THE INTERNATIONAL CENTRE OF
INSECT PHYSIOLOGY AND ECOLOGY
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HOST RESISTANCE - INTEGRATED TICK CONTROL.
SEPTEMBER 1983 - AUGUST 1985

1.0. INTRODUCTION.

1.1. This report covers the scientific aspects of the research programme on Host Resistance - Integrated Tick Control during the period 1st September 1983 to 31st August 1985. (Grant No. DAN-4083-G-SS-3063-00).

1.2. Research Trends. During the period under review attention has focussed on the artificial induction of immunity either to reinforce naturally acquired immunity arising from the tick feeding process, or to induce completely new humoral immune responses in the host that will significantly interfere with the tick life cycle. Encouraging progress in the latter area has been achieved with Boophilus sp. in Australia.

Partly as a result of our own field studies and partly because of studies in Australia, the direction of the programme has tended to shift from reinforcing natural immunity to the artificial induction of new humoral immune mechanisms.

The improvement in ECF vaccination procedures has continued to a point where fairly major interventions in tick management regimens can now be envisaged. Some initial collaborative studies in this area of research are described, and future programmes outlined.

It is becoming evident that in areas where vaccination has controlled E.C.F. there is an urgent and increasing need for prophylactic methods to control anaplasmosis, babesiosis, and cowdriosis.
1.3. *ICIPE research trends.* Over the period under review there has been a significant increase in laboratory based staff (see 2.0 below) enabling studies on the isolation of specific immunising antigens to be initiated. These studies have become more directed towards antigens not normally presented to the host during the attachment/feeding period as the review period progressed.

The location of four African Regional Post Graduate Programme (ARPPIS) students within the programme has allowed the programme to complete two studies of significance: laboratory/field strain comparisons of *Rhipicephalus appendiculatus*; and on the effect of host resistance on the toxicity of *Rhipicephalus evertsi*.

2.0. **PERSONNEL:**

2.1. The number of graduates attached to the programme increased during the period from eight to thirteen including ARPPIS graduates. The period was marred by the death of the Programme Leader and Deputy Director Dr. M.P. Cunningham in August 1985.

2.2. The present staff are:

- Dr. P.B. Capstick: Programme Leader
- Dr. R.M. Newson: Senior Research Scientist
- Dr. M.B.A. Nyindo: Senior Research Scientist
- Dr. C.K.A. Mango: Research Scientist
- Dr. J.J. de Castro: Research Scientist
3.0. PROGRESS WITH AGREED OUTPUTS

3.1 Rusinga Island

One Scientific Officer with an assistant has been located at Mbita Point Field Station since February 1985 and has commenced a survey of the ticks present on the island.

The island has been divided into ten areas and 3-5 farms identified in each area for study. Seven of these areas (28 farms) have been csampled to date. On each farm a preliminary questionnaire has been completed detailing the number of people; cattle, sheep and goats, supported by the farm. Simple productivity data has been acquired (See Appendix I). Five adult cattle, five immature cattle, 5 sheep, and 5 goats have been examined and whole body tick collections made from each
animal made and subsequently identified in the laboratory. Preliminary results are attached (Appendix II).

Rainfall data accumulated over 10 years by the meteorological station on the island has been obtained. The farms on the present 10 selected sites will be used as the basis for selection for the next stage of the project.

3.2. Intona Ranch.

Are naturally tick-resistant cattle able to control ticks?

3.2.1. Introduction

Work carried out at the Kenya Agricultural Research Institute in Muguga suggested that when *Rhipicephalus appendiculatus* ticks were allowed to feed exclusively on resistant hosts; tick numbers were depressed to such levels that the population of ticks in the paddock where resistant animals were; almost disappeared. A similar paddock, where only tick-naive cattle were present, showed an almost uncontrolled tick population growth.

An experiment was designed to evaluate these findings in an area enzontic for tick-borne diseases and where several tick species and where alternative wild hosts are present. Intona ranch fulfilled these conditions and, apart from very good collaboration from the owners, a great deal of work has been carried out on ticks and tick-borne diseases in the area.
The ranch is situated on a ridge between 2 rivers. It is triangular in shape, bounded at its base on the Kilgoris-Lolgorian road to the East and on either side by Migori and Mintangwa rivers. It had not been grazed for some years except by game until a group of 62 indigenous cattle were introduced in 1979. The ranch is not fenced and continues to carry large herds of game; especially African buffalo (100), zebra (400), Impala (200), topi (100), waterbuck (50), oribi, bushbuck, etc. The ranch is being developed and has water laid on to high security bomas (stockades), to animal housing and to a plunge dip. It is also equipped with a weighbridge; electricity generator, yards and crushes.

The experiment

1. A paddock of approximately 32 hectares was demarcated and double-fenced with an outer conventional fence reinforced with 3 electrified wire strands and an electrified inner fence which also sub-divides the paddock in 4 sub-units (1; 2; 3 and 4; see diagram). A stockade also sub-divided in 4 independent areas, each with its own water source and crush, was also built in the centre of the paddock where the 4 sub-units converge. No wild animals have been in the paddock since October 1984. (See appendix IV).

2. Boran *Bos indicus* cattle were purchased from an area known to be free of *Rhipicephalus appendiculatus* and East Coast fever. They were shown to be susceptible to *R. appendiculatus* by feeding nymphs on them and after boosting resistance of 10 animals (by application of
Irradiated ticks) 2 groups of 5 cattle were placed in paddocks 1 and 3 and have remained there up to the present time. Susceptible animals have occupied paddocks 2 and 4 and they have been replaced at 3-4 monthly intervals.

3. The ticks present in the paddock are:

- *Rhipicephalus appendiculatus*
- *Rhipicephalus evertsi*
- *Amblyomma variegatum*
- *Amblyomma cohaerens*
- *Boophilus decoloratus*

and the transmission of the following diseases have been confirmed:

- East Coast fever (*Theileria parva parva*)
- Corridor diseases (*T. p. lawrencei*)
- Benign theileriosis (*T. mutans*)
- Gall sickness (*Anaplasma marginale*)
- Redwater (*Babesia bigemina*)
- Heartwater (*Cowdria ruminantium*).

Other diseases present are trypanosomiasis, anthrax, FMD, ephemeral fever, etc. There is a continuous 100% lethal challenge of ECF (Young and de Castro, unpublished).

4. The purpose of the experiment is to demonstrate the
feasibility of tick and ECF resistant cattle to thrive under extremely adverse conditions and assess their impact in the tick populations of the paddocks as compared with the 2 paddocks occupied by tick susceptible animals.

Results up to August 1985

Successful immunizations against theileriosis have been achieved in all the experimental cattle by infection with local isolates of *Theileria parva parva* and *Theileria parva lawrencei* and treatment with either parvaquone or short-acting oxytetracycline. The cattle have survived a lethal theileriosis challenge (confirmed in November 1984 to be 100% lethal for susceptible cattle). Of the other tick-borne diseases present, heartwater was responsible for the deaths of 5 cattle (1 resistant and 4 susceptible) and tick toxicosis killed 3 susceptible cattle.

Statistically significant differences have so far not been recorded in the number of ticks of all species feeding on the cattle and in the vegetation. All 4 paddocks show a progressive increase in numbers of ticks in the absence of wild hosts since no alternative hosts, apart from the experimental cattle, are present in the paddock.

Weight gains of tick-resistant undipped, tick-naive undipped and tick-naive dipped animals are being monitored. All groups have shown similar weight gains during the first 3 months when the total number
of ticks was not higher than approximately 100 adults per animal on both undipped groups. However as the total number of adult ticks rose to 250 per animal, the undipped naive group showed reduced weight gains whereas the undipped resistant group continued gaining weight until the tick challenge reached 600 adults per animal when negative weight gains were observed. Undipped-naive cattle continued to show negative weight gains at all levels of tick challenge.

This experiment has so far demonstrated that successful ECF vaccination can be achieved in a remote ranching situation. In spite of continual mixed tick infestation and artificial (gamma irradiated) tick stimulation of immunity; it has proved impossible to induce protection against a mixed field tick challenge, once a defined tick threshold level has been reached.

Valuable results on weight gains in the three groups relative to tick challenge numbers are being accumulated.

Depending on the breed of cattle used, a degree of tick resistance was induced by a combination of irradiated and natural tick infestations and a significant reduction in the number and weight of nymphs of *R. appendiculatus* was achieved. However, very little cross-resistance appears to exist between *R. appendiculatus* and the other ticks present in the experimental paddock.
3.3. Kuja River Station

The area was visited and a preliminary survey made. Tick collection on 50 native zebu cattle of all ages revealed *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Amblyomma variegatum* and *Boophilus decoloratus*.

Further visits during the rainy period revealed major deficiencies in the site. The principal access roads were impassable and the major part of the proposed area for the study became flooded and impossible to use for grazing. It was concluded that the main objectives of the output were unachievable and the project was abandoned.

3.4 Laboratory experiments on cattle acquired resistance and effect of resistance on feeding ticks

3.4.1. Tick Survival ability from resistant and susceptible hosts

Nymphs fed on susceptible and resistant hosts were allowed to moult and the survival of males and females over a period of approximately 100 weeks was recorded. Adult survival was inversely related to the degree of host resistance. The mean survival time of females was significantly better than that of males if the nymphs from which they moulted had fed on susceptible or poorly resistant hosts, but similar if the hosts were highly resistant. However, the effect on tick reproduction was unlikely to have a significant effect on the field tick population.
3.4.2. Cross resistance between Amblyomma and Rhipicephalus sp

This study was commenced by feeding Amblyomma and Rhipicephalus nymphs on Rhipicephalus resistant cattle and comparing percentage engorging, female fed weights and eggs mass weights. There appeared to be definite evidence that Rhipicephalus resistance also affected Amblyomma female fed weight and egg mass weights. However, the severe drought in 1984 resulted in termination of the cattle controls and results had to be inferred from the subsequent use of rabbit controls, while these results appear to be valid they require reconfirmation in cattle.

3.4.3: Effect of tick resistance on Theileria parva infection

I. parva susceptible Boran cattle were obtained from an area of Kenya where Rhipicephalus appendiculatus is not present. The animals were serologically negative. Immunisation against R. appendiculatus was attempted by test feeds on Days 0, 34; 55, and 114. On days 14 and 75, 1000 and 500 irradiated adults respectively were fed on the ears of each animal (see later for details of irradiation). The fourth test feed on day 114 indicated that although these animals developed resistance, it is much more difficult to induce resistance in Bos indicus than in Bos taurus.

In collaboration with KARI Muguga, the animals were then submitted to challenge with ECF infected nymphs. Unfortunately due to drought and other uncontrollable factors only two resistant and three
susceptible animals were available for challenge. Both resistant cattle failed to contract the disease or to seroconvert. Of the three controls, one died of ECF and the other two failed to contract the disease or to seroconvert: the results can only be described as inconclusive.

4.0 NEW OUIFUS

4.1: Comparison of *Rhizophalus* field and laboratory strains

Previous experiments using the Muguga laboratory strain of *R. appendiculatus* had demonstrated that tick immune cattle placed in paddocks seeded with this strain appeared to control and eventually almost eradicate the tick population compared to paddocks infested with susceptible cattle: A study was commenced in rabbits and cattle to compare the susceptibility of the lab strain (which has been on rabbits for over 30 years) with two recently collected field strains.

Results to date in rabbits indicate that the laboratory strain is superior in its reproductive performance on susceptible rabbits compared to the field strains. When compared on resistant rabbits the laboratory strain showed greatly reduced reproductive ability.

This finding may be highly significant if confirmed in cattle. The lab strain of ticks has been used almost exclusively to assess the development of immunity, both artificial and natural in rabbits and cattle for many years. It is possible that experimental resistance levels have thus been estimated as higher than would be shown by cattle in the field.
4.2. Use of irradiated *R. appendiculatus* to induce tick-resistance in cattle

Preliminary experiments had shown that doses of 1.2 and 2.4 krad of gamma irradiation prevented breeding but did not interfere with tick feeding. Further experiments in rabbits with ticks subjected to 2.4 krad of irradiation showed that irradiated males resulted in total female sterility and no eggs were produced whether the females were irradiated or not. Irradiated females showed normal conversion of engorged weight to eggs but these were subsequently sterile. The engorged weights of irradiated adults was reduced by 33% compared to non irradiated controls.

In subsequent experiments (see 3.4 and 3.2) irradiated adults have been used to induce immunity in Boran cattle.

4.3. Assessment of host resistance to *R. appendiculatus* in the field

The application of 100 nymphs to one ear has been used to assess immunity in the field. The test has been further quantified by assessing the differences between tests on both ears. No significant differences between ears was demonstrated.

4.4. Assessment of host resistance by intradermal test

Following initial studies by Binta M.G. and Cunningham M.P. in which an extract of larval *Rhipicephalus appendiculatus* was used, further studies were carried out with larval extract, salivary gland
extract and extract of sonicated tissue culture cells of a cell line derived from embryonated *Rhipicephalus appendiculatus* eggs.

Each extract was titrated in 10 fold dilutions in both exposed and non exposed cattle. Both larval extract and tissue culture extract in exposed cattle reacted to a dilution of $10^{-2.5}$ per 0.1 ml inoculated. There was no reaction to undiluted extracts in non-exposed (naive) animals. Extract of salivary gland reacted to the same dilution ($10^{-2.5}$ approx.) in both naive and immune animals.

This reaction could form the basis of a most useful field test to discriminate between tick naive and immune animals.

4.5. Inducement of artificial host immunity with selected antigens

4.5.1. Rabbits: Groups of rabbits were immunised with the following antigens

a) Crude midgut homogenate

b) Fraction I protein of midgut fractionated through Sephadex G-100.

The rabbits were challenged with 35 female and 35 male ticks, 100 nymphs and 200 larvae. In the Crude Midgut homogenate group there were reductions in the mean fed adult weight (27%), mean engorged nymphs weight (12%) and neon larval fed weight (6.4%). Egg production in vaccinated rabbits was reduced by 31% and hatchability by 11%. Adult and larval mortalities were increased by 12% and 7% respectively.
In rabbits immunised with Fraction I midgut protein; egg production was reduced by 20% and hatchability decreased from 75% to 49%. Mortality was significantly increased from 7.1% to 40.4% in adult females; from 1.5% to 61.5% in nymphs; and from 9.4% to 69% in larvae.

On the basis of these results further studies have commenced in rabbits using the following antigens:

a) Aqueous extract of midgut (fed for 5 days)

b) Solubilised midgut membrane extract

c) Insoluble midgut membrane

d) A series of 12 fractions of different molecular weights derived from aqueous extracts of 5 day fed midgut.

Group e), b), and c) will be challenged in the near future; meanwhile double immunodiffusion tests show the development of four or more antibodies in some groups.

Group d) antigens are now ready for immunisation or ravolts.

A5:2. Tissue culture and vitellin antigens

Antigens prepared by sonicating a *Rhipicephalus appendiculatus* embryonic tissue culture cell line were used to immunise rabbits. Although antiserum from immunised rabbits has been shown to react in the double diffusion test with adult fed female tick extract; egg extract; midgut and tissue culture cell extract; there was no evidence of adverse biological activity on larvae, nymphs and adults when rabbits were challenged.
Tick vitellin isolated from eggs was similarly used to immunise rabbits without significant biological effects when challenged.

4.5.3: Goats

Groups of goats have been immunised by natural exposure to ticks; crude midgut homogenate; and by a combination of both.

These goats will be subjected to 100 larvae; 50 nymphs and 35 adult female and 35 adult male ear challenge in the near future. They will then be released onto clean pastures seeded with *Rhipicephalus appendiculatus* and the degree of immunity and its effect on whole body tick counts and pasture tick population assessed.

4.5.4: Sheep

A study in sheep has commenced to assess the immune response following vaccination with the following *Rhipicephalus* antigens.

- **Group I**: Soluble midgut antigens in PBS
- **Group II**: Solubilised midgut membrane antigens plus membranes
- **Group III**: Haemocytes washed in PBS and sonicated
- **Group IV**: Haemocytes plus Group II antigens.

4.5.5: Cattle

Work has commenced on the collection of midgut from ticks fed on cattle. Following assessment of the results of 4.5.1, suitable antigens will be used to vaccinate susceptible cattle for subsequent challenge and isolation of biologically significant antigens.
5.0. FUTURE PLANS FOR THE REST 12 MONTHS

5.1. Rusinga Island

5.1.1. The current survey will be completed in approx. 3 months. Ten farms will then be used to carry out a year long survey of the effect of climate variation on host tick populations.

5.1.2. A disease survey of approx. 500 cattle, sheep and goats on the island will be carried out to detect I. *parva* and I. *mutans* infection/carrier rates. Animals will be tagged, aged and the farms used as a nucleus for further selection for entry into a vaccination-productivity-upgrading scheme.

5.1.3. *Hippoceltes appendiculatus* ticks will be collected to produce stabilates for subsequent ECF vaccination of upgraded immature stock.

5.1.4. A tsetse fly survey of the Island will be carried out: The neighbouring island (MFANGAND) is heavily infested.

5.1.5. Identification of sociological and animal production characteristics for permanent recording of the effects of improved genetic potential will be undertaken.

5.1.6. Further detail of farmer selection, pasture improvement, animal gene input, nutritional requirements, method of introducing new potential (e.g. artificial insemination, bull camps) will be clarified.
5.2. **Intona Ranch**

5.2.1. Continue present experiment for at least 12 months.

5.2.2. Introduce a further breed of cattle to the expt.; possibly Masai to compare performance.

5.2.3. Assess the naturally acquired resistance to other tick species in the paddocks.

5.2.4. Introduce new animals immune to non feeding antigens when these become available.

5.3. **Assessment of host cattle resistance**

5.3.1. **Tick challenge** - The 100 nymphal test will be expanded to include larvae and adults as the effects of resistance on both these stadia will be needed to assess the efficiency of artificially induced ranch antigen resistance.

5.3.2. **Intradermal test** - Tests to correlate the development of a humoral immune response with exposure to feeding ticks will commence if truly tick naive animals can be found.

The effect of the test itself on humoral immune response will be assessed by Eliza, double diffusion; and haemagglutination techniques. It would be counter productive if a test to demonstrate tick naivete converted such animals to the equivalent of tick exposed animals.

5.3.3. **In vitro feeding**. Studies have shown that *Boophilus* sp. can be induced to feed artificially on bovine blood in an in vitro
situation through artificial membranes. Devices to study possible *Babesia* sp feeding through similar devices will be initiated. If successful the technique should enable the degree of host humoral resistance to be assessed without inducing further changes in the resistance status such as occur with direct tick feeding assessments.

5.4: **Laboratory studies of host resistance**

The studies to isolate and characterise antigens from different tick organs will continue. Depending upon the results of crude antigen preparations already in progress; further potentially lethal targets will be included in future immunisation experiments in all host species.

6.0: **PUBLICATIONS**

The following publications are relevant to the report:

6.1: **Published**


6.1.3. Chiera, J.W.; Cumulative effects of host resistance on
Rhipicephalus appendiculatus in the laboratory.
Proceedings of Parasitology (1985) 90, 401-408.

6.1.4. Punyuaj, D.K.; Newson; R.W.; and Mutinda, M.J., Diurnal
and seasonal activity of unfed adult Rhipicephalus
appendiculatus in relation to some intrinsic and extrinsic

6.1.5. Newson, R.W.; Chiera; J.W.; Young, A.S.; Dolan; T.I.;
Cunningham; M.P.; and Radley; D.E.; Survival of
Rhipicephalus appendiculatus and persistence of Theileria
parva in the field. Int. Jour. Parasitol. 14(5) 483-489
1984:

6.1.6. Punyuaj, D.K.; Development period of Rhipicephalus
appendiculatus under field conditions. Insect Sci.

6.1.7. de Castro; J.J.; Cunningham; M.P.; Dolan; T.I.;
Dransfield; R.D.; Newson; R.W.; and Young, A.S.; Effects
of cattle of artificial infestations with the tick
Rhipicephalus appendiculatus. Parasitology. (1985) 90,
21-33:
PRELIMINARY PRODUCTIVITY DATA FROM 28 FARMS

RUSINGA ISLAND

(Questionnaire elicited)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MILK YIELD (ml)</th>
<th>PROGENY PER ANIMAL</th>
<th>BREEDING FREQUENCY (YRS)</th>
<th>TIME TO MATURITY (YRS)</th>
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<tr>
<td>CATTLE</td>
<td>718</td>
<td>8.1 ± 2.5</td>
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<td>GOATS</td>
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<td>9.5 ± 2.3</td>
<td>1.5</td>
<td>1.5 ± 0.6</td>
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D.K. FUNYUA
31 AUGUST 1985.
## APPENDIX II

### NUMBER AND SPECIES OF TICKS FOUND ON

#### 28 FARMS ON RUSINGA ISLAND

<table>
<thead>
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<tr>
<td></td>
<td>Total (mean)</td>
<td>Total (mean)</td>
<td>Total (mean)</td>
<td>Total (mean)</td>
<td>Total (mean)</td>
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</tbody>
</table>

| PHIPICHELUS | Adults | 6082 (71.6) | 2698 (37.5) | 760 (15.2) | 1072 (8.7) |
|            | Nymphs  | 367 (4.3)   | 152 (2.1)   | 23 (0.5)   | 260 (2.1)  |
|            | Larvae  | 22 (0.3)    | 6 (0.1)     | 0 (0.0)    | 8 (0.1)    |

| APPENDICULATUS | Adults | 571 (6.7) | 343 (4.8) | 394 (7.9) | 665 (5.4) |
|                | Nymphs | 13 (0.2)  | 9 (0.1)   | 113 (2.3) | 1005 (8.1) |
|                | Larvae | 11 (0.1)  | 1 (0.0)   | 482 (9.6) | 1480 (11.9) |

| PHIPICHELUS | Adults | 2959 (34.8) | 1612 (22.4) | 101 (2.0) | 81 (0.6) |
|            | Nymphs | 1858 (21.9) | 750 (10.4)  | 177 (3.5) | 634 (5.1) |
|            | Larvae | 729 (8.6)   | 431 (6.0)   | 7 (0.1)   | 341 (2.8) |

| ANBLYOMMA | Adults | 115 (1.4) | 70 (1.0) | Nil | 4 (0.0) |
|          | Nymphs | 48 (0.6)  | 34 (0.5) | 2 (0.04) | 3 (0.02) |
|          | Larvae | 12 (0.1)  | 3 (0.04) | Nil | Nil |

| BOOPHILUS | Adults | 2 (0.02) | 1 (0.01) | Nil | Nil |
|          | A. GEMMA | Adults | Nil | 1 (0.01) | Nil |

| R. FULCHELLUS | Adults | 1 (0.01) | Nil | Nil |
| HYALOMMA RUJIPES | Adults | 1 (0.01) | Nil | Nil |

[ ] = SAMPLE SIZE

() = MEAN NO. TICKS PER ANIMAL

D.K: PUNYUA 31 AUGUST 1985
LEGEND
1 and 3 resistant paddocks
2 and 4 susceptible paddocks
5 Stockade
6 Tent
7 Ecology arena
8 Crush
9 Inner fence and sub-divisions
10 Outer fence
11 Gate