TITLE   .

STUDIES OF BIOLOGICAL DIVERSITY OF THE TICK <u>RHIPICEPHALUS</u>

<u>APPENDICULATUS</u> (ACARINA: IXODIDAE) IN RELATIONSHIP

TO TRANSMISSION OF <u>THEILERIA</u> <u>PARVA</u> (APICOMPLEXA:
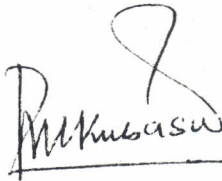
THEILERIDAE) IN KENYA

BY

SAMMY SHIMENGA KUBASU

A thesis submitted in part fulfilment for the Degree of

Master of Science (Entomology) in the University

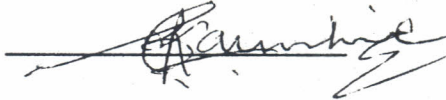of Nairobi.

1990

DECLARATION. -

I, SAMMY SHIMENGA KUBASU, hereby declare that this thesis is my original work and has not been presented for a degree in any other university.
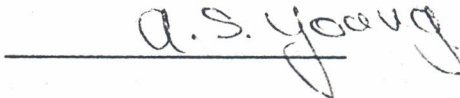
Sammy Shimenga Kubasu.

This thesis has been submitted for examination with our approval as university supervisors.

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ACKNOWLEDGEMENTS .

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ABSTRACT   .

This study was undertaken to examine whether different
populations of Rhipicephalus appendiculatus,the African brown ear
tick, found inhabiting different geographically isolated zones in
Kenya are biologically different to such an extent that they may be
assigned subspecies status.

The biological characteristics investigated included size of
unfed females, engorgement weights of larvae, nymphs and females, egg
production, duration of moulting of larvae and nymphs, isoenzyme
studies and comparative transmission of Theileria parva stocks by R.
appendiculatus. Ticks were collected from five geographically
isolated zones in Kenya for investigation. The work was carried out
to provide epidemiological information for East Coast fever (ECF)
control.

In this three-host tick species, some significant differences
in weights of engorged nymphs and females, size of unfed females,
weights of eggs per tick and moulting duration were noted between the
different tick stocks. In the case of engorged females, statistical
differences were noted between Kilifi (FS1) and Kiambu (FS2); Kilifi
and South Nyanza (FS4); and, Uasin Gishu (FS3) and Muguga laboratory
(MLS) tick populations.

Significant differences in size of unfed females were noted
between FS2 and FS3; and, FS2 and MLS. In the case of the mean weight
of eggs, significant differences were shown between FS1 and MLS only.

Significant differences in moulting duration was also noted in larvae but not in nymphs. In the former, statistical differences were recorded between FS2 and FS3; FS3 and FS4; FS1 and FS3; and, FS2 and MLS.

Out of the eleven enzymes examined, ten showed polymorphism in most of the five tick populations while only one,i.e. peptidase 7, was monomorphic.Four enzymes (peptidase 1, PEP 2,malate dehydrogenase and glucosephosphate isomerase) showed differences in the tick populations. This confirms further that the tick populations, under investigation do differ.

Differences were also noted in the five populations as regards to their efficiency to become infected with Theileria parva parasites. It was concluded that R. appendiculatus found in different geographical zones show differences in some important biological characteristics. These features that showed differences in the tick populations might be important in the control of the vector and also might have an influence on ECF transmission.

CHAPTER 1

INTRODUCTION

Rhipicephalus appendiculatus Neumann 1901, also known as the African brown ear tick, belongs to the order Acarina and family Ixodidae, which represents a group of ticks commonly known as hard ticks. It has three different instars during its life cycle, i.e. larvae, nymphs and adults of both sexes, and each instar feeds on separate host, hence the tick is referred to as a three host tick. Ticks are ectoparasites, sucking blood and other tissue fluids over a period of several days of attachment to the host before they drop engorged to the ground to continue their developmental cycle (Arthur, 1962; Halpin, 1975). Although the main host of R. appendiculatus is cattle, they also commonly infest sheep, goats, camels and many wild bovids especially the African buffalo, Syncerus caffer, and the eland, Taurotragus oryx (Walker, 1974). Birds, rodents, hares and hedgehogs may act as intermediate or incidental hosts and help in maintaining and disseminating ticks (Lewis, 1939, Theiler, 1959, Karra et al.; 1963; Sutherst et al., 1978).

R. appendiculatus is found inhabiting a variety of altitudes in Kenya. It is found most frequently in areas which fall between 1200 metres and 2100 metres above sea level, with an adequate vegetation cover and a well distributed rainfall of about 635mm per year or more. However, it is often capable of living in places which do not

come into this category, i.e. in areas with lower annual rainfall, provided the dry season is not too long, or at higher or lower altitudes than given above (Walker, 1974). It is not found in forested areas above 2300 metres as the tick cannot withstand prolonged low temperatures. In Kenya, the tick may be found along the coastal strip and Taita Hills in Coast Province due to the moist conditions in these areas. In the Eastern Province, it is found in the wetter parts of Machakos, Kitui, Embu and Meru districts. Elsewhere, it is found in all the districts in Central Province, all the wetter parts of Kajiado, Narok, Kericho, Elgeyo Marakwet, Baringo, West Pokot, Trans-Nzoia and Uasin Gishu Districts in Rift Valley Province. The tick is also found in all parts of Nyanza and most parts of Western Province (Walker,1974; Wishitemi, 1983). The tick therefore occurs in all Kenyan provinces except the dry North Eastern Province. Distribution of the tick in Kenya is shown in Figure 2.

The major importance of the tick R. appendiculatus is the role it plays in the transmission of the haemoprotozoan parasite, Theileria parva Theiler, which causes theileriosis, the most important disease of cattle in East and Central Africa. The tick transmits three forms of the disease theileriosis, caused by the parasite in the regions. These three forms have, for convenience, been described as three subspecies of the parasite (Uilenberg, 1981). The tick transmits T.p. lawrencei from African buffalo (S. caffer) to cattle, causing Corridor disease. A milder form of theileriosis is caused by T.p. bovis, transmitted by the same tick, and the third form of the East Coast fever (ECF),is caused by T.p. parva which appears to be maintained only within cattle population

(Grootenhuis et al., 1987). Miller et al., (1977) reported that half a million cattle die due to ECF per annum in East Africa. Moreover, Robson et al. (1961), working in Tanzania, found that in endemic areas, morbidity due to the disease is 100%, mortality 25-50% and, in fully susceptible bovines, the mortality may rise to 95%. Similar findings have been reported by Duffus (1977). In addition, losses in productivity in recovering cattle, the cost of control, as well as the exclusion of cattle of higher potential productivity from endemic areas are often not considered (Dolan and Young, 1981). Therefore, ECF control merits a high priority for all East and Central African governments because if left unattended to, it is likely to seriously affect the economic development in these regions.

In addition, R. appendiculatus transmits T. taurotragi which may also be involved in cattle theileriosis (Young et al., 1981). The tick also transmits the virus which causes Nairobi sheep disease, and also rickettsia, which causes tick bite fever in man. Moreover, the tick may cause toxicosis and hypersensitivity when present in large numbers and their bites may allow secondary bacterial infection. Furthermore, high tick infestation may also debilitate cattle and also cause considerable damage to the skin directly lowering the quality of hides, hence their value. Of great importance, Duffus (1977) estimated that 52.8% of cattle in Kenya are in areas where R. appendiculatus is present, while Dolan and Young (1981) reported over 80% of cattle in Kenya were in such areas. Recently, Kariuki (1989) reported that in 1985, just over 67% of all cattle in Kenya are covered by the Government tick control programme. In view of this fact, and the economic importance of the tick highlighted above, it

Fig.1:   Proportion of exotic breeds of dairy and beef cattle in

Kenya

Legend:

- 70% AND ABOVE
- 50% — 69%
- 10% — 49%
- 0% — 9%

0   50   100   150   200   250   300 km
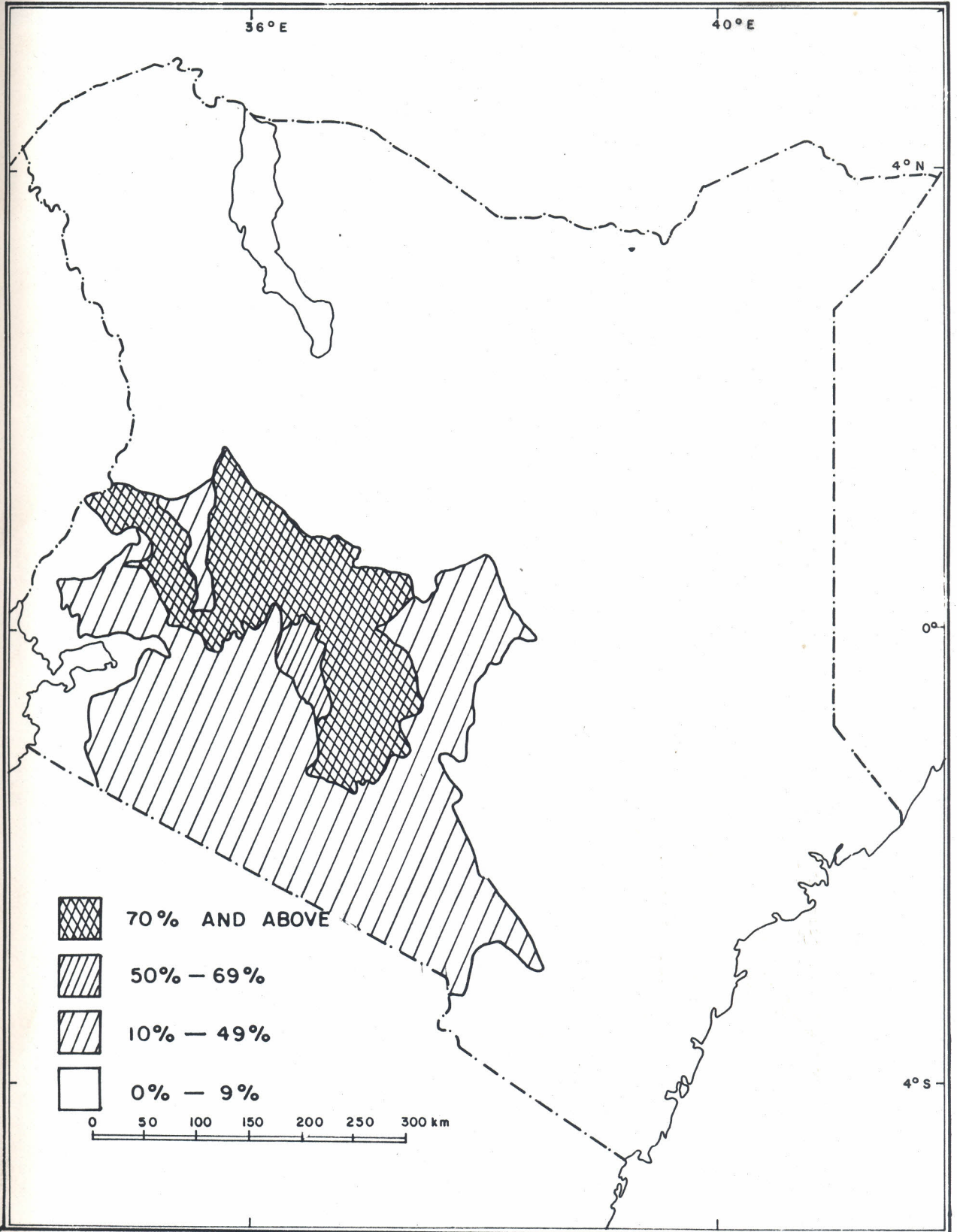
FIG·I· DITRIBUTION OF EXOTIC BREEDS OF DAIRY AND BEEF CATTLE·
(Source: FAO,1975)·

Fig 2:   Distribution of the tick R. appendiculatus in Kenya.

Note that the distribution of this tick concides with

that of exotic breeds of cattle (Fig.1)
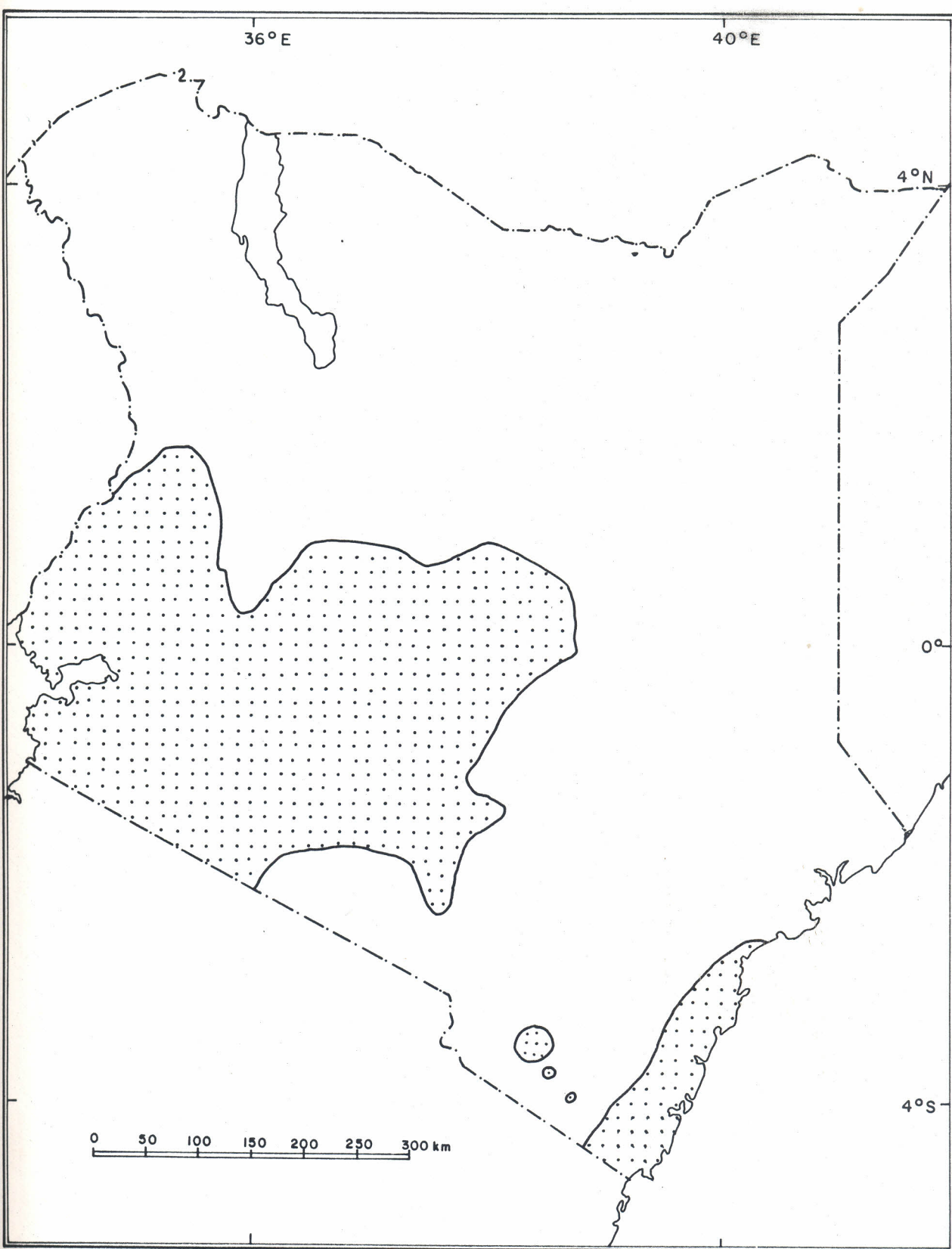
FIG·2: DISTRIBUTION OF R· APPENDICULATUS IN KENYA·
(Source: FAO, 1975)·

Fig 3:   Percentage of cattle serologically positive to
          theileriosis (Source: FAO, 1975)
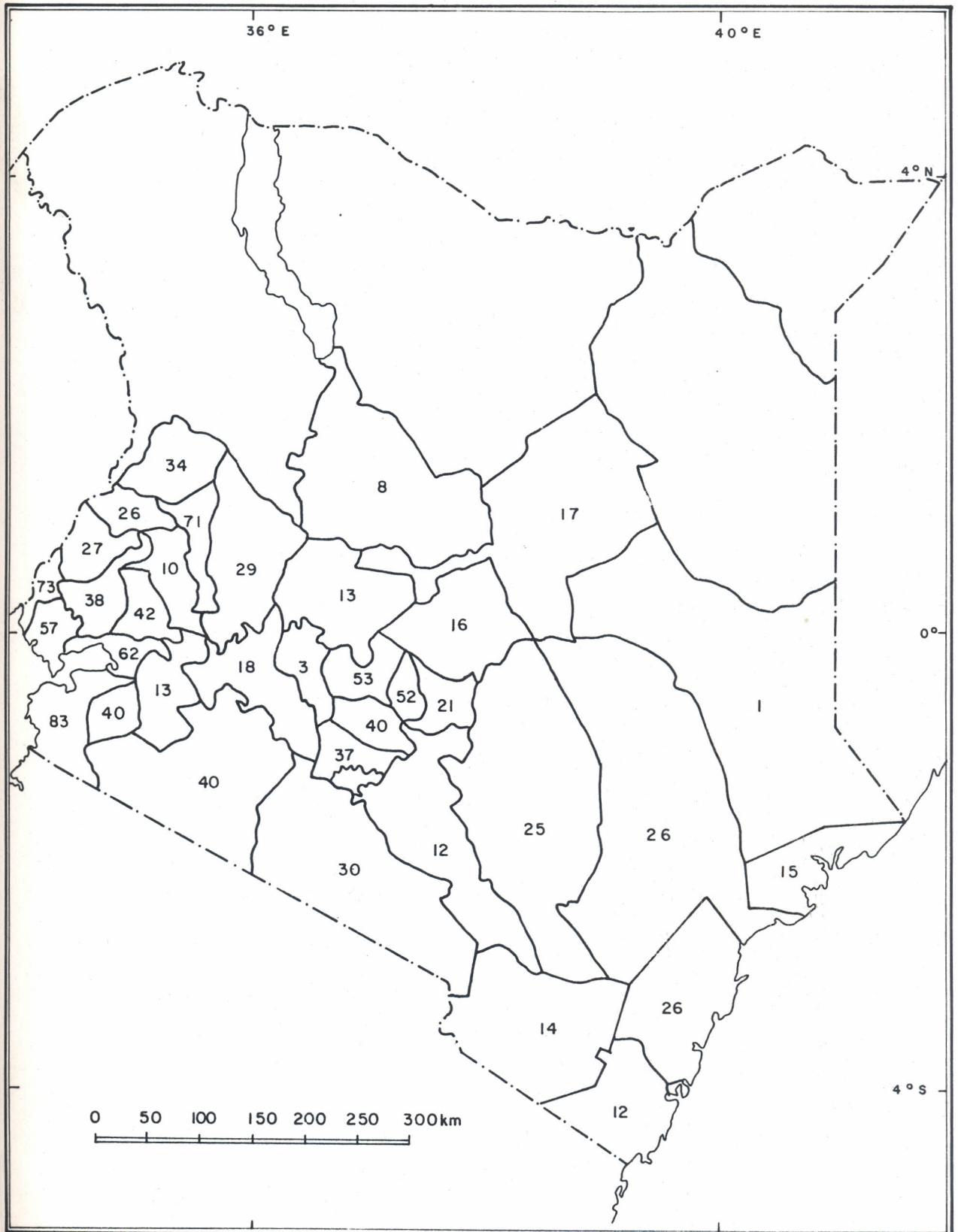
FIG·3: PERCENTAGE OF CATTLE SEROLOGICALLY POSITIVE TO THEILERIOSIS·(Source:FAO,1975)·

is imperative that control of ticks and tick-borne diseases must receive high priority, both in control activities and research, since ticks are widespread in areas of high livestock potential and productivity (see Figures 1,2 and 3).

The current tick and tick-borne disease control measures in Kenya are based on either acaricide application to cattle by dipping or spraying to control ticks, or chemotherapy and vaccination to control the disease although both these have their drawbacks. The methods are costly and their effectiveness is jeopardised by the ticks developing resistance to the acaricides (Wharton and Roulston, 1970; Cunningham, 1981 and Keating, 1983). Chemical acaricides are also toxic to livestock and man and may cause environmental pollution. Another problem is that there may be need to apply these chemicals, up to twice a week in some areas, making it a labour-intensive task, while communal dips can provide a focus for disease transmission. Another factor to be considered is that the costs drug companies incur during developing new acaricides and drugs are continuously increasing and if resistance develops quickly, profit from sales is likely to be less than the cost of development, particularly if the region where these acaricides and drugs can be used is limited. With the inevitability of rising prices, and the uncertainty that new compounds will be forthcoming, the use of chemicals as the sole method of control cannot be supported. This therefore calls for alternative cheaper methods for tick and tick-borne disease control. This could best be realized through better understanding of tick biology and ecology, which may help to improve the efficacy of chemicals.

Alternatives to the use of acaricides which have been considered include sterile male technique, use of the tick's natural parasites and predators and pasture spelling which, as a matter of fact, involves habitat management (Wilkinson, 1957). Use of host resistance to tick infestation has been suggested (Roberts, 1968; Seifert, 1971). This method involves the genetical selection of host populations. The variation of the acquired host resistance to ticks (Roberts, 1968; Seifert, 1971) within and between different host species allows the selection of individuals or host species which have the ability to limit the numbers of ticks which mature on them. For instance, the more resistant Zebu cattle (Bos indicus) and their crosses with European (Bos taurus) cattle provide such an opportunity where ticks and disease cause problems with European cattle (Wharton et al., 1973). This method can be used with some success, for instance, in the control of Boophilus microplus and B. annulatus (Cunningham, 1981). This method might not however be practicable in control of a 3-host tick such as R. appendiculatus because its adults can survive for up to two years or more (Young et al.,1983). Involved in this approach also is clearing of vegetation to control ticks. This method has been used to control the lone star tick, Amblyomma americanum, in the United States of America by Hair and Howell (1970). On the other hand, the sterile male technique and use of parasites and predators have not shown much success in controlling ticks. The current picture of tick ecology suggests little potential for biological control (Cole, 1965) because the fecundities of parasites and predators appear to be far below the level required to respond to the explosive increases in tick numbers which follow certain types of weather. However, introduction and encouragement of

specific predatory birds (e.g. Buphagus spp.) may have a role in increasing the efficacy of integrated control programmes (Sutherst et al., 1978).

Availability of specific drugs and vaccines has been an important alternative in the control of tick-borne diseases. Parvaquone, a curative drug for ECF, which was developed by Wellcome (K) Limited in collaboration with Muguga, is now on sale under the trade name Clexon, though expensive to a small scale-farmer. Further research by Cooper Animal Health and Muguga has identified another analogue which is more effective for ECF treatment, called buparvaquone under the trade name Butalex. A drug from the quinolinone group, halofuginone, is also used for ECF treatment under the name Terit. Furthermore, exciting research carried out at Muguga recently has shown that low oral doses of interferon - a natural substance produced by the body, which is now cheap, can also prevent the development of ECF (Young et al., 1990a). Moreover, a vaccine is being developed by the Kenya Agricultural Research Institute (KARI) Muguga which employs infection of the cattle and treatment, and results have been quite encouraging (Radley, 1978, 1981; Morzaria et al., 1985; Young, et al., 1990b).

The present study of R. appendiculatus was undertaken to obtain further biological information on the relationship between stocks of the tick found in different geographically and ecologically isolated zones. This could be important in designing control measures aimed at the tick vector and the associated tick-borne diseases. It is also hoped that the very sensitive isoelectric focusing technique employed in this study will provide a way by which the tick vector from different areas may be identified and characterized, a factor which

may provide us with epidemiological and taxonomic information about the tick vector.

Objectives of the study

There are two main objectives of the study:-

1. To determine whether biological characteristics of R. appendiculatus from geographically isolated areas in Kenya differ.

2. To determine and compare the ability of R. appendiculatus from geographically isolated areas in Kenya to transmit T. parva.

CHAPTER 2

LITERATURE REVIEW

Development of the tick

During its life cycle, the tick passes through four main developmental stages, i.e. egg, larva, nymph and adult. Sexual differentiation and reproduction occur only in the adult stage. A specific pecularity of some ixodid species and less often of argasids, is the tendency to change from temporary to more continuous parasitism. Blood sucking in larval, nymphal and adult stages results in very complicated life cycles involving regular alternation of free-living and parasitic existence. Tick life cycles are divided into four types on the basis of the number of host changes and moults: multi- host, 3-, 2-, and 1- host (Nuttall, 1911). In ixodid ticks, the number of blood meals during the developmental process has been reduced to three (larval, nymphal and adult feeding); thus the maximum number of hosts required is found in the 3- host development type. Ticks remain on the host only while feeding. This classification of ixodid ticks based on host numbers is quite deficient because it doesn't give a clear, elaborate picture of the biology of the tick. The developmental details of individual ixodid species, therefore, need to be explored in order to understand clearly the biology of the tick.

Studies on the biological characteristics of ticks including duration of feeding, duration of egg hatching and duration of moulting of larvae and nymphs have been scanty, only a few workers having carried out investigations on some of the aspects.

Duration of feeding in some Rhipicephalus spp. has been investigated by various workers. Orago et al., (1983) while using sheep, noted that engorgement of R. appendiculatus nymphs started on day 4 with a peak attained on day 6. The adults started engorging on day 6 and higher engorgement percentages were realized between day 7 and 9. Wellcome Kenya Ltd. (1980) reported that the feeding periods for nymphs and adults of R. appendiculatus were 3-7 days and 5-7 days respectively. From studies by Irvin and Young (1981), it was reported that adult females of R. appendiculatus engorged with blood drop from their host and lay eggs in grass and herbage, which hatch into larvae after about two weeks. The larvae attach and feed on a new host and when engorged in about five days, later fall to the ground and moult into nymphs. These in turn attach to fresh hosts, feed over about seven days and then moult to adults. The female adults according to Newson et al., (1984) complete engorgement in 7-10 days, but these workers recorded shorter feeding duration for nymphs than their counter parts above, i.e. four days. In addition, Young and Leitch (1981) looked at the moulting behaviour of four strains of R. appendiculatus (i.e. Mbita, Ukunda, Muguga paddock and Muguga laboratory) nymphs from Kenya at a temperature of $28^{o}C$ and constant relative humidity of 85%. They noted that the mean times in days post-repletion on rabbits to the completion of the moult were different between the respective strains. This diversity of results on duration of feeding, duration of egg hatching and duration of moulting of R. appendiculatus reported by the different workers would suggest that the generation time of R. appendiculatus is poorly known. The generation time of each tick species is of considerable significance as it affects the tick's population dynamics, a factor,

which if known, would help during vector control. It would be

possible to know for instance, at what stage or stadium acaricidal

application should be done to be cost effective in its control,

according to the tick stock in question.

Like the other biological aspects mentioned above, little

information is known about detailed studies on size of R.

appendiculatus ticks. Although weight has been in the past used as a

convenient method of assessing tick size, Chiera et al., (1985)

recommended use of scutal length as this does not alter even after

engorgement. The authors noted a high degree of correlation of scutal

length of unfed male and female ticks and their engorged body

weights. It would therefore be reasonable to combine both weight of

engorged ticks and scutal length for better comparison of tick

populations. Weight of engorged ticks is important as this is

directly related with egg output by adults. For instance, Randolph

(1979) suggested that low engorgement weight may result in reduced

egg output by adults.

Factors affecting the development of the tick

Various workers have carried out investigations on the factors

which affect the development of ticks. Evidence collected by

Hoogstraal (1956) and Arthur (1962) indicated that the rate of

development of R. appendiculatus would-like other ixodids-be governed

by temperature. This has been confirmed by Punyua (1984). In

addition, Branagan (1973) while looking at the survival and

development of all the three instars of R. appendiculatus noted the

dominance of temperature on the rate of development, i.e. the rate

of development was accelerated by raising the temperature and
retarded by lowering it. The author noted that humidity had no
influence on the rate of development. These findings have been
supported by Tukahirwa (1976). Before then, evidence concerning the
influence of humidity on the development of R. appendiculatus was far
from being clear because, on one hand, Wilson (1944, 1946, 1950)
associated the engorgement and oviposition of females with wet
seasons. When the relative humidity was above 75%, on the other hand,
neither Yeoman (1966a,b) nor McCulloch et al. (1968) made a firm
correlation between high humidities and evidence of accelerated
developement in R. appendiculatus. Arthur (1962) referring to ixodid
ticks in general considered that relative humidity and precipitation
have little or no influence upon the duration of developmental
periods.

Use of isoenzymes in characterization of organisms

Isoenzymes have been defined as enzymatically active proteins,
catalysing the same reactions and occurring in the same species but
differing in certain of their physio-chemical properties (Wilkinson,
1970). A good example of an enzyme composed of isoenzymes is lactate
dehydrogenase of various human and animal tissues. This consists of
several different isoenzymes which can be separated by conventional
starch-gel or acrylamide-gel electrophoresis.

Since the discovery of the heterogenicity of lactate
dehydrogenase, the multiple forms of this enzyme have become the
subject of extensive literature and the techniques introduced have
been applied to investigations of other isoenzymes (Wilkinson, 1970).

Morphological characteristics have traditionally been used by taxonomists in identification and classification of organisms. There are, however, instances where organisms having different behavioural characteristics are morphologically indistinguishable, thus posing epidemiological and taxonomic problems. This led Rausch (1967) to question the recognition of intra specific variants in the past, based primarily on morphological criteria. In such cases, isoenzyme analysis by starch gel electrophoresis has been a most widely used technique (Gibson et al., 1980; Godfrey, 1979).

Isoenzyme analysis has for example been used to characterize some East African Theileria spp. isolates with particular reference to T. parva by Allsopp et al., (1985). By looking at 24 enzymes, the authors distinguished between T. parva and T. taurotragi by twelve enzymes. Five enzymes showed variation within the T. parva population.

Melrose and Brown (1979) reported isoenzyme variation in piroplasms isolated from bovine blood infected with T. annulata and T. parva. This technique has been used as an aid in the classification of Plasmodia, Trypanosoma, Eimeria, Babesia and Entamoeba spp. (Carter and Walliker, 1977) but the method has not been fully evaluated with vector species.

The questions of how much genetic variability occurs in species of ectoparasites and how that variation is distributed among populations have received little attention. For instance, Takeshi and Leonard (1988) while looking at isoenzymes of natural populations of the tsetse Glossina pallidipes from an area free of sleeping sickness and an area where the sickness is endemic in Kenya, found out that out of the 12 enzymes they examined, two enzymes showed high

polymorphism in the two populations, while the other 10 enzymes were all monomorphic. Furthermore, Van Etten (1982) had analysed eight populations of G. pallidipes in Kenya using starch gel electrophoresis and found that three of the 11 enzymes examined showed polymorphism with significant heterogeneity of allelic composition among the populations. Little work is known to have been done on ticks in general and R. appendiculatus in particular. One notable work was by Hilburn and Sattler (1986). These workers employed electrophoresis technique to detect protein variation in natural populations of the lone star tick Amblyomma americanum. The authors investigated nine populations for variation of 21 enzymes. Only three enzymes were found not to be polymorphic. In another investigation by Sattler et al., (1986), four natural populations and four laboratory colonies of Boophilus microplus and a laboratory colony of B. annulatus were subjected to electrophoretic analysis to determine levels of genetic variability. Results showed that populations of B. microplus exhibited high genetic similarity, indicating that all populations share a relatively undifferentiated gene pool. The two contradictory results in the investigations above are a good indicator that there is little basis upon which to predict expected levels of genetic variation between populations, due to limited studies on ectoparasites using electrophoretic technique. This therefore calls for much more work so that more information can be made available before firm conclusions can be drawn from the results of related investigations.

Transmission of T. parva and epidemiology of ECF

The tick R. appendiculatus has been proven to be the only
vector for all forms of T. parva in the field, although other tick
species have been shown to transmit the parasite experimentally
(Lawrence et al., 1983). The role of the tick R. appendiculatus as
the vector of T. parva begins when larvae or nymphs get infected by
feeding on infected cattle. They transmit the parasite only in the
next instar, transstadially. The seriousness involved in this
transmission is that a single tick with only one salivary gland
acinus infected is capable of causing the death of a cow (Lewis,
1950). It therefore becomes increasingly important to understand the
biology of the tick, so as to gain information that could be used in
ECF control measures.

The life cycle of T. parva involves development of the parasite
in both the tick vector and cattle host. Considerable work has been
done on the development of T. parva in both the tick and cattle.
Under normal conditions, the maturation of T. parva to form
sporozoites is induced by feeding of the infected ticks on a
mammalian host (Martin et al., 1964; Purnell et al., 1973). The
parasite undergoes a sexual stage in the gut of the tick and enter
the salivary glands where they form an elaborate intracellular
sporoblast. There have been several laboratory studies on the
survival and development of T. parva in the tick (Lewis and
Fotheringham, 1941; Lewis, 1950; Martin et al., 1964). Young et al.,
(1983) and Newson et al.,(1984) have carried out such work under
field conditions at high altitude (2100m) at Muguga, Kenya, using
locally isolated stocks of T. p. parva in adult R. appendiculatus.

During tick feeding, the sporoblast gives rise to 30,000 to 50,000 infective sporozoites. These are injected into cattle in tick saliva. In cattle they attach to and enter lymphocytes, the white blood cells of the immune system. Within the lymphocytes, the sporozoites develop into schizonts, and the first parasite stage seen is the macroschizont. This is the pathogenic stage of the parasite and the infected lymphocytes are transformed into enlarged lymphoblastoid cells which multiply together with the macroschizonts, resulting in a rapidly expanding population of parasitized cells. The final stage of disease is characterized by large-scale cell destruction, often leading to death (ILRAD, 1987; Fawcett et al., 1985). Microschizonts develop from macroschizonts, these produce micro-merozoites which invade erythrocytes to form piroplasms which are then available to infect feeding tick.

Apart from feeding on mammalian host, current research has shown that other factors can also induce T. parva maturation. Samish (1977) demonstrated that a temperature of $37^{o}C$ for several days, probably coupled with a high relative humidity, stimulated the non-infective particles of T. annulata in the salivary glands of unfed Hyalomma ticks to become infective without prior stimulation of feeding. Similar findings have been recorded for T. parva (Young et al., 1979). The authors in their studies have demonstrated that 2 strains of T. parva i.e T. parva Kiambu 5 and T. parva Muguga, in adult ticks maintained at $37^{o}C$ for 6 days or more could mature to infective stage in the salivary glands without feeding on a mammalian host. Work by Irvin et al., (1981) has also shown that the maturation of T. parva in unfed R. appendiculatus incubated at $37^{o}C$ is possible. Ochanda et al., (1988) have also demonstrated the same.

It is not easy to eradicate theileriosis, unless its epidemiology is clearly understood. There have been few such studies in East africa. Studies of Yeoman (1966a, b) and McCulloch et al., (1968) investigated the cause of the large epidemics of ECF in Sukumaland, Tanzania. However, these workers concentrated on the population of the tick vector R. appendiculatus. The Food and Agriculture Organisation (FAO, 1975) undertook an enormous survey of the tick populations and T. parva antibodies from cattle in Kenya. Different disease prevalence was noted in different endemic areas (see Figure 3). Blouin and Stoltsz (1989) carried out studies on comparative infection rates of T.p. lawrencei in salivary glands of R. appendiculatus and R. zambeziensis. Results from their study revealed significantly higher infection rates in the salivary glands of R. zambeziensis than in R. appendiculatus. Despite these studies, no work is known to have been carried out to look into the biological characteristics of R. appendiculatus from geographically separate areas in Kenya, to establish whether the differences in epidemiology in different areas may be related to the tick strain. This formed one of the main objectives of the current study.

CHAPTER 3

MATERIALS AND METHODS

Description of study areas

The study was carried out in the following geographically and
ecologically isolated areas in Kenya:

(a)   Kilifi

The district stretches from coastline to 400m in altitude.It
records a minimum annual temperature of 22.8°C and a maximum annual
temperature of 30.4°C. The average annual rainfall ranges from
500mm-1100mm. The mean relative humidity is 72%. Vegetation cover is
bushland and thicket and some grassland with scattered trees, and dry
bush with trees interrupted by coconut, cassava and cashewnut crops.
Ticks collected from this area were designated field stock 1 (FS1).

(b)   Kiambu

The district stretches from 1000-3000m above sea level.It
records a minimum annual temperature of 12.8°C and a maximum
temperature of 23.5°C. Its mean annual rainfall ranges between
600-2000mm. The mean relative humidity is 68%. Vegetation cover is
mainly forests and grassland. Tall grass and scattered trees may
occur in some areas.
Ticks collected from this area were designated FS2.

(c)   Uasin Gishu

The district is formed mainly by highland plateau which is
between 2000-3000m above sea level. Mean annual minimum and maximum
temperatures are 17.5°C and 26°C respectively. Mean annual
rainfall is between 500-1500mm. The mean relative humidity is 59%.
Vegetation is mainly mountain forests and grassland.
Ticks collected from this area were designated FS3.

Fig. 4:   Map of Kenya showing districts studied .

STUDY AREA
1 UASIN GISHU
2 SOUTH NYANZA
3 KIAMBU
4 KILIFI

0 50 100 150 200 250 300 km
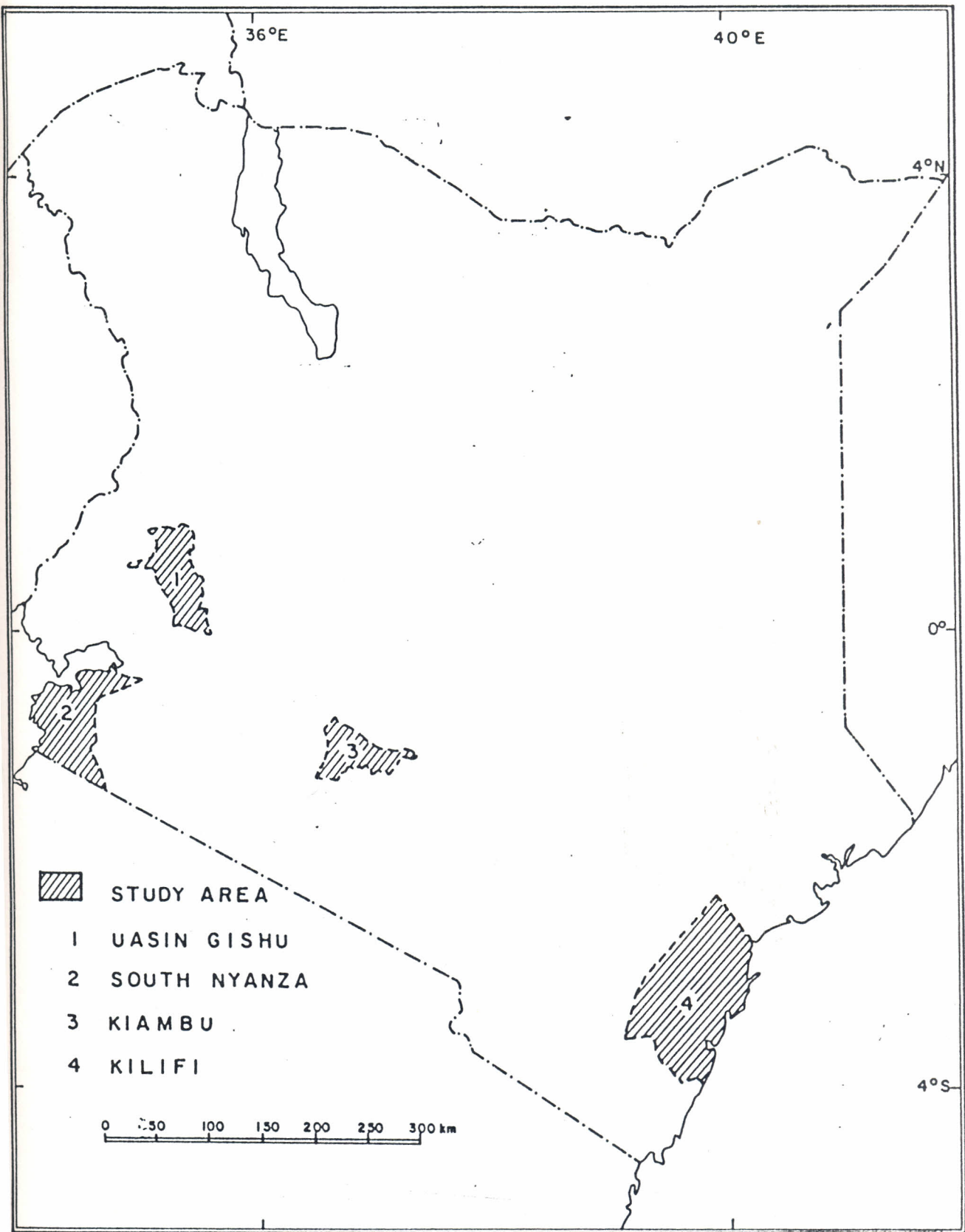
FIG· 4· MAP OF KENYA SHOWING STUDY AREAS·

(d)   South Nyanza

Stretches from L. Victoria, southwards. It lies between 1000-4000m above sea level. Mean annual rainfall is between 500-2000mm.   Temperature ranges between 18.2^{o}C and 31.2^{o}C. The mean relative humidity is 75%. Vegetation is mainly savanna and wooded savanna. Ticks collected from this area were designated FS4.

Figure 4 shows areas from where R. appendiculatus was collected,whereas Table 1 gives a summary of geographical conditions of the areas.

Together with ticks collected from the above regions, R. appendiculatus stock that has been kept on rabbits by Bailey's (1960) method, at NVRC laboratory, Muguga, since 1952, herein designated Muguga Laboratory stock (MSL), was used in the study.

Collection of ticks from the field

R. appendiculatus used in the experiments were collected from the four named geographical zones in Kenya. The ticks were from collections of partially engorged or engorged females hand-picked directly from cattle from the four natural populations in Kenya. The ticks were kept in glass sample bottles (7.5 x 3.5cm) with perforated lids and submerged in moist sand, then kept in a cool box and transported to National Veterinary Research Centre Muguga, where together with the Muguga laboratory stock, colonies were raised to be used in the experiments. Rearing was done on rabbits (Bailey, 1960) in the laboratory for one generation and cohorts from the resultant ticks were used. The tick colonies were established from a group of 120 engorged females from each stock.

Table 1. <u>A summmary of geographical conditions of the study</u>

<u>areas</u>

Area	Altitude (m)	Annual temperature			Mean annual rainfall range (mm)	Mean annual R.H.(%)
		Min. ($^\circ$c)	Max. ($^\circ$c)	Mean ($^\circ$c)		
Kilifi	0- 400	22.8	30.4	26.6	500-1100	72
Kiambu	1000-3000	12.8	23.5	18.2	600-2000	68
U.Gishu	2000-3000	17.5	26.0	21.8	500-1500	59
S.Nyanza	1000-4000	18.2	31.2	24.7	500-2000	75

Laboratory maintenance of the tick colonies and storage for
experimental purposes

Adult ticks were kept in 80x25mm flat bottomed glass tubes.
Each engorged female was placed in one glass tube on a piece of
absorbent paper or filter paper on which to lay eggs. This was to
prevent the eggs from sticking to the glass.Batches of 200 engorged
larvae were contained within 40 x 10mm flat bottomed glass tubes,
and batches of 100 engorged nymphs within similar 80 x 25mm tubes.
In all cases, inside each tube was placed a strip of paper, on which
pencilled details were clearly recorded to show date of incubation,
tick stock, developmental stage and sex (where appropriate). The
tubes were plugged with gauze - covered cotton wool and kept in a
dark incubator at approximately 28°C and relative humidity (R.H.)
of approximately 85% for engorged females to lay eggs and the laid
eggs to hatch and the resultant larvae and nymphs to moult to nymphs
and adults respectively. Unfed forms were stored in an illuminated
cooled incubator run at approximately 18°C and 85% R.H., to be used
in later experiments. The laboratory tick cultures were maintained at
constant temperatures by the use of two types of incubators
thermostatically controlled (Models B&T cooled and Gallenkamp
illuminated cooled). For constant humidity, the cultures were kept
above saturated solutions in sealed desiccators, according to methods
described by Winston and Bates (1960) and Solomon (1956). A Lovibond
1000 Comparator Kit was used to measure relative humidities and
thermometers were used to measure the temperatures.

Rabbits for tick rearing

The rabbits used were naive male adult New Zealand whites (Oryctolagus cuniculus Linnaeus) from the Small Animal Unit at NVRC and supplemented at times of shortages by the same breed purchased from Njoro and Molo Farms in Nakuru. Prior to using the rabbits, thorough examination was done to ensure that there were no lesions indicating ectoparasite infections. Both the ears of each rabbit were shaved with scissors ready for tick application for feeding. Each rabbit was used only once in the tick feeding procedure, in order to rule out the possiblity of developing host resistance to tick attachment. Rabbits were used because they are comparable to cattle as hosts for R. appendiculatus (Branagan, 1974; Tukahirwa, 1976).

Tick feeding

R. appendiculatus ticks were fed on the ears of rabbits inside sleeves made of white cloth (ear bags). Two feeding bags were used on each rabbit, one bag on each ear. The ear bags were stuck to the ear with elastoplast bound round the base and the distal ends of the bags were sealed with a strip of elastoplast and stuck down to the collar. The ear bags were tightened only at the ends thus allowing free tick and attachment at sites of their choice on the ear, but the space remained small enough to confine the ticks close to the rabbit skin. By using open ended bags, it was easy to open one end to inspect the ear or collect engorged ticks. Special wide collars of thin leather were used on the necks of the rabbits to protect the ear bags from the feet of rabbits, which could be used to rub off the bags. These were attached to the rabbit by means of a harness prepared from a one

inch wide elastic as follows: Two bands of elastic were passed round the body, one round the neck and one round the thorax behind the front legs; these bands were joined dorsally and ventrally by short strips of elastic, the whole being held together with staples. The collar was then attached to this harness by tapes. All stages of the ticks were successfully fed in this way.

For tick application for each feeding, 6.5×10^3 larvae, 3.5 $\times 10^3$ nymphs, and 240 adults (120 males and 120 females) were used. Ticks from the five populations were fed in different ear bags. All stages of ticks used in the experiments were of similar age. The rabbits after tick application for feeding, were restrained individually inside specially designed feeding cages (2ft x 2ft x 2ft). Details of the culture methods and feeding techniques are given by Bailey(1960), Irvin and Brocklesby (1970) with modifications by Branagan (1969) and summarised by Irvin et al., (1973).

Infecting ticks

T. parva parasites used in the experiments are those that had been isolated from ticks fed on infected cattle, and the isolates stored in appropriately ground up tick form (stabilates) to be used in future experiments.

Cattle were inoculated with tick-derived stabilates to infect them. The cattle used were steers of European breed, Bos taurus type which were shown to be negative for antibodies against T. parva using the indirect fluorescent - antibody (IFA) test as described by

Burridge and Kimber (1972). Eight <u>Bos</u> <u>taurus</u> steers were used,
divided into two groups of four animals each:-

Group 1:   Animal Number

                955A

                959A

                961A

                970A

Group 2:   974A

                976A

                983A

                984A

Two stocks of <u>T. parva</u> (Muguga and Kilae) were used to infect
nymphal <u>R. appendiculatus</u>. Animals in Group 1 were subcutaneously
inoculated with 0.5ml of neat (undiluted) tick-derived <u>Theileria</u>
<u>parva</u> <u>parva</u> Muguga stabilate No. 147, under the left ear on day
zero.Animals in Group 2 were similarly inoculated with 1ml of neat
tick-derived <u>T</u>. <u>p</u>. <u>parva</u> Kilae stabilate No. 187 under the left ear
on day zero as described by Young et al., (1987). Both stabilates
were from the bank at NVRC - Muguga where they have been kept in
liquid nitrogen. The stabilates were thawed at 37°C after which
they were left at room temperature for 45 minutes to equilibrate
before inoculation.

The cattle were washed after the start of the experiment and
the areas where the patches for tick feeding were to be applied
shaved using a sharp pair of scissors. The shaved areas were washed
for three consecutive days whereby, during the first two days, the

washing was done using Teepol detergent dissolved in water and the last washing was done using plain tap water. This washing was necessary to remove any traces of acaricide chemicals that could have been acquired during dipping, which could affect tick attachment. The animals were monitored for ECF infection as follows. Rectal temperatures of the cattle were taken daily, starting one day after the inoculation of the animals, and plotted for each animal. Any animal whose temperature exceeded $39.5^{\circ}C$ was regarded as showing a febrile response on that day, indicating infection. From the fifth day of inoculation of the stabilate, needle biopsies of the left parotid lymph nodes of each animal were taken daily. Smears were prepared, stained with 5% Giemsa in 95% buffer solution for 40 minutes, and examined for the presence of Theileria schizonts. Once these were noticed, smears of the right prescapular lymph node biopsies and peripheral blood collected from a needle prick, were prepared daily from each animal, and examined at a magnification of x 100, for schizonts and piroplasms respectively. The number of red blood cells infected with piroplasm was counted and expressed as the percentage of the total number of red blood cells in the observed field and this gave the percentage parastaemia. This was done using a light binocular compound microscope (Wild Heerbrugg 139052 model). Ticks were applied on cattle once piroplasms were noticed. All the eight animals died and full post-mortem examinations confirmed the deaths as caused by ECF.

Two days before tick application, white cloth-sleeved porous patches were glued to the skin, enclosing shaved areas. 2,000 nymphs were introduced for feeding in each patch on day fourteen after

stabilate inoculation, so that the nymphal ticks completed their
repletion when the cattle had high piroplasm parastaemia. Areas on
individual cattle where the nymphal ticks from the five stocks were
applied were as follows:-

site of application	Tick stock applied
Right patch (R.P.)	MLS
Left patch (L.P.)	FS1
Back patch (B.P.)	FS3
Left shoulder (L.S.)	FS4
Right Shoulder (R.S.)	FS2

The patches were inspected daily from day one after nymphal
application, and all the detached nymphs were collected in glass
tubes of the size 80 x 25mm, each containing at most 100 engorged
nymphs. These were incubated at 28°C and approximately 85% relative
humidity, for 26-29 days during which time they completed moulting.
Thirty tick drops were chosen from six cattle, whose resultant adults
were fed on rabbits for three days for sprozoites to mature (Young
and Leitch, 1981). For all the experiments, nymphal tick batches
which had completed repletion on an animal on the same day were used.
One hundred ticks (50 males and 50 females) were randomly selected
from each of the thirty tick drops, for salivary gland examination
for infection rate and level of infection determination.

Infection rate and level of infection

The ticks selected as mentioned above were dissected for
salivary glands. The ticks were embedded in molten paraffin wax and
the dissection was done under physiological saline. Using a sharp
scalpel blade, a slit was made along the posterior lateral margin of

the scutum along the ventral line. The scutal covering of the tick was carefully lifted using fine forceps and cut off to expose the internal organs. Using the fine forceps, the gut was carefully separated and removed from the salivary glands and their connective tissues, and the latter two carefully cut and removed and put in phosphate buffered saline (PBS) of pH 7.2. The glands were removed as described by Purnell and Joyner (1968) and Walker et al., (1979). Paired salivary glands from male and female ticks from the same stock were placed in different small glass tubes, that were clearly labelled. These were then transfered to small tubes containing Carnoy's fixative. Different salivary glands from ticks from different stocks were likewise put in separate glass tubes under the stated condition. The salivary glands were fixed in Carnoy's fluid for 20 minutes. The glands were then transferred separately as above, into 100% ethanol for two hours to dissolve excess Carnoy's, then transfered to 5M hydrochloric acid (HCl) for 20 minutes for the glands to be macerated. The glands were then rinsed twice with distilled water to wash out excess HCl, and then transfered into Schiff's stain overnight at room temperature. The following day, the salivary glands were rinsed in tap water, three times, to remove excess stain. The glands were then dehydrated in a graded series of alcohols after which they were cleared in xylene from where salivary glands from individual ticks were mounted on separate microscope slides, that had been labelled using a diamond pen, to show the area of tick collection, cattle number on which the nymphs fed, site of feeding, sex of the tick and date of dissection. Mounting was done under cover-slips in DPX and the salivary gland slides observed under compound microscope at x 100 magnification for detection of infected

salivary gland acini cells; these were then examined at x 400
magnification to determine the degree of parasite infection. For
infection rate, the number of ticks found infected was expressed as a
percentage of the total number of ticks examined in a batch, and for
the level of infection, the number of salivary gland acini infected
per tick in the ticks was examined.

Weights of engorged ticks, duration of moulting, weights of eggs and size of unfed females

An experiment was done to determine engorgement weights of
larvae, nymphs and females, using only ticks that attached within 24
hours after application. Ticks which had attached within this period
were discarded. Engorged ticks were collected each morning and the
afternoon of the same day during a feeding process and were subjected
accordingly to various experiments that same morning and/ or
afternoon. The engorged larvae and nymphs were counted, weighed in
bulk and allowed to moult and duration of moulting recorded. The
ticks were monitored daily from the time of engorgement until
moulting started, and the time was recorded when about 75% larvae or
nymphs had moulted to nymphs or adults respectively . This was taken
as the duration of moulting/ecdysis. As mentioned earlier, engorged
females were weighed individually and then kept in separate tubes to
lay eggs. The eggs produced by each female were weighed separately.
Size of unfed females from the different tick stocks was recorded (in
microns) by measuring the lengths of scuta of 30 females from each
stock. Prior to the scutal measurement, the ticks were fixed and
preserved in 70% ethanol (Chiera et al., 1985).

All weighings were done on Oertling - OB 52 balance at NVRC and
measurement of size of females was done using an Olympus objective
micrometer together with eyepiece micrometer under a light Leitz
Wetzlar microscope at the Department of Zoology, University of
Nairobi.

Isoelectric focusing (IEF) for isoenzyme analysis

Isoenzyme characteristics of eleven enzymes of the five tick
stocks were investigated. The enzymes which were investigated
included glucosephosphate isomerase (GPI), adenylate kinase (ADK),
phosphoglucomutase (PGM), malic enzyme (ME), malate dehydrogenase
(MDH), isocitrate dehydrogenase (IDH), diaphorase (DIA), peptidase 1
(leucyl-glyclyl-glycine) (PEP1), peptidase 2 (leucyl-alanine)(PEP2),
peptidase 7(tyrosyl-tyrosyl-tyrosine)-(PEP7) and lactase
dehydrogenase (LDH). Investigations on these enzymes were done on
ground up tick homogenates.

Preparation of tick homogenates for isoelectric focusing (IEF) enzyme analysis

The isoelectric focusing (IEF) technique was done on ground up
ticks from each of the five stocks.The ticks were washed under
running tap water to remove surface dirt and debris (FAO, 1984). Each
tick stock was ground thoroughly by hand in a separate pestle and
mortar in 7ml of distilled water. The ground up tick (GUT) materials
were suspended in phosphate buffered saline (PBS), pH 7.2, and
transferred into separate plastic conical tubes and centrifuged at
1100g force at 4°C for one hour in an International Portable
Refrigerated Centrifuge, model PR-2, at NVRC laboratory. The

supernatant was recovered in separate clean conical tubes, and
separately aliquoted into 10µl using micropipettes and dropping these
into liquid nitrogen ($-176^{\circ}C$) in a 250ml flat bottomed flask, to
form `beads' of the above stated volume. The `beads' from the
different stocks were put in small plastic conical tubes with
perforated lids, and stored in liquid nitrogen for use in the
isoelectric focusing experiments. The preparation of the 10µl
aliquots was done and the `beads' stored as described by Gibson et
al., (1978) and Allsopp et al, (1985).

Isoelectric focusing was performed on agarose gel by standard
methods (Wraxall and Culliford, 1968; Godfrey and Kilgour, 1976;
Gibson et al., 1980; Allsopp and Gibson, 1983) with electrophoretic
conditions being modified accordingly to maximise band resolution.
Separation and staining conditions were optimized as described by
Harris and Hopkinson (1976) for all enzymes.

Statistical analysis

Comparisons were made using a single classification (one way)
analysis of variance (ANOVA),Chi-square and G- tests. Means were
compared by the multiple comparison test using the least significance
difference (LSD) method (Steel and Torrie, 1987; Parker, 1979).
Computations were done using a Casio College fx-100B 10-digit
scientific calculator .

CHAPTER 4

RESULTS

Feeding performance of the five tick populations

To study the feeding performance of the five tick populations, the mean engorgement weights of larvae, nymphs, and females were investigated.

There were small differences in the mean weights of engorged larvae (Table 2) but these were not statistically different (Anova: F=1.792, df = 4,145, P> 0.05).

Results from the mean weights of nymphs indicated that FS1 were the heaviest, followed by FS3, FS2, FS4 and MLS (Table 2). By performing analysis of variance on the results, significant differences were revealed in the ticks collected from the five populations (Anova: F = 173.587, df = 4,145, P<0.05). For the mean engorged weights of females (Table 2) results indicated that FS1 were the heaviest ticks, followed by FS3, FS4, FS2 and MLS in that descending order. The ticks from the five populations were shown to be statistically different (Anova: F = 20.077, df = 4,145, P<0.05). LSD revealed significant difference between FS2 and FS1, FS1 and FS4, and, FS3 and MLS.

From the results on the weights of engorged nymphs and females, it can be observed that the five tick populations may be different in their feeding ability.

Table 2: <u>Mean engorgement weight (mg. ± S.E) of larvae, nymphs and</u>
<u>females of R. appendiculatus from five different geographical</u>
<u>zones fed on susceptible rabbits</u>

Tick strain	Mean weight of larval batches	Mean weight of nymphal batches	Mean individual weight of females
FS1	124.667+6.464	1049.000+8.845	448.333+13.847
FS2	117.000+3.495	952.333+9.131	292.667+17.073
FS3	119.333+3.250	998.333+7.491	334.667+16.861
FS4	115.333+3.579	829.000+8.3370	331.333+14.197
MLS	109.333+3.214	769.000+10.398	278.333+11.901
	n=30	n=30	n=30

Table 3: <u>Mean weight (mg) of egg batches of</u> R. <u>appendiculatus from</u>
          <u>five different geographical zones</u>.

<u>Tick strain</u>	<u>Mean weight of 30 egg batches+ S.E</u>
FS1	176.000 ± 12.444
FS2	152.333 ± 8.118
FS3	137.467 ± 11.044
FS4	$140.500 + 9.383$
MLS	119.200 ± 7.865

n=30

Reproductive performance of five tick populations

During this part of the study, reproductive performance was investigated by making observations on female engorgement weights and weights of egg batches. The trend from results obtaind by investigating mean weights of engorged females (Table 2) has been discussed above.

Results from mean weights of egg batches from females fed on susceptible rabbits revealed that FS1 produced the heaviest weight, followed by FS2, FS4, FS3 and MLS in that descending order (Table 3). Their weights were shown to be statistically different (Anova: F = 4.459, df = 4,145, P<0.05). LSD test revealed that significant difference existed only between the FS1 and MLS.

The respective egg batch weights (Table 3) and the weights of engorged females (Table 2) were related, except for FS2 and FS3 egg batch weights which related inversely to their respective weights of females. This would imply that blood meal is important in egg formation.

Development performance of the five populations

Results from mean duration in days post-repletion on rabbits to completion of the moult (Table 4) showed that FS1 larvae took the longest period, followed by FS4, MLS and FS2 all of which took similar period. FS3 took the least duration. These differences were shown to be significant (Anova, F=14.509, df=4,145,P<0.05).

Results from the mean duration in days post-repletion on rabbits to completion of the moult for nymphs showed that FS1, FS2 and FS4 took the longest duration followed closely by MLS. FS3 took the least duration. Despite the observed apparent differences, the

Table 4: <u>Mean duration (days ± S.E) of larval and nymphal moulting</u>

Mean duration (days) post repletion

to the completion of the moult

<u>Tick strain</u>	<u>larval batches</u>	<u>nymphal batches</u>
FS1	13.000+0.392	16.100+0.088
FS2	11.000+0.173	16.030+0.112
FS3	10.930+0.166	10.930+0.113
FS4	11.530+0.115	16.030+0.058
MLS	11.470+0.124	15.970+0.112
	n=30	n=30

populations were not statistically different (Anova,

F=0.227,df=4,145,P>0.05, Table 4).

Size of unfed females

To understand the size of unfed female ticks, mean length of

scuta of unfed females from the five populations was investigated

(Table 5). The ticks from the five populations were different in

size. The FS2 ticks were the largest, followed by FS4, FS1, MLS and

FS3 ticks in that descending order. From the analysis of variance

test, the differences were shown to be significant (Anova, F=5.809,

df=4,145, P< 0.05, Table 5). LSD test revealed significant

differences between FS2 and FS3, and, FS2 and MLS.

Comparative infection rates and levels of Theileria parva strains in R. appendiculatus populations from five ecological zones in Kenya

The infection rates in salivary glands of R. appendiculatus

adults infected with T.p. parva Muguga are shown in Table 6. FS1 and

FS3 ticks showed a high infection rate, with FS1 having the highest.

MLS, FS4 and FS2 showed lower infection rates with the latter showing

the least infection. There are statistically significant differences

in the infection rates of the T. p. parva Muguga in the five

populations by applying Chi- square test (X^2 =24.03, df=4, P <

0.001, Table 7).

Generally, MLS showed the highest mean number of infected acini.

This population also showed the highest number of acini infected with

the T.p.parva Muguga per tick examined. This was followed by FS1. The

averages of the rest of the populations were not very different from

each other. However, from the analysis of variance, these differences

Table 5:   <u>Summary of mean scutal lengths (size) of unfed females</u>

<u>of</u> <u>R</u>. <u>appendiculatus</u> <u>from the five geographical zones</u>

Scutal length

Tick strain	No. of ticks	(microns+ S.E)
FS1	30	1267.900+14.815
FS2	30	1358.833+28.863
FS3	30	1220.367+23.145
FS4	30	1281.000+19.849
MLS	30	1258.600+14.609

Table 6:   Infection rate of T. p. parva Muguga in salivary glands

            of R. appendiculatus adults collected from different

            ecological zones in Kenya

	Females		Males	
Tick strain	examined	infected	examined	infected
FS1	80	26	98	15
FS2	128	11	74	2
FS3	98	25	98	13
FS4	88	23	102	3
MLS	90	22	148	11

Table 7:   Infection rate of <u>T. p. parva</u> Muguga

               in salivary glands of <u>R. appendiculatus</u> adult

               populations in Kenya

Tick Population	Total No. Infected	Total No. not infected	Totals
FS1	41 (26.77)	137 (151.23)	178
FS2	13 (30.38)	189 (171.62)	202
FS3	38 (29.48)	158 (166.52)	196
FS4	26 (28.58)	164 (161.42)	190
MLS	33 (35.79)	205 (202.21)	238
Totals	151	853	1004

<u>Note-:</u> The numbers in brackets represent the frequencies

        expected.

        Significant at $X^2 = 24.03$, df=4, P<.001.

Table 8:   Infection levels of T. p. parva Muguga in salivary

glands of R. appendiculatus adult populations in

Kenya

Tick population	No. of ticks examined		mean infected acini	No. of infected acini per tick examined
	Females	Males		
FS1	80	98	36.500+11.390	0.820
FS2	128	74	17.750+15.755	0.351
FS3	98	98	19.400+7.991	0.495
FS4	88	102	16.800+6.010	0.442
MLS	90	148	52.000+31.842	1.092

Note:

Not significant. Anova, $F=0.768$, $df=4,18$, $P>0.05$.

Table 9:   <u>Infection rate of T. p. parva</u> Kilae in salivary glands

of <u>R</u>. <u>appendiculatus</u> adults collected from different

ecological zones in Kenya

Tick Population	Females examined	infected	Males examined	infected
FS1	164	15	126	1
FS2	140	7	134	0
FS3	132	17	120	16
FS4	150	19	150	1
MLS	148	8	140	0

Table 10:   Infection rate of T. p. parva Kilae in salivary glands

of R.appendiculatus adult populations in Kenya

Tick population	Total No. infected	Total No. not infected	Totals
FS1	16 (17.35)	274 (272.65)	290
FS2	7 (16.39)	267 (257.61)	274
FS3	33 (15.08)	219 (236.92)	252
FS4	20 (17.95)	280 (282.05)	300
MLS	8 (17.23)	280 (270.77)	288
Totals	84	1320	1404

Significant, G-test, X^2 =31.18, df=4, P<0.001.

Table 11:   Infection levels of <u>T</u>. <u>p</u>. <u>parva</u> Kilae in salivary glands

of <u>R</u>. <u>appendiculatus</u> adult populations in Kenya

Tick population	No. of ticks examined Females	Males	Mean infected acini	No.of infected acini per tick
FS1	164	126	9.500+3.884	0.131
FS2	140	134	18.000+8.718	0.197
FS3	132	120	24.600+7.004	0.488
FS4	150	150	20.500+16.292	0.273
MLS	148	140	15.500+14.500	0.108

<u>Note:</u>

No significant differences, Anova, $F=0.442$, $df=4,17$, $P>0.05$.

Table 12:   Comparison of infection rates of T. p. parva Muguga and

T. p. parva Kilae in salivary glands of R.

appendiculatus populations in Kenya

Tick population	No. infected with T.p. parva Muguga	No. infected with T.p. parva Kilae	Totals
FS1	41 (26.77)	16 (17.35)	57
FS2	13 (30.38)	7 (16.38)	20
FS3	38 (29.48)	33 (15.08)	71
FS4	26 (28.58)	20 (17.95)	46
MLS	33 (35.79)	8 (17.23)	41
Totals	151	84	235

Note:

Significant, G-test, X^2 =51.16, df=4, P<0.001

were not significant (Anova, F=0.768, df=4,18, P> 0.05, Table 8).

The infection rates of T.p. parva Kilae in R.appendiculatus adult salivary glands are shown in Table 9. For this T. parva strain, the highest infection rate was in FS3 ticks followed by FS4. In FS1 and FS4 ticks the infection rates was about half that of FS3 with MLS and FS2 showing the lowest infection rates. By applying G- test, statistical differences in infection rates of T. p. parva Kilae were shown in the five populations (X^2=31.18, df=4, P< 0.001, Table 10).

FS3 showed the highest mean infected acini with T. p.parva Kilae, followed by FS4, FS2 and MLS (Table 11). FS1 showed the least mean infected acini. There is however no statistical differences between the populations (Anova, F=0.442, df=4,17, P> 0.05, Table 11).

This is an interesting observation because FS1 showed the highest mean infected acini with T. p.parva Muguga. In addition, it seems as if the tick populations generally showed averagely higher numbers of infected acini with T. p. parva Muguga compared with T. p. parva Kilae.

Generally, the tick populations show a higher ability to become infected with T.p. parva Muguga than T.p. parva Kilae. These differences in infection rates of T.p. parva Muguga and T. p.parva Kilae in the five tick populations, were statistically significant, by using G-test (X^2 =51.16, df=4, P<0.001, Table 12).

Isoenzyme studies of R. appendiculatus stocks

Eleven enzymes were investigated for isoenzyme profiles for the five different R. appendiculatus tick stocks in order to find out whether the tick populations differ. The study was also carried out to see whether isoenzyme profiles may be used as a marker in characterizing different tick populations. Zymograms of resultant bands are shown in figures 5-15.

Four of the eleven enzymes, i.e. peptidase 1, peptidase 2, malate dehydrogenase and glucosephosphate isomerase revealed that one out of the five tick populations studied, that is FS3, differs from the other four which are very similar. The results are shown below. In the description of the band profile, the band closest to the point of origin, is designated as `a' followed by `b', `c' etc .

Note: On the zymograms, the numbers represent the following tick strains used:

1 - FS3

2 - MLS

3 - FS2

4 - FS1

5 - FS4

Peptidase 1 (Fig.5)

The migration of isoenzymes was anodal i.e. towards the positive electrode and they were fairly well resolved. Two major bands were noticed and each contained a single band. The bands obtained from MLS and FS2 were the clearest. Both bands from FS3, MLS, FS1 and FS4 had the same mobility but were slower than from FS2. The FS2 ticks seem, therefore, to be different from all the rest in this aspect. In addition, the profile from FS3 lacked the `a' band which appeared in all the rest. This enzyme is, therefore, polymorphic in these populations except for FS3 which is monomorphic.

Peptidase 2 (Fig.6)

The migration of isoenzymes was also towards the anode. Two bands were noticed in MLS and FS2. Band `a' had the same mobility in both cases, but `b' was present in all five populations and showed a difference in mobility, being faster in FS2 than the rest. In this respect, FS2 therefore is different from the other stocks.

Malate dehydrogenase (MDH)(Fig.7)

The migration of isoenzymes was towards the cathode (-). Two distinct bands were noticed from all five populations. The enzyme is therefore, polymorphic in the tick populations investigated. Band `a' had the same mobility in all cases. Band `b' was fastest in FS3 and slowest in FS1 and FS4.

Fig. 5:   PEP 1 zymogram.

Note the anodal (+) migration of isoenzymes as indicated

arrow. The isoenzyme bands were fairly well resolved in a

five populations. Note that both the isoenzymes in FS2 t:

faster movers and distinguished this tick population fror

the rest.

Fig. 5: PEP 1 zymogram

Under 53 header: 53
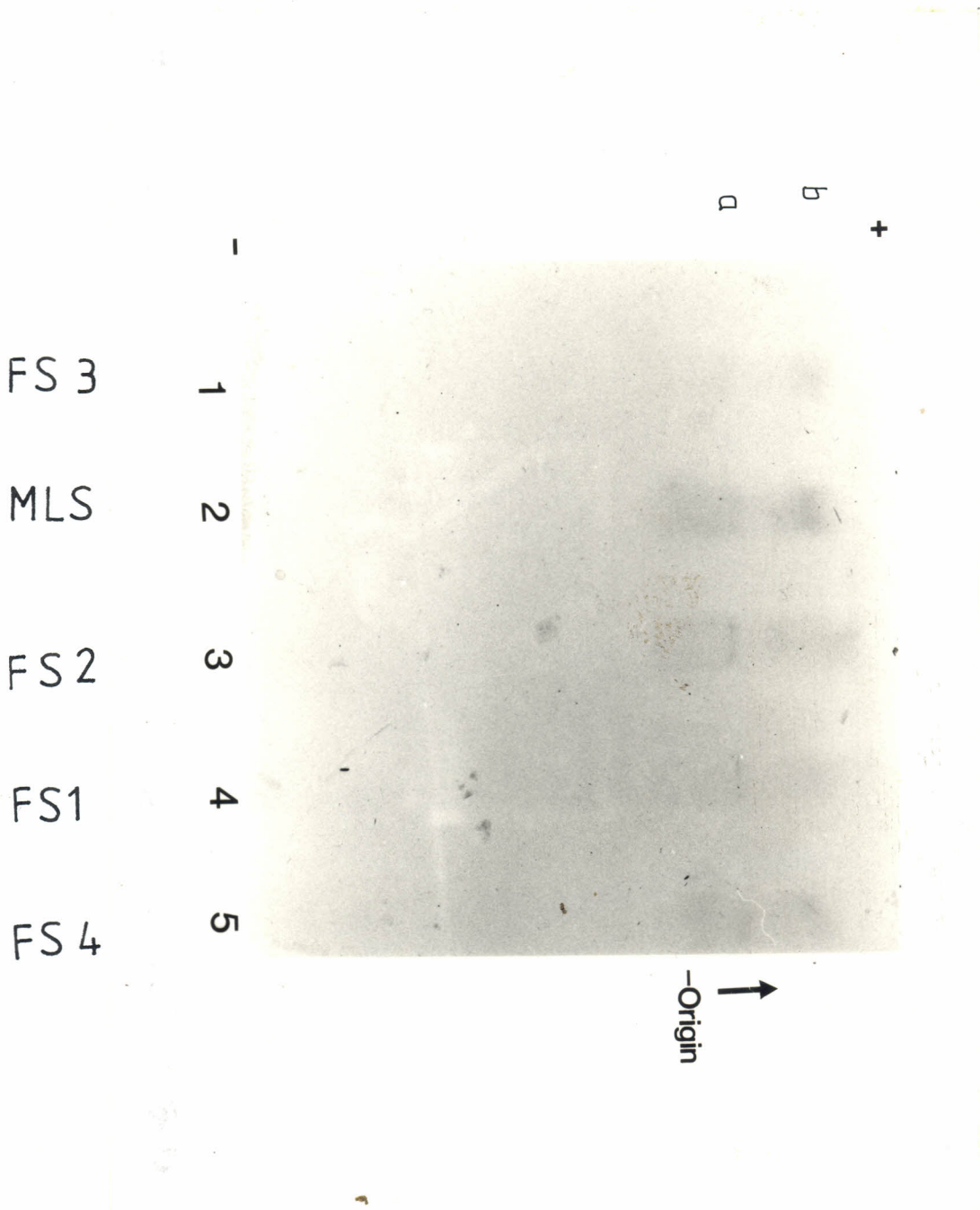
Fig. 6:   PEP 2 zymogram

Note that the migration of isoenzymes was towards

electrode. Two isoenzyme bands were well resolved

Also note that the second isoenzyme band (`b') was

faster in mobility in FS2 than the rest, suggestir

further that this population may be different from

rest.

54
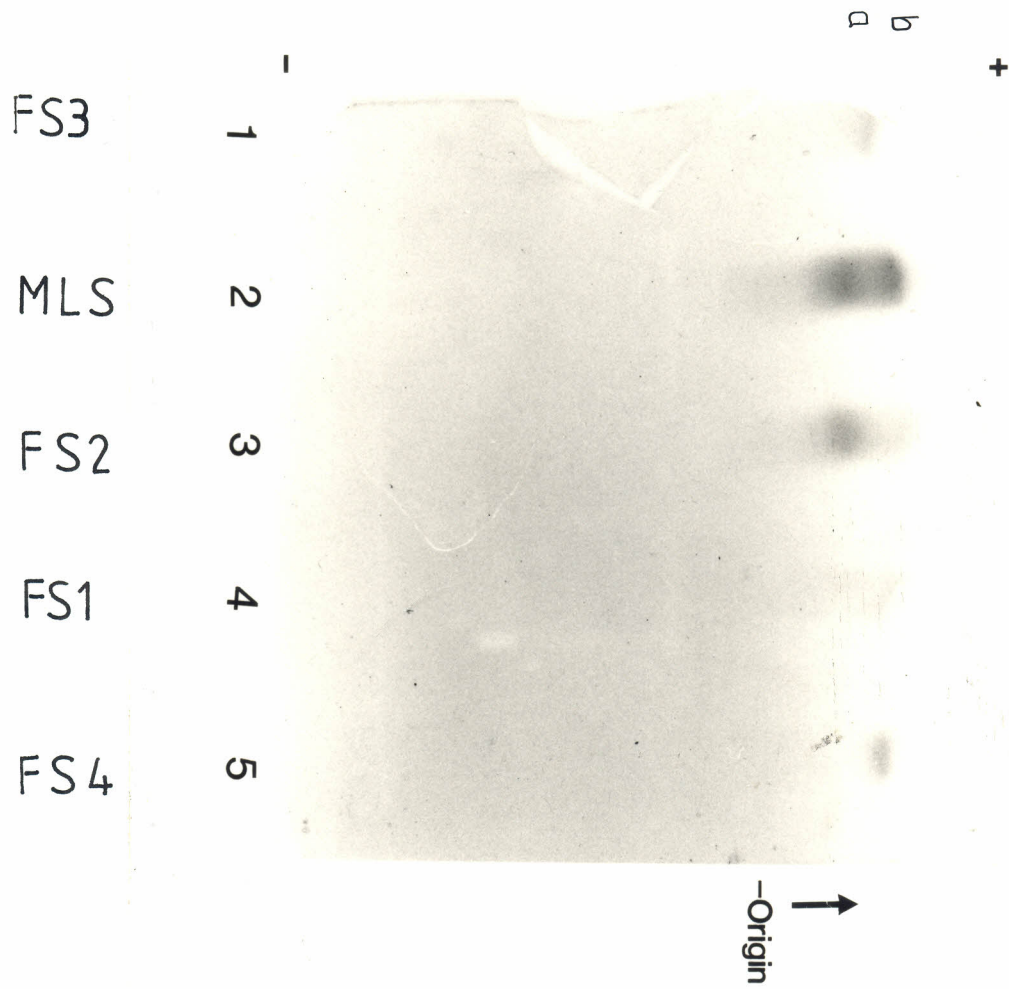
Fig.6:   PEP 2 zymogram

Fig. 7: MDH zymogram.

The isoenzymes migrated towards cathode electrode (-). Not

two distinct isoenzyme bands in all the populations. Also

that the second band (`b') is faster in mobility in FS3; s

in MLS and FS2; and slowest in FS1 and FS4. These results

further suggest that the tick populations are different.
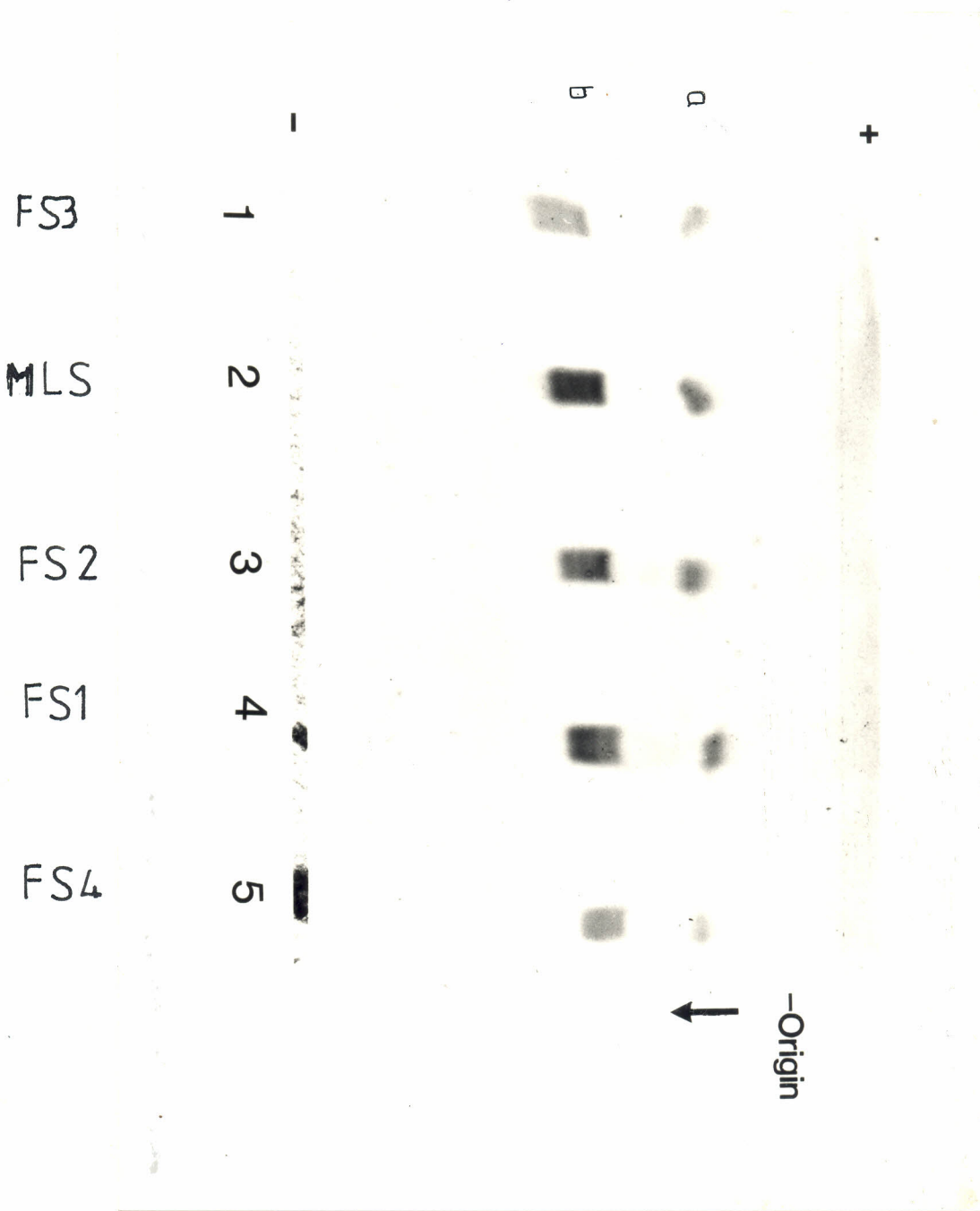
Fig. 7: MDH zymogram

Glucosephosphate isomerase (GPI)(Fig.8)

The migration of the isoenzymes was cathodal. Three single bands were noticed. Mobility of all three bands was the same in all cases where the bands were observed. Band `a' was present, but faint, in FS3, MLS and FS2 stocks. It was absent from FS1 and FS4. Band `b' was very distinct in all the five populations. Band `c' was fairly faint in all cases. This enzyme, therefore, revealed differences between the five stocks. FS1 and FS4 were shown to be similar, but distinct from the other three stocks.

Malic enzyme (ME)(Fig.9)

Migration of the isoenzymes was cathodal. Band `a' has three bands of the same mobility in all cases. Both `a' and `b' have a single band of the same mobility in all the five stocks. The second isoenzyme band ` b' exists as three bands (alloenzymes). This enzyme is known to be dimeric because it shows one band in homozygotes and three bands in heterozygotes. Therefore it expresses itself in two ways, and the results here fit the latter condition. In the present study, malic enzyme does not show any differences in the five tick populations.

Phosphoglucomutase enzyme (PGM)(Fig. 10)

Migration of the isoenzymes was towards cathodal. Three bands were noticed in all the five populations. All the three bands had the same mobility. The enzyme did not reveal differences among the five tick stocks.

Adenylate kinase (ADK)(Fig.11)

Migration of the isoenzymes was cathodal. Two bands were seen. Both `a' and `b' have a single band of the same mobility in all the five cases.

Band `a' was however not clear but it appeared as if it had more than one band which had fused together; a point which would suggest that the enzyme is dimeric. The enzyme did not reveal differences among the five tick populations.

Isocitrate dehydrogenase (IDH)(Fig. 12)

Migration of isoenzymes was cathodal. Bands `a' and `b' had single bands of the same mobility in all the five cases. The enzyme did not reveal differences between the five populations.

Diaphorase (DIA)(Fig.13)

Migration of the isoenzymes was cathodal. Two single bands were noticed. Both `a' and `b' had single bands and were of the same mobility in all the five cases.

Peptidase 7 (PEP7)(Fig.14)

Migration of the isoenzyme was cathodal. One single band was noticed in all cases. This enzyme is therefore monomorphic in the investigated populations. Mobility was the same in all the five populations.This enzyme, therefore, did not reveal differences between the five tick populations.

Lactate dehydrogenase (LDH)(Fig. 15)

Migration of the isoenzymes was cathodal. Two single bands were observed in all cases. The bands had the same mobility in all the five populations. This enzyme did not reveal differences between the five tick populations.

Fig. 8:   GPI zymogram.

Isoenzymes migrated towards cathode electrode. Note the tl

isoenzyme bands (`a',`b' and `c'). Note that the first bai

(`a') was present in FS3, MLS and FS2 but absent in FS1 ai
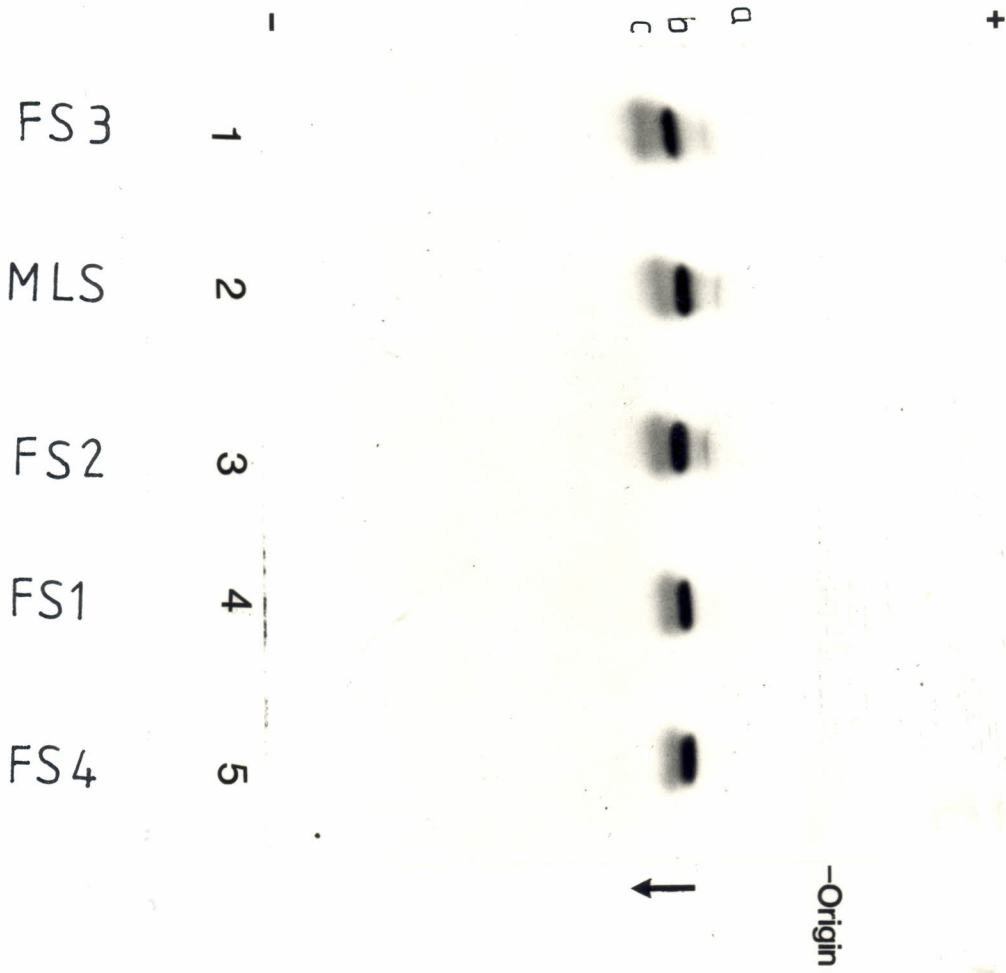
Fig. 8: GPI zymogram

Fig. 9:   ME zymogram.

The isoenzymes migrated towards cathode electrode. Note th

distinct isoenzyme bands of same mobility in all the

populations. No differences were noticed among the tick

populations.
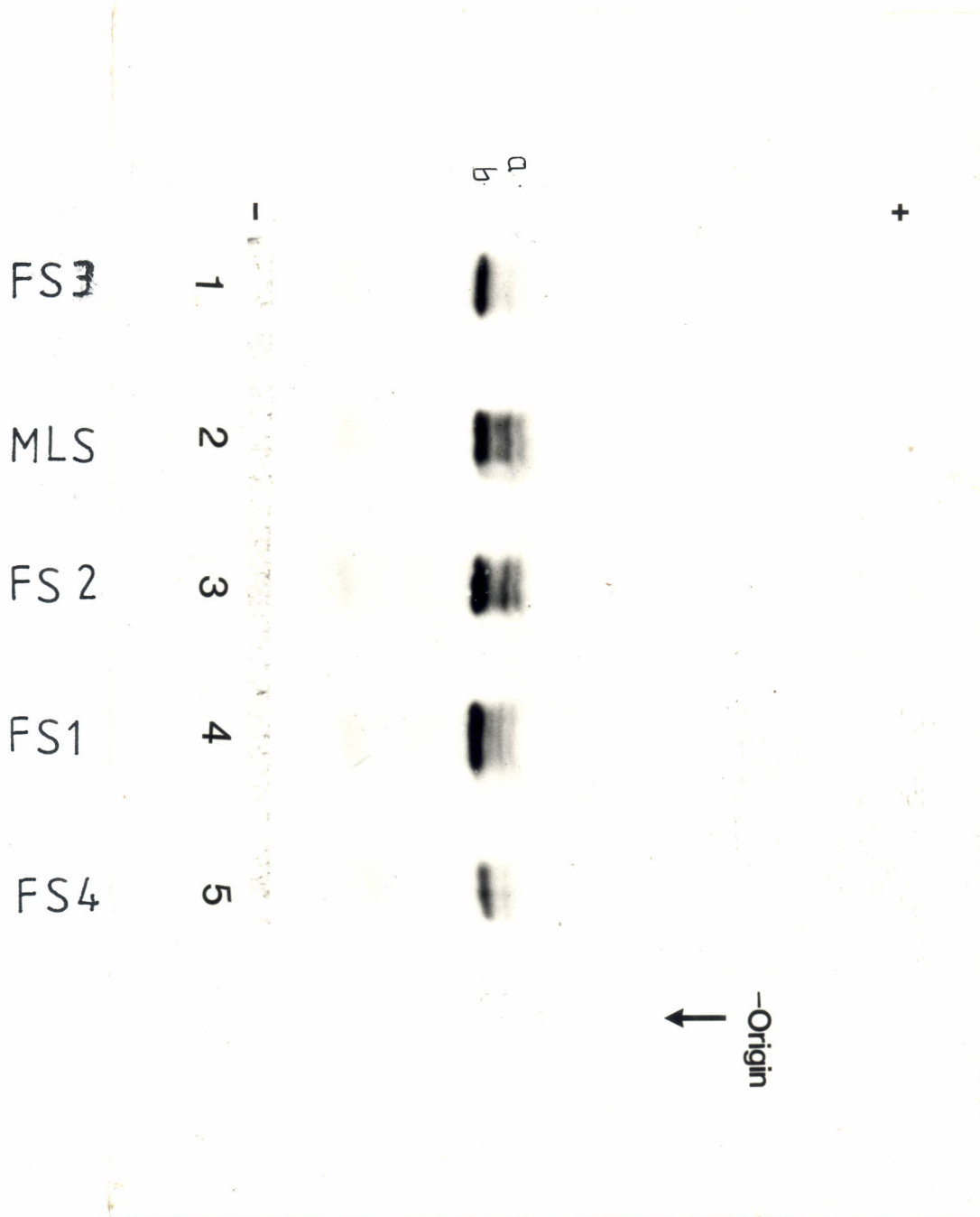
Fig.9: ME zymogram

Fig. 10: PGM zymogram.

Isoenzymes migrated towards cathode electrode (see arrow)
the three isoenzyme bands which are well resolved. Also
that the bands have same mobility in all the five tick
populations. The enzyme therefore revealed no differences
the populations.
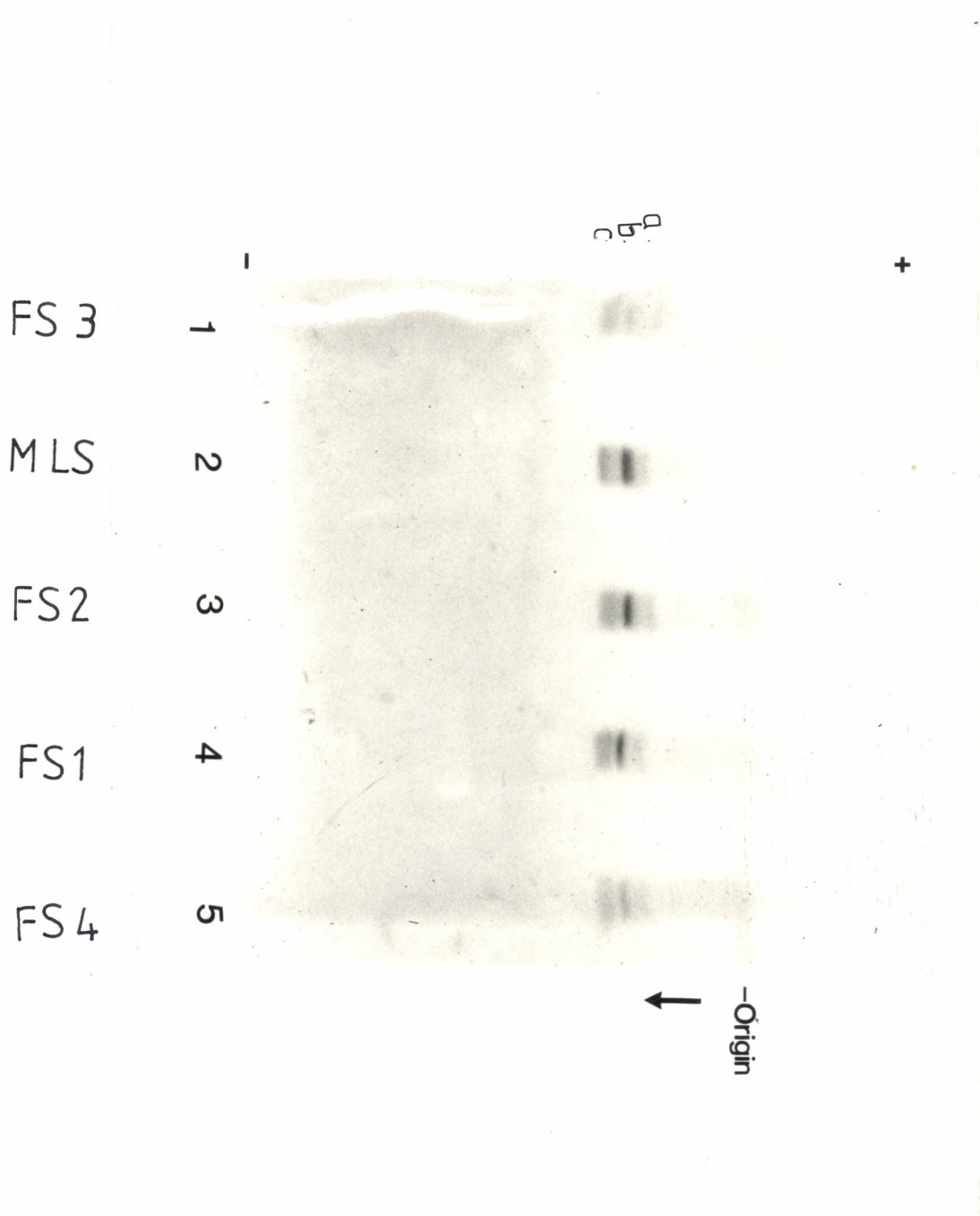
Fig. 10: PGM zymogram

Fig. 11: ADK zymogram.

Note the migration of isoenzymes towards cathode electrode.

isoenzyme bands (`a' and `b') can be noticed, though not we

separated. The bands have same mobility in all the five ti

populations. No differences among the populations are reve

by the enzyme.
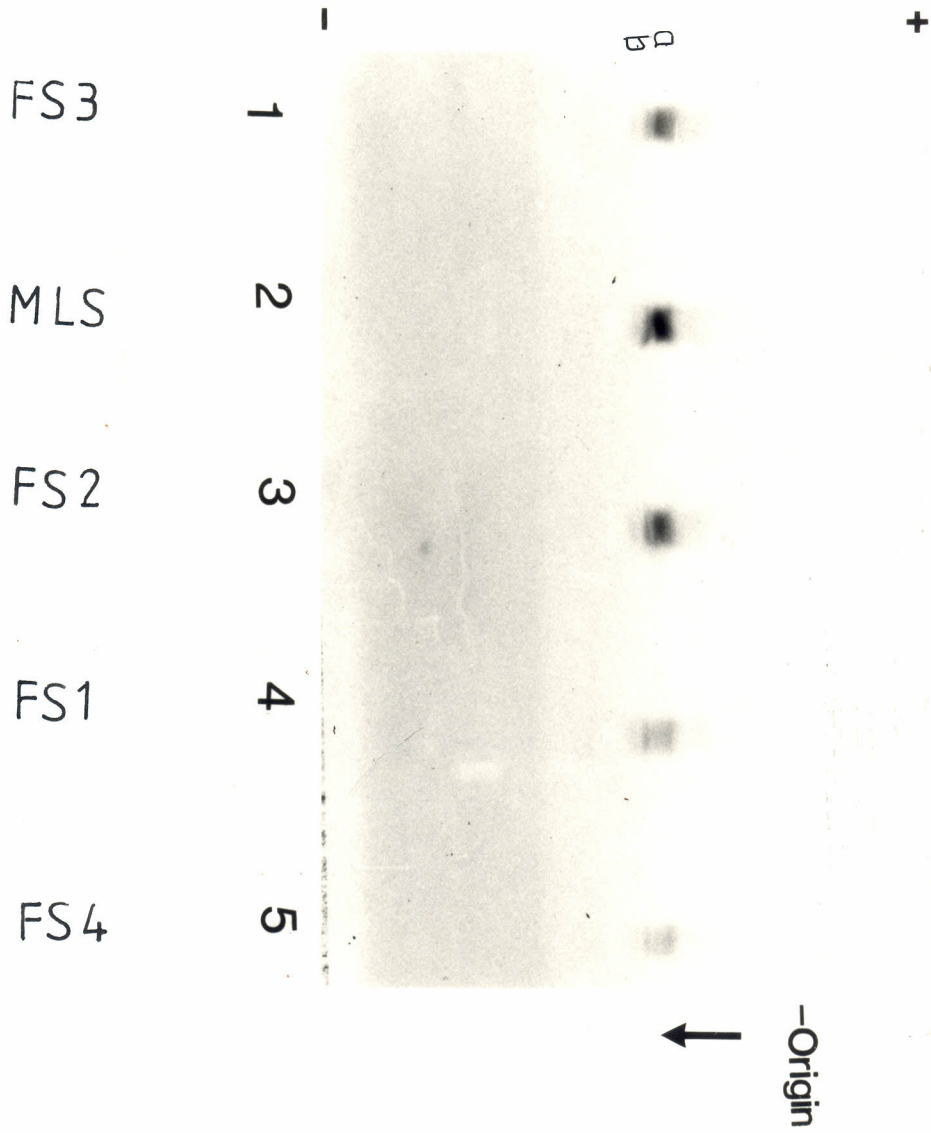
Fig.11: ADK zymogram

Fig. 12:   IDH zymogram.

Migration of isoenzymes is towards cathode electrode (see

arrow). Note the two isoenzyme bands which are clear and w

resolved. The bands have same mobility in all the five

populations investigated. No differences among the populat

are revealed by the enzyme.
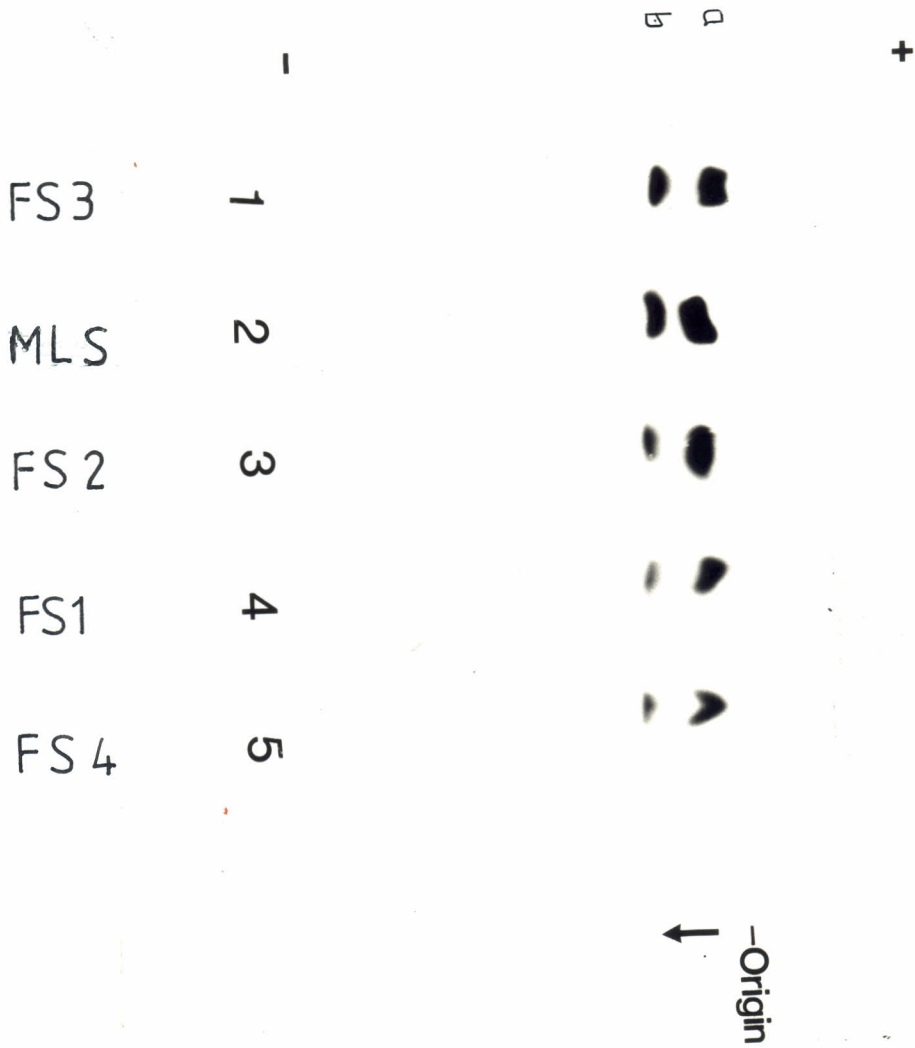
Fig.12:IDH zymogram

Fig. 13:   DIA zymogram.

Isoenzymes migrated towards cathode electrode. Note the tw

bands which have same mobility in all the tick populations

studied. No differences are revealed among the populations

the enzyme.
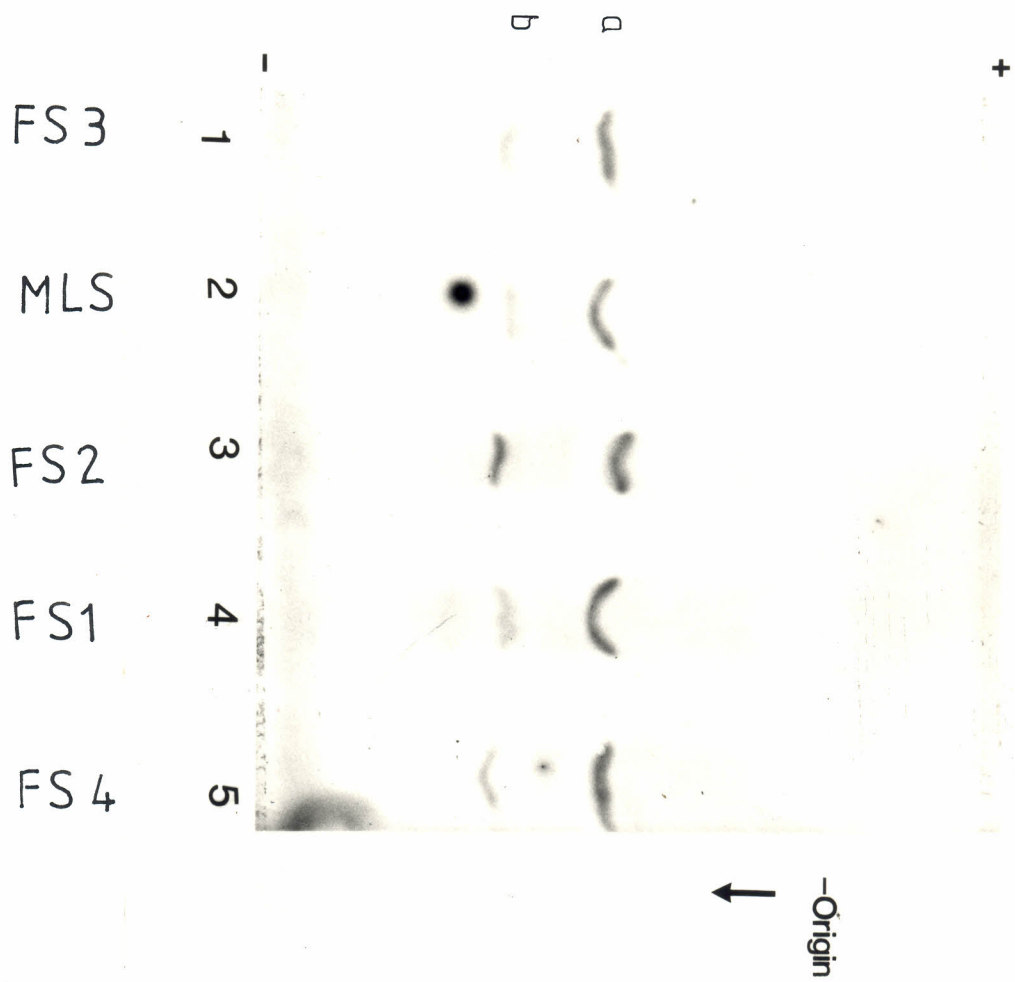
Fig.13: DIA zymogram

Fig. 14: PEP 7 zymogram.

Isoenzymes migrated towards cathode electrode. Note that t

single band in all the tick populations has same mobility.

differences among the populations are revealed by the enzy
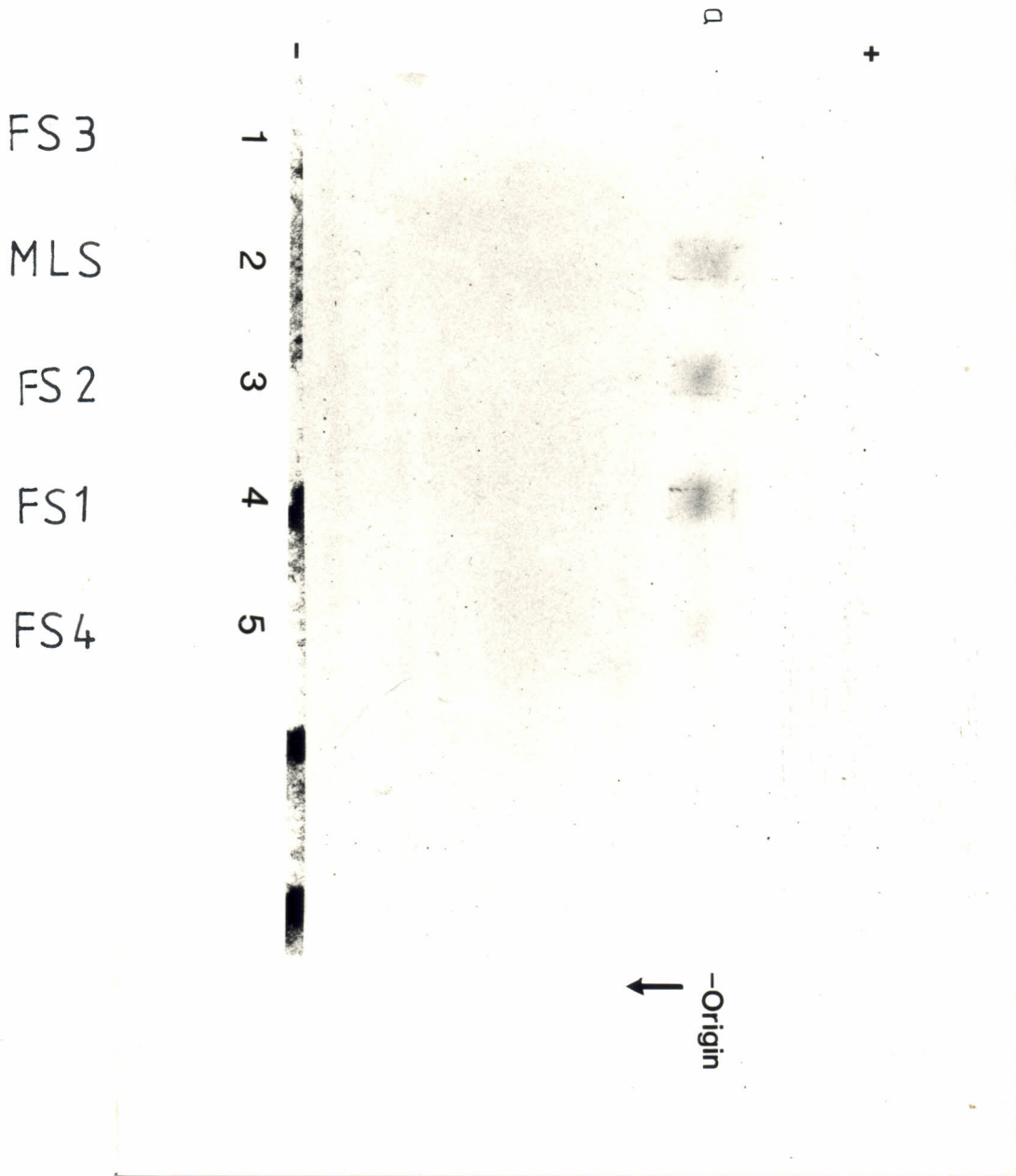
Fig.14: PEP 7 zymogram

Fig. 15: LDH zymogram.

      Migration of isoenzymes is towards cathode electrode.Two dist

      isoenzyme bands (`a' and `b') are noticed. The bands have sam

      mobility in all the tick populations investigated.No differen

      among the populations are revealed by the enzyme.

CHAPTER 5   ·

### DISCUSSION AND CONCLUSIONS

Generally, different tick species inhabit a variety of climatic regions (Walker, 1974). Within the same species, ticks occasionally are found distributed in areas which are geographically isolated (Walker, 1974; Theiler, 1959). Within these areas, the ticks occupy terrestrial habitats with adequate vegetation cover where they are directly exposed to the vigorously fluctuating microclimatic conditions, of which temperature and relative humidity are of paramount importance (McCulloch et al., 1968; Tukahirwa, 1976; Branagan, 1973; Punyua, 1984). These variations may individually or cumulatively affect the behaviour as well as survival or development of the ticks and as a result, the ticks may undergo various modifications in both their physical and physiological nature in order to adapt themselves to these unfavourable environmental conditions (Andrew, 1980).

Differences in the characteristics of tick populations have so far been recorded in ticks which occur in various countries. For instance, it has been reported that R. appendiculatus populations in South Africa have an adult diapause and are larger in size than the East African R. appendiculatus ticks which do not undergo diapause (Pegram and Banda, 1990; Rechav, 1981). Therefore, in the case of the S. African ticks, larval, nymphal and adult cycles do not overlap. It has also been recorded that in non-diapausing ticks the climate

controls the cycle. For the Zambian R. appendiculatus, it has been noted that the ticks are changing from diapause to non-diapause behaviour (Rechav, 1981; Pegram and Banda, 1990). Another notable observation is that R. zambeziensis which is related to R. appendiculatus (Walker et al., 1981) thrives in drier areas (Norval et al., 1982); and has been shown to be better vector of ECF and corridor diseases that R. appendiculatus (Norval et al.,1982; Blouin and Stoltsz, 1989). It may therefore be assumed that the two are strains of the same species but adapted differently to drier areas. since the distribution of ticks tend to be controlled by climate (Theiler, 1959; Walker, 1974), it could be assumed that diffferences in populations occur between habitats which differ in microclimatic conditions.

Different tick stocks may therefore differ in their ability to re-produce and develop, due to the influence of the forces of natural selection pressure during the course of evolution. Since the epidemiology of ECF is intimately connected with the ecology of R. appendiculatus, there is a need to analyse and characterize the R. appendiculatus populations from different geographically and ecologically isolated areas. This will help to elucidate the epidemiology hence might give insights into the control of ECF. Some important biological differences in the tick populations have been revealed in the present study.

Results in the present study suggest that different R. appendiculatus populations differ in weights of engorged nymphs and females. Furthermore different tick populations are hereby reported to differ in the weights of egg batches laid by respective females.

Food intake in haematophagous arthropods is an essential component of egg maturation and reproduction (Engelman, 1968; Johansonn 1964). Results in the present study indicate that ticks from geographical zones which are relatively hotter have higher mean engorged weights. It is worth mentioning at this juncture that the variations in the quantity of blood meal ingested are significant in elucidating the transmission of pathogenic organisms by larvae and nymphs. Efficient blood ingestion, digestion and absorption of nutrients is important because the two (i.e. blood and nutrients) serve various functions of which the most important is egg yield. It is therefore not surprising that the egg yield reflected in the mean weight of eggs was related to the mean engorged weight of females. These observations conform well to those by Snow and Arthur (1966), Sweatman (1968), Hafez et al., (1972), Branagan (1973) and Tukahirwa (1976) reported earlier. It also appears that heavier ticks have more nutrient store for egg maturation.

The efficiency of nutrient conversion into eggs is a useful parameter for assessing the extent of blood meal utilization by ticks. In the R. appendiculatus populations under study, the best performance was recorded in FS1 ticks suggesting that the environmental conditions to which the tick population is adapted are the best for egg development. Cases of ticks with poor egg yield observed in this study could be due to poor adaptation of the ticks in efficiency of nutrient conversion into eggs.

Development performance of the ticks from different sources was determined by looking at mean duration of larval and nymphal moulting. No significant difference exists in moulting duration of nymphs from the five populations. These results conform to those recorded by Young and

Leitch (1981) while working on moulting behaviour of four strains of R. appendiculatus nymphs from Kenya. The observation of non-significant difference in nymphal moulting duration recorded in this study may have been due to the fact that the ticks used in the study were the same species and although ticks are widely distributed, different species of ticks have optimal conditions of temperature and relative humidity which favour maximum development and survival. Although found in different geographical zones, one expects, basing on this argument, ticks of the same species to choose conducive microclimates that would be similar, though in different zones. This however, would not be practical. The most likely explanation of the observed similarity would be that the prevailing environmental conditions in the studied geographical zones, may not yet have exerted enough pressure to regulate the biological activities to which the tick populations have adapted, as seems to be the case with the larvae from the same populations. The observations further throw light on the fact that the environmental conditions influence different tick stadia differently. Otherwise we would have observed a significant difference in the moulting durations of nymphs from the five populations since a significant difference had been noted in case of the larvae.

The effects of environmental conditions, especially temperature, on the moulting activity in ticks, were reported by Feldman - Muhsam and Muhsam (1966), Hafez et al., (1971) and Branagan (1973). These authors in their work reported that temperature among other processes in ticks greatly affects moulting activity. Evidence gathered by some earlier workers (Hoogstaal, 1956; Arthur, 1962; Yeoman, 1966a,b; McCulloch et al., 1968) had similarly correlated development of R. appendiculatus with various climatic factors, more specifically temperature (Branagan, 1973; Punyua, 1984). Results from the current study agree with these findings,

and further bring out clearly the fact that the climatic factors may have a long-lasting effect in such a way as to change the genetic composition of the species.

Different tick populations show different efficiency of transmission for T. parva stocks as has been shown by results in the present study. Ticks of FS3, FS4 and FS1 were more efficient transmitters of the two T. parva stocks that were investigated, than the rest. On average, the FS2 tick population was the poorest in the ability to become infected with parasites. Blouin and Stoltsz (1989), while investigating differences in infection rates of T.p. lawrencei by comparing R. appendiculatus and R. zambeziensis, suggested that R. zambeziensis which is generally found in hotter, drier areas (Norval et al., 1982), may be a more efficient transmitter of at least certain isolates of T. p. lawrencei than R. appendiculatus. The results in this study were in close agreement with those reported above because the FS3, FS4 and FS1 tick populations which recorded high infections are found in areas where temperature is high. In this study, the tick population from FS2 collected from an area which had comparatively low temperatures was the least likely to become infected with the parasite. Apart from temperature, there seem to be other factors which influence infection rates in tick vectors. This is because the FS3 population which came from an area which had comparatively lower temperature than those of both FS1 and FS4, performed better than both the latter. Furthermore, differences in T. parva strains have been shown in the present study to be picked at different levels by the vectors, an indication that the epidemiology of ECF may be related to the parasite strain involved. As mentioned earlier, the tick populations consistently showed higher infection rates and levels with T. p. parva Muguga than with T. p. parva Kilae. Factors other than climatic and strain of the parasite

could be used to explain the transmission of ECF. This is evident from the fact that FS3 in this study is seen to be equally efficient in becoming infected with both the strains.

Climatic conditions of the habitats, strain of T.parva and other factors, most probably genetic, therefore, seem to be exerting an influence on the ability of the tick vectors to transmit the parasite. These differences in transmission efficiency seem to give us a clue with regard to the tick populations from different geographical zones being biologically different. This is a very important finding as it may be used to help explain ECF occurence and different levels of prevalence of the disease recorded in different localities in the country.

Out of the eleven enzymes studied, ten showed polymorphism in most of the five populations, while only one i.e. PEP 7 was monomorphic. On the basis of results from PEP 1, PEP 2, MDH and GPI enzymes, R. appendiculatus populations from different geographical areas seem to be different. Similar differences in intra-specific populations have been reported in other ticks as well as other vectors. These results for instance conform to those by other workers such as Hilburn and Sattler (1986) while working on the lone star tick Amblyomma americanum; Sattler et al., (1986) while working on four natural populations and four laboratory colonies of Boophilus microplus and a laboratory colony of B. annulatus. Small levels of genetic differentiation such as reported in the current study for R. appendiculatus were also observed between geographically isolated populations of Aponomma hydrosauri and Amblyomma albolimbatum (Bull et al., 1984). These results also agree with those reported by other workers while investigating other vectors such as results by Takeshi and Leonard (1988) while looking at the isoenzymes of two natural populations in Kenya of the tsetse Glossina pallidipes and Van Etten's (1982) study of eleven

populations of G. pallidipes by means of starch gel electrophoresis.He
reported that polymorphism was demonstrated in.three of the eleven systems
tested. The present study has therefore clearly demonstrated that only
four enzymes, PEP 1 (fig. 5), PEP 2, (fig. 6), MDH (fig. 7) and GPI (fig.
8) may be used for characterization of R. appendiculatus populations.
These four enzymes can be used in this way because their isoenzymes are
not present in all populations.

Variation in allopatric populations such as those studied may come
about as a result of either genetical influence due to mutation, genetic
drift, founder effect or environmental effects resulting in natural
selection or can be the product of a combination of these forces.
What this implies is that the genetic composition of a population changes
gradually due to environmental pressure until the entire population
evolves. If this is left for several generations, there may be a constant
refinement of the genetic composition of the population, as the genes
selected for are those best adapted to the conditions in which the
population lives.

The samples of R. appendiculatus under study were taken from areas
with different conditions which may have contributed to the variations
observed and it was not surprising that they differed in certain of their
alleles thereby exhibiting the observed different biological
characteristics. This could be due to selection for different homozygotes
under varying environmental conditions.

In such widespread panmictic populations as in this study, selection
may produce differential survival in different regions and result in
different allelic variation. Since these differences among localities
must arise by divergence from a common gene pool of zygotes, the
differential survival ability in response to an environmental variable

which changes with latitude (e.g. temperature) seems a likely explanation (Andrew, 1980). This is just one of the factors that may be used to explain the observed differences in the tick populations as we shall see later.

There is also another way of explaining the genetic divergence observed among the tick populations studied. Since the ticks are relatively inactive when they are off the host, the dispersion of these vectors, and therefore, the rate of gene flow, depends on host mobility. The presence of the observed genetic divergence between the geographically isolated R. appendiculatus populations can be explained by the lack of extensive mobility of its hosts, including cattle, all of which can disperse ticks rather broadly. Moreover, lack of substantial movement of domesticated animals by man over long distances, within the range of this tick, could also have a negligible effect on dispersion of ticks, resulting in insufficient gene flow between the study areas, thus promoting genetic divergence between the geographically isolated tick populations.

The results strongly suggest the enzymes selected could be used to illustrate the geographical and ecological diversity of R. appendiculatus hence the epidemiology of ECF. This is the first time diversity of the tick in Kenya has been reported. The information could be used in structuring appropriate vector and disease control measures depending on the ecology of the vector in question. For instance, higher yields of tick -derived parasite material for serological and immunological studies have been obtained by feeding R. zambeziensis on experimental carrier animals (Blouin and stoltsz, 1989). Such results as presented here suggest that it would be possible to select R. appendiculatus population with better ability to pick T. parva infection for producing maximum numbers of

sporozoites of _Theileria_ species to be used in immunization studies and practical application in infection- treatment method of cattle vaccination which is currently under trial (Radley, 1978, 1981; Morzaria _et al._, 1985; Young _et al._, 1990b). Furthermore, having the knowledge that _R. appendiculatus_ populations are diversified according to their geographical and ecological zones, it would be possible to carry out further studies to determine the levels of resistance and/or susceptability to acaricides used for control. This would enable the governments concerned to structure appropriate control measures aimed at specific _R. appendiculatus_ populations.

Isoenzyme analysis, therefore, coupled with the other biological characteristics, may provide us with a good marker for further analysis of genetic differentiation among natural populations of the tick vector. This could help to explain the differences in the transmission of different _T. parva_ strains by different tick populations.

From the results of the present study, it is suggested that the MLS ticks, on average, performed more poorly than the field populations studied. This tick population is a closed population which has been bred and fed on rabbits for 37 years now and it is possible that this inbreeding has resulted in the smaller size of the laboratory strain. Most of the information on the biology of _R. appendiculatus_ in Kenya, for instance their ability to transmit _T. parva_ parasite, has been obtained using the laboratory strain. From the results, it therefore becomes quite questionable, how much reliance we should continue to place on data obtained using the laboratory strain, when it comes to the application in the field.

Although the biology and ecology of _R. appendiculatus_ has been studied in some detail, many aspects of its biology have not been fully

related to its ability to transmit theileriosis. In the earlier studies, investigators have tended to study the biology of the tick, while veterinary scientists have studied the role of the tick in transmission of theileriosis. The present study reported here has attempted to put such studies together. This study attempted to relate the biological diversity of R. appendiculatus to its ability to become infected with T. parva, which is obviously a very important yet inadequately studied aspect. Studies of the type reported here are important because using such results, it may be possible to select R. appendiculatus, with better ability to transmit T. parva, on the basis of their specific biological characteristics. This aspect would be very important for both practical studies aimed at producing maximum numbers of sporozoites of Theileria species for immunization studies as mentioned earlier, and theoretical understanding of the genetics of the tick vector for control purposes. Such studies would make it possible to understand the effects of different strains of tick populations and the transmission of tick-borne diseases.

CHAPTER 6

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