAUL
Studies on the Effect of Treatment with Anthelmin-
tics on Weight Gain in Calves, Naturally Infected with Gastrointestinal Nema-
todes


AUTHORS' SUMMARY: Calves naturally in-
fected with gastrointestinal nematodes were treated with anthelminitics after stabling and the effect of the weight gain during the in door season was studied. Other groups of calves were treated 3 weeks after turning out on infected pastures in May and the weight gain and number of eggs in faeces were studied during the grazing season.

IBAR/1981 LAGOIN, Y. 39 Actual Data on a Nema-
todosis of Man, Anisakiasis or « Herring Worm Dis-
eease »


AUTHOR'S SUMMARY: Anisakis simplex, parasitic nematode of various sea fishes has recently been found in smoked herrings on sale in a French wholesale market. This note aims at bringing up to date the main data on the cycle of this worm, its pathogenicity to man and technological treatments used to inactivate this nematode.

IBAR/1981 REMOND (MRS MICH-40 LE), PERRET (MRS CA-
THERINE) and SOULE, C.
Conditions of in vitro Surviv-
al of Cysticercus bovis


AUTHORS' SUMMARY: The authors report the results of a study on the in vitro main-
tenance of cysts of Taenia saginata. Suitable procedures for the extraction of the cysts and their in vitro maintenance are described. Cysts can be kept alive for 5 weeks in NCTC 135 medium with foetal calf serum and 2 weeks in phosphate buffered saline supplemented with glucose.

Cysts treated with Hanks trypsin solution can develop until the strobilar stage in NCTC 135 medium with foetal calf serum.

IBAR/1981 F. W. DOUVRES, M.J. 41 THOMPSON and W.E. ROBBINS
In vitro Cultivation of Ost-
tertagia ostertagi the Me-
dium Stomach Worm of Cattle. II. Effect of Insect-Growth-Disrupting Amines and Amides on Development

*Veterinary Parasitology, 7* (1980) 195–205

AUTHORS' SUMMARY: Thirteen secondary and tertiary amides and amines that disrupt growth and development in certain insects were tested at concentrations of 0.25-5.0 p.p.m. for their effects on development of *Ostertagia ostertagi*, form infective larvae to egg-laying adults, in a two-step roller culture system. These compounds did not affect exsheathment of infective larvae. However, some of these compounds inhibited development or killed 100% of the exsheathed larvae at concentrations of 1.0-2.5 p.p.m. Minimum inhibitory concentrations of several of the amines and amides affected the nematodes in one or more of the following ways: decreased motility or paralysis; reduced survival; delayed or blocked third or fourth ecdysis; lowered yields of advanced stages; decreased production of fertile and non-fertile eggs. The results also indicated that, as in insects, these chemicals exerted their lethal effects against *O. ostertagi* at the time of molt.

**IBAR/1981**

H.D. PATTISON, R.J.

**THOMAS, W.C. SMITH**

A survey of Gastrointestinal Parasitism in Pigs

*The Veterinary Record*, November 1, 1980. 107. 415–418.

AUTHORS' SUMMARY: Over a period of two years 1980 pork pigs, 144 baconers and 144 sows from two slaughterhouses in northern England were examined for gastrointestinal parasites. Sows showed the highest level and intensity of infection, 85 per cent harbouring the dominant parasites *Oesophagostomum dentatum* and *O. quadrirspinulatum*; the highest worm burden recorded was 21,000. *Hyostomum rubidum* was present in 28.5 per cent of sows but virtually absent from younger pigs. Small numbers of *Ascaris suum* and *Trichurus suis* were recorded, 16 per cent and 23 per cent respectively in pork pigs and lower in older animals. Levels of parasitism appeared to be influenced by a number of management practices, in particular the housing system.

**IBAR/1981 D.R. NAWATHE and A. ABEGUNDE**

Egg drop syndrome 76 in Nigeria: Serological evidence in commercial farms

*The Veterinary Record* (1980) 107. 466–467

AUTHORS' SUMMARY: A serological survey for egg drop syndrome 76 (EDS 76) virus, using the haemagglutination inhibition test, was conducted on commercial poultry flocks in Nigeria. Antibody was detected in layers on most of the farms. EDS 76 poses a potential threat to the Nigerian poultry and possible means of prevention and control are discussed.

**IBAR/1981 S.M. LAL, S. VASANTHA, B.B.L. MATHUR, B.K. KATHURIA B.S. NEGI and M.C. PANDEY**

Immune and Antibody Response of Cattle to Foot and Mouth Disease type A vaccine


AUTHORS' SUMMARY: Formalin inactivated saponified Foot and Mouth Disease vaccine prepared from type 'A' virus in BHK21 suspension cells grown as monolayers was formulated containing 70% virus and 30% alhydrogel. Vaccine was concentrated by sedimentation. Potency of vaccine was tested by actual challenge with 10,000 bovine ID50/ml virulent type 'A' virus in Jersey cross-bred male calves by PD50 method and in guinea pigs by measuring the antibody concentration present in the vaccinated cattle under field conditions over a period of 6 months duration. Vaccine had 12 PD50 values in cattle and protection 'C' index 3.87 in guinea pigs. Serum antibody concentration of cattle vaccinated against type A vaccine had a titre of 1.35 or greater which probably is suggestive of having a satisfactory level of protection under field conditions where the dose of actual challenge is supposed to be smaller than the actual challenge.
IBAR/1981 TOMA, B. and MORAIL- LON ANNE
Infection of the Dog by a Virus Antigenically Akin to the Virus of Transmissible Gastro-enteritis of the Pig.


AUTHORS’ SUMMARY: Serum neutralization of the virus of transmissible gastro-enteritis of the pigs has been used to study the antibody response in puppies infected with gastro-enteritis and to carry out a serological survey on 417 dogs living in Paris area.

It enabled to demonstrate the responsibility of a virus antigenically akin to that transmitted to puppies from a spontaneous outbreak and to reveal the presence of antibodies in 75 per cent of the 417 dogs studied. The virus responsible for the synthesis of antibodies is widespread in the dog population studied and is likely to be canine coronavirus.

IBAR/1981 LEFEVRE, P.C.
Lumpy Skin Disease. II. Production of a Freeze Dried Vaccine.


AUTHOR’S SUMMARY: A freeze dried attenuated virus vaccine is produced to protect cattle against lumpy skin disease. The technics of production, the stability of the freeze dried product and the stability of the reconstituted vaccine are studied.

No loss of titer is seen after freeze drying whatever the protective medium used. The medium with neopepton (11 p. 100) assures a better protection of the freeze dried vaccine against heat.

Once reconstituted the vaccine is relatively thermostable but must be protected against sunlight.

IBAR/1981 BHOOP SINGH, ABDUL SAMAD, L.G. ANANT- WAR and V.O. BHONSLE
Chemotherapeutic Activity of Oxytetracycline Against Clinical Cases of *Theileria annulata* infection in Exotic and Cross-breed Cattle

*Indian vet. J. 57 October 1980: 849–852*

AUTHORS’ SUMMARY: Clinical trials were undertaken to study the chemotherapeutic efficacy of oxytetracycline (Oxytetracin) against 19 cases of *Theileria annulata* infection in exotic and cross-bred cattle reported for treatment at Veterinary Clinics, Marathwada Agricultural University, Parbhani (Maharashtra).

Seventeen clinical cases of *T. annulata* infection treated with Oxytetracin at 10-15 mg/kg body weight daily for 4-6 days were cured, whereas two cross-breds treated at 4mg and 7 mg/kg succumbed of infection showing typical signs of theileriosis. Supportive treatment like CalMag, Esgiprin, Belamyl or Mifex and blood transfusion to highly anaemic patients proved very beneficial.

IBAR/1981 H.V. WHITLOCK, J.D.
KELLY, C.J. PORTER, D. L. GRIFFIN and I.C.A. MARTIN
In vitro Field Screening for Anthelmintic Resistance in Strongyles of Sheep and Horses


AUTHORS’ SUMMARY: Simplified in vitro field methods are described for the detection and assay of benzimidazole-resistance in sheep trichostrongylids and horse strongyles. Worm eggs are recovered from fresh faeces, within 1 hour of collection, by flotation in sugar solution. The separated eggs are then incubated for 20–24 hours at 27-30°C in solutions of thiabendazole in distilled water ranging from 0.1 to 1.1 p.p.m. for sheep trichostrongylids and from 0.05 to 0.5 p.p.m. for horse strongyles. Under controlled conditions, eggs from thiabendazole-susceptible individuals of both sheep and horse nematodes rarely hatch at thiabendazole concentrations of 0.1 p.p.m. Eggs from resistant individuals will hatch at 0.1 p.p.m. and above. Semi-quantitative estimates of the level of resistance can be determined by measuring the % egg-hatch at varying concentrations of thiabendazole. A field method for selecting test animals with
low egg-counts, and an in vitro method for the culture of eggs of first-stage larvae to third stage for identification are described.

IBAR/1981 BHOOP SINGH, D.P.
49 BANERJEE and O.P. GAUTAM
Comparative efficacy of Diminazene Diaceturate and Imidocarb Dipropionate Against Babesia equi Infection in Donkeys

Veterinary Parasitology, 7 (1980) 173–179

AUTHORS’ SUMMARY: Diminazene diaceturate (Berenil, Hoechst) at 12 mg/kg intramuscularly (i/m) and repeated after 24 hours controlled the rising parasitaemia of Babesia equi infection in four out of five splenectomised donkeys. The drug was more effective in the early stages of the disease and had a prophylactic effect for at least 30–35 days.

A new babesicide, imidocarb (Imizol, Burroughs Wellcome), was 100% effective in three splenectomised donkeys at 5 mg/kg, i/m and repeated after 48 hours. However, imidocarb at 5 or 2 mg/kg i/m with a single injection was only partially effective or ineffective.

Influence of the Nature of the Excipient on the Elimination of the Antibiotics Administered Intramammary.


AUTHORS’ SUMMARY: The authors carried out a study on the influence of the excipient on the elimination of cloxacillin and neomycin residues in the milk of cows treated by intramammary route.

In total, nine different types of excipients were tested, allowing classification into three groups according to elimination times of the active principles. In the case of administration during the lactation period, the first group, including oily excipients whose composition is given, obtained a withdrawal time compatible with the economic requirements of the dairy industry.

Evaluation of Oxfendazole Against Natural Infections of Gastrointestinal Nematodes and Cestodes in Egyptian Camels.


AUTHORS’ SUMMARY: A single oral administration of oxfendazole liquid suspension at 4.5 mg/kg to 15 adult camels naturally infected with worms of the species Haemonchus, Oster tagia, Trichostronglus, Nematodirus, Cooperia, Bunostomum, Chabertia, Oesophagostomum, Trichuris and Moniezia resulted in reductions of faecal egg counts of 82 to 99% within three days of treatment and 78 to 98% by the tenth day after treatment. The failure of hatching of eggs found in the faeces of treated animals demonstrated the ovicidal properties of oxfendazole.

IBAR/1981 PULLANI, N.B.
52 Productivity of White Fulani Cattle on the Jos Plateau, Nigeria. III. Disease and Management Factors.


AUTHOR’S SUMMARY: The importance of disease and management factors in relation to the productivity of traditionally managed White Fulani cattle herds on the Jos plateau is described. Disease per se was not considered to be a major factor limiting productivity with the reduction in importance of the major epizootic diseases of rinderpest and contagious bovine pleuropneumonia. Diseases which were considered of some importance were streptothricosis and liver fluke in adult cattle and cocidiosis and possibly parasitic gastroenteritis in younger animals.

One of the management factors limiting productivity was the keeping of older castrates
mostly in the Sulani-owned herds. The most important management practice affecting the productivity of the herds was the provision of dry season supplementation. Only one herd received substantial amounts and its productivity was much greater than that of the other study herds.

IBAR/1981 MISRA, R.K., RAINA, B. L. and BHAT, P.N.
Studies on the Genetic and Non-genetic Factors Affecting Inter-calving Period in Pure-bred Indigenous Cattle and Their Crosses with Friesian.


Authors’ Summary: Data on reproduction traits of 1364 individuals belonging to Sahiwal, Red-Sindhi, Gir, Tharparkar and Hariana breeds and their crosses with Friesian were analysed. The effects of genetic and non-genetic factors on inter-calving period were studied. The period of calving was found to affect significantly the inter-calving period in cross-breds, but not in the indigenous breeds. Service period significantly affected the trait in the indigenous breeds as well as in crossbreds. Preceding dry period had no effect. The magnitude of heritability estimates varied from very low to not significant either in the indigenous breeds or their Friesian crosses. The results indicated that this trait is largely controlled by non-genetic factors and can be improved to a large extent by controlling environmental factors.

IBAR/1981 ROEDER, P.L.
Effects of Copper and Cobalt Treatment of Cattle in the Ethiopian Rift Valley.


Authors’ Summary: The effects of treatment of young Borana-type zebu cattle with copper and cyanocobalamin by injection are described. Cattle receiving copper gained a mean of 9.18 kg more than cobalt treated and control cattle over a 23-week period of the dry season. Anaemia was not present initially nor in control animals and blood parameters, with the exception of serum copper content, were low initially in all cattle and, although increased in the copper treatment group, remained below normal levels.

IBAR/1981 EDELSTEN, R.M.
55 Chronic Copper Poisoning of Sheep in Nigeria


Author’s Summary: An outbreak of chronic copper poisoning in sheep is described. At least 145 out of 687 sheep (21.1%) on a government farm died during a period of 2 months. The clinical and post-mortem picture was one of sudden, profound intravascular haemolysis and subsequent jaundice. Mortality approached 100%. Plasma copper levels ranged from 22 to 76μmol/litre. There was no obvious source of exogenous copper. Possible causes of the elevated copper levels are discussed.

IBAR/1981 N. B. WILLIAMSON, F.W.
56 QUINTON and G.A. ANDERSON.

The Effect of Variations in the Interval between Calving and First Service of the Reproductive Performance of Normal Dairy Cows

*Australian Veterinary Journal,* Vol. 56, October, 1980 Page 477–480

Authors’ Summary: Reproductive records from 8 commercial dairy herds in a herd health scheme were examined to determine the effect of the calving to first service interval on breeding performance. A reduction in the first service conception rate was observed in cows bred within 60 days of calving. Early breeding did not appear to have any adverse effect on subsequent reproductive performance.

A linear relationship between days to first service and intercalving interval was demonstrated. A 1 day reduction in the interval to first service reduced the intercaving interval b̂ 0.86 of a day. This paper shows that a reduction in calving to service intervals to achieve annual calving produces high economic gains.
IBAR/1981 SERRES, H. and BERTAU-DIERE, L.

Discontinuous Natural Phosphate Rock Intakes in Tropical Cattle Feeding


AUTHORS’ SUMMARY: The authors have studied the effect of natural phosphate rock used as an inexpensive calcium phosphate supplementation despite its fluorine content, in tropical cattle, by using discontinuous supplies so as to allow the elimination of the fluorine component.

Sixty young zebu cattle were part of an experiment carried out in Chad. For 3 years in a row, they were given on a limited period of time, 50 g of natural phosphate per head and per day. Such an intake entails skeletal and tooth lesions because of the fluorine content in phosphate. Tooth lesions are not serious in the majority of cases but they don’t heal. On the other hand, bone and joint lesions can be severe and entail functional diseases such as limping, but after a few month without phosphate, they heal almost completely. During the experiment no animal died of fluorine poisoning and the animals which came through the experiment were very sound. Fluorine titration in the skeleton shows that the amount of fluorine rises sharply when phosphate is given but it decreases between two intakes as it is eliminated up to a rate compatible with the bone normal structure. Meanwhile the fluorine rate remains very low in soft tissue and milk.
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