Genetic Variation in *Rhipicephalus appendiculatus* (Acari: Ixodidae) Populations in Kenya, Assessed by two-Dimensional Gel Electrophoresis

F. N. Baliraine[1,2], E. O. Osir[2], S. B. Obuya[2] and F. J. Mulaa[1]
[1]Department of Biochemistry, University of Nairobi, P. O. Box 30197, Nairobi, Kenya;
[2]International Centre of Insect Physiology and Ecology (ICIPE), P. O. Box 30772 Nairobi, Kenya

Abstract—The study of protein variation is an indirect, relatively inexpensive approach to genetic analysis. In this study, the protein profiles of two distinct populations of the brown ear tick (*Rhipicephalus appendiculatus*) in Kenya, which differ in their *Theileria parva* vector-competence were compared by two-dimensional gel electrophoresis. A majority of the proteins were homologous to both populations. However, a few proteins were found to be population-specific. Since proteins are gene products, the presence of population-specific proteins suggests genetic differences between the two populations. It is speculated that some of these population-specific proteins might be related to the observed differences in *T. parva* vector competence.

Key Words: *Rhipicephalus appendiculatus, Theileria parva*, Vector-competence, Two-dimensional gel electrophoresis, Genetic variation

Introduction

The brown ear tick, *Rhipicephalus appendiculatus* Neumann (Acari: Ixodidae) transmits *Theileria parva*, which causes East Coast Fever (ECF), the most economically important disease of cattle in Kenya and other afflicted areas of Africa (Norval *et al*., 1991). Norval *et al*. 1991 and Kubasu, 1992 reported that brown ear ticks from geographically isolated areas differ in their *T. parva* vectorial capabilities. It would be of epidemiological interest to determine whether tick populations which differ in their *T. parva* vector competence also differ genetically. This study was therefore designed to compare the genetic variation between two distinct *R. appendiculatus* tick populations in Kenya, and to possibly relate this information to the observed differences in their *T. parva* vectorial capacities. Two-dimensional gel electrophoresis (2-DE), a powerful and relatively inexpensive method of genetic analysis (O'Farrell, 1975; Dunbar, 1987; Hames and Rickwood, 1990), was used to compare the protein profiles of the two brown ear tick populations.

Materials and Methods

Ticks, protein extraction, and electrophoretic analysis

Rhipicephalus appendiculatus Neumann ticks were obtained from Muguga, near Nairobi, and Rusinga Island, in the North eastern corner of lake Victoria, in Kiambu and Suba districts of Kenya, respectively (Fig. 1). Only adult ticks were used in this study. Tick rearing, protein extraction, quantification and 2-D gel electrophoresis were carried out as reported by Baliraine *et al*. (2000).

Results and Discussion

Two dimensional electrophoresis successfully and reproducibly revealed similarities and differences between the protein profiles of the two tick populations. A total of at least 48 strongly-staining spots, ranging from about 13.5 to 54.0 KDa were detected from the two populations (Table 1). A majority of the proteins (~66.7%) were common to both populations (Table 1). However, about 12.5% of the spots were specific to the Muguga populations, while

Fig. 1. Map of Kenya, showing the two sampling sites (Muguga and Rusinga Island) from where *R. appendiculatus* used in this study were obtained

20.8% of the protein spots were observed only in the Rusinga tick population.

It is probable that some of the proteins that are common to both populations are species-specific. However, their absence in other tick species needs to be confirmed before a firm conclusion can be reached in this regard. Since all proteins are gene products (Suzuki *et al*., 1986), the presence of population-specific proteins in the Muguga and Rusinga populations (Table 1) suggests the presence of genetic differences between the two tick populations. Comparative DNA analysis studies on brown ear ticks from the same localities also revealed population-specific alleles (Baliraine, 1999), which also suggests that these populations are genetically different.

Kubasu (1992) carried out infection rate studies on *R. appendiculatus* populations in Kenya and found the ticks from Suba district to be more *T. parva* vector-competent than those from Kiambu. Recent infection rate studies on ticks from Muguga and Rusinga Islands also concur with the above observation (Osir and Kiara, unpublished data). Since the susceptibility of haematophagus arthropods to pathogens is genetically controlled (Gooding, 1996; Mutebi *et al.*, 1997; Roit *et al.*, 1993), it is reasonable to speculate that some of the population-specific proteins may be related to the observed differences in vector competence. Nevertheless, the present data alone are not sufficient to conclude that the population-specific proteins are responsible for vector competence. Some of the observed differences in the protein profiles could also arise from genes other than those responsible for vector competence (Baliraine *et al*., 2000). Further detailed genetic analysis (involving qualitative trait loci analysis/genetic mapping) of these populations are therefore needed, in view of isolating the gene(s) responsible for both *T. parva* susceptibility and refractoriness. Once the genes are isolated and their sequences determined, it can be possible to determine the corresponding proteins and use them as standards in trait diagnostic 2-DE assays, or design primers based on the gene sequences to

Table 1. Molecular weights (Kilo Daltons, KDa) of the 2-DE-resolved shared and population-specific *R. appendiculatus* proteins from the Muguga and Rusinga, Kenya. Numbers of spots sharing the same size are indicated in parentheses.

Protein spot size (~KDa)	Muguga Population	Rusinga population
13.5	-	+
13.6	-	+
14.0	-	+
14.4	+	-
15.8 (2)	+	+
18.0	+	+
18.3	+	+
18.7	+	+
19.2	+	-
21.6	-	+
23.3	+	+
24.0	+	-
24.6	+	+
25.6	+	+
25.9	+	-
26.8	+	+
27.7	+	+
28.5 (2)	+	+
29.0	-	+
29.4	+	+
30.3 (2)	+	+
31.0 (3)	+	+
31.5	+	+
31.8	+	+
32.0	+	+
33.0	+	+
34.0	+	+
34.5	+	+
35.0	+	+
36.0	+	+
36.7	-	+
37.0	+	+
39.0	+	+
40.0	+	+
41.0	-	+
44.6	-	+
45.0	+	+
46.5	-	+
47.0	+	-
50.0	+	-
48.0	-	+
52.0	+	+
54.0	+	+

+, present; -, absent

PCR-identify the *T. parva* susceptible and refractory ticks. This would have at least four practical advantages. First, the use of trait-specific primers would help avoid the labour-intensive and time-consuming infection-rate studies, and thereby enable *T. parva* vaccine researchers to quickly establish *T. parva* refractory and *T. parva* susceptible tick colonies. Highly susceptible ticks are needed for growing sporozoites for the infection and treatment method (IAM) of vaccination, and for the isolation of candidate molecules for novel molecular vaccine development (Nyindo, 1992; de Castro, 1997; Prichard, 1997). Second, lowly infected ticks are needed in the assessing of the levels of protection afforded to the animals by experimental vaccines. Third, *T. parva*-refractory ticks could be used to induce tick resistance in cattle, as an alternative approach to ECF control (Nyindo *et al*., 1996). Fourth, it would facilitate geographic mapping of vector competent and incompetent tick populations, disease risk assessment, and limiting of acaricide use, especially in areas where natural tick populations are *T. parva* refractory.

Acknowledgements— This work was funded by the Toyota Foundation and a German Academic Exchange Service (DAAD) Fellowship to FNB through the University of Nairobi. The work was carried out in the Biochemistry and Molecular Biology Unit of the International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

References

Baliraine, F. N. (1999) *M.Sc. Thesis.* University of Nairobi, Kenya.

Baliraine, F. N. et al. (2000) *Insect Sci. Applic*. **20:** 227-231.

de Castro J. J. (1997) *Vet. Parasitol.* **71:** 77-97.

Dunbar B. S. (1987) *Two-Dimensional Electrophoresis and Immunological Techniques*. 204 pp. Plenum Press, New York.

Gooding R. H. (1996) *Clin. Microbiol. Rev.* **9:** 301-320.

Hames B. D. and Rickwood, D. (1990) *Gel Electrophoresis of Proteins: A practical approach*, 2nd edn, pp. 1-270. IRL Press, Oxford.

Kubasu S. S. (1992) *Bull. Entl. Res.* **82:** 349-353.

Mutebi J. P. et al. (1997) *J. Heredity* **88:** 489-494.

Norval R. A. I. et al., 1991) *Parasitology* **102:** 347-356.

Nyindo M. (1992) Animal disease due to protozoa and rickettsia. pp 47-97. English Press, Nairobi, Kenya.

Nyindo M. et al. (1996) *J. Med. Entomol*. **34:** 559-656.

O'Farrell P. H. (1975) *J. Biol. Chem.* 250, 4007-4021.

Prichard R. (1997) *Vet. Parasitol.* **71:** 155-175.

Roit I., Brostoff, J. and Male, D. (Eds) (1993) *Immunology* pp. 5.1-14.7. Mosby -Year Book Europe Ltd., London.

Suzuki D. T., Griffiths, A. J. F., Miller, J. H., and Lewontin, R. C. (1986) *An Introduction to Genetic Analysis*, 3rd edn, pp. 504-563. W. H. Freeman and Company, New York.