Ann. Soc. belge Méd. trop.
1987, 67, 267-270

SPECIES CHARACTERISATION OF *RHIPICEPHALUS APPENDICULATUS* AND *RHIPICEPHALUS ZAMBEZIENSIS* (IXODIDAE: ACARINA) BY ENZYME-ELECTROPHORESIS

by

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Summary — Two species *R. appendiculatus* and *R. zambeziensis* are compared isoenzymatically for 7 enzymes. Because of difficult morphological identification, glucose-phosphate-isomerase and mannose-phosphate-isomerase isoenzymes can be used for identification of the two recently described species. The other five enzymes do not show a species specific pattern.

KEYWORDS: *Rhipicephalus* spp.: Species Differenciation: Isoenzymes.

Introduction

R. zambeziensis sp., a new tick described by Walker (4) occurs in Eastern and Southern Africa. The ecology differs from the ecology of *R. appendiculatus* as described by Norval *et al.* (3). In general *R. zambeziensis* is found in hotter and drier areas than *R. appendiculatus.* It has been shown experimentally by Lawrence *et al.* (2) that *R. zambeziensis* is also able to transmit *Theileria parva* in cattle.

The new tick has morphological similarity with *R. appendiculatus*. Both species show a wide range of morphological variation, so that it can be extremely difficult, even impossible to identify individual field collected specimens.

Material and methods

Ticks

Two laboratory strains of *R. zambeziensis* were obtained from Zimbabwe (West Nicholson 1976) and South Africa (Killkenny), here after NIC and KIL respectively. *R. appendiculatus* is originated from Kenya. EAVRO (Muguga 1952), hereafter MUG.

Sample preparation

Extracts of unfed adult ticks were prepared for electrophoresis by homogenising individual ticks in 300 μl of an enzymstabilisator (0.05 mol/l Tris. pH 7.5, 0.1 mol/l NaCl, 0.02 mol/l MgCl2, 0.5 mmol/l, EDTA, 0.25 mol/l sucrose, 10^{-3} mol/l 2-mercaptoethanol), 30 individuals of each strain were compared. The homogenate of each tick was ultracentrifugated at 80.000 *g* (30 min). For electrophoresis sucrose (30 μl, 20 %) was added to the sample.

Electrophoresis

Isoenzyme separations were done at 5 °C on polyacrylamidegels with the mini-slab electrophoresis system 1001 (Idea Scientific Company). Iso-electric-focusing was used with a pH range from 3 to 10. Preparation of the acrylamide gels: To 10 ml of distilled water 8 mg ammonium persulphate, 0,55 ml of carrier ampholytes (40 %, LKB, pH range 3-10), 2.5 ml of an acrylamide stock solution (400 g acrylamide and 10 g bisacrylamide per liter) and 10 μl N, N, N, N-tetramethylenediamine (TEMED) were added. This gel solution was poured in 3 frames ($10 \times 8 \times 0.5$) and polymerised in 30 minutes. A vertical system was used with two buffer compartments: 0.02 mol/l H_3PO_4 at the anode and 0.02 mol/l NaOH at the cathodal side. A constant voltage of 120 V was used with an initial current of 12mA. Table 1 summarises the revelation conditions for 7 enzymes: GPI (EC.5.3.1.9); Glucose-phosphate-isomerase, AK (EC.2.7.4.3); Adenylate kinase, MPI (EC.5.3.1.8.); Mannose-6-phosphate-isomerase; PGDH (EC.1.1.1.44); 6-Phosphogluconic dehydrogenase, EST (EC.3.1.1.); Esterase, CA (EC4.2.1.1). Carbonic anhydrase, PEP B (EC.3.4.11), Peptidase B. The duration of electrophoresis is 3 h for GPI, EST, 2.5 h for MPI, PGDH, 2 h for AK, PEP B and 75 min for CA. The incubation was carried out at 38 °C during 10 minutes to 1 hour.

TABLE 1
Electrophoresis recipes for 7 enzymes

Enzyme	Developper buffer	Activator	Coenzyme	Linking enzyme	Substrate
GPI	Tris 0.1 N pH 8 5.7 ml	MgCl 0.1 M 1 ml	NADP 5 mg	G-6-PDH5 μl	Fructose-6-P 5 mg
AK	Tris 0.1 N pH 7.4 6 ml	MgCl 0.1 M 1 ml	NADP 3 mg	G-6-PDH 10 μl Hexokinase 1 mg	ADP 12 mg Glucose 25 mg
MPI	Tris 0.06 M pH 8 6.5 ml		NADP 5 mg	G-6-PDH 10 μl GPI 10 μl	Mannose-6-P 5 mg
PGDH	Tris 0.06 M pH 8 10 ml	MgCl 0.1 M 1 ml EDTA 10 mg	NADP 5 mg		Gluconate-6-P 10 mg
PEP B	Na_2HPO_4/NaH_2PO_4 pH 7.5	MnCl 0.1 M 150 μl		L-aminoacid oxidase 0.5 mg Peroxidase 0.25 mg	L-Lencyl- L-alanine 5 mg
EST	Tris 0.1 M pH 8.8 50 ml	$MgCl_2$ 10 % 40 μl			1-naphtyl-acetate 50 mg
CA	Tris 0.06 M pH 8 25 ml				1 % 2-naphtyl-acetate solution in aceton 200 μl

Visualisation for GPI, AK, MPI and PGDH; to the revelation solution add 1.5 ml 3-(4,5-dimenthyl-2-thiazolyl-2H-tetrazolium bromide (MTT), 2 mg/ml. 30 ul Meldola Blue (6mg/ml) was added to this solution just before mixing with 5 ml agarose (2 %) at 60 °C. For PEP B add 4 mg 3-amino acid oxidase to the revelation solution. For EST and CA add Fast Blue BB (50 mg and 10 mg respectively).

Results

The characteristic enzyme phenotypes occurring in these two species are these of MPI and GPI. The species specific isoenzymes have a different

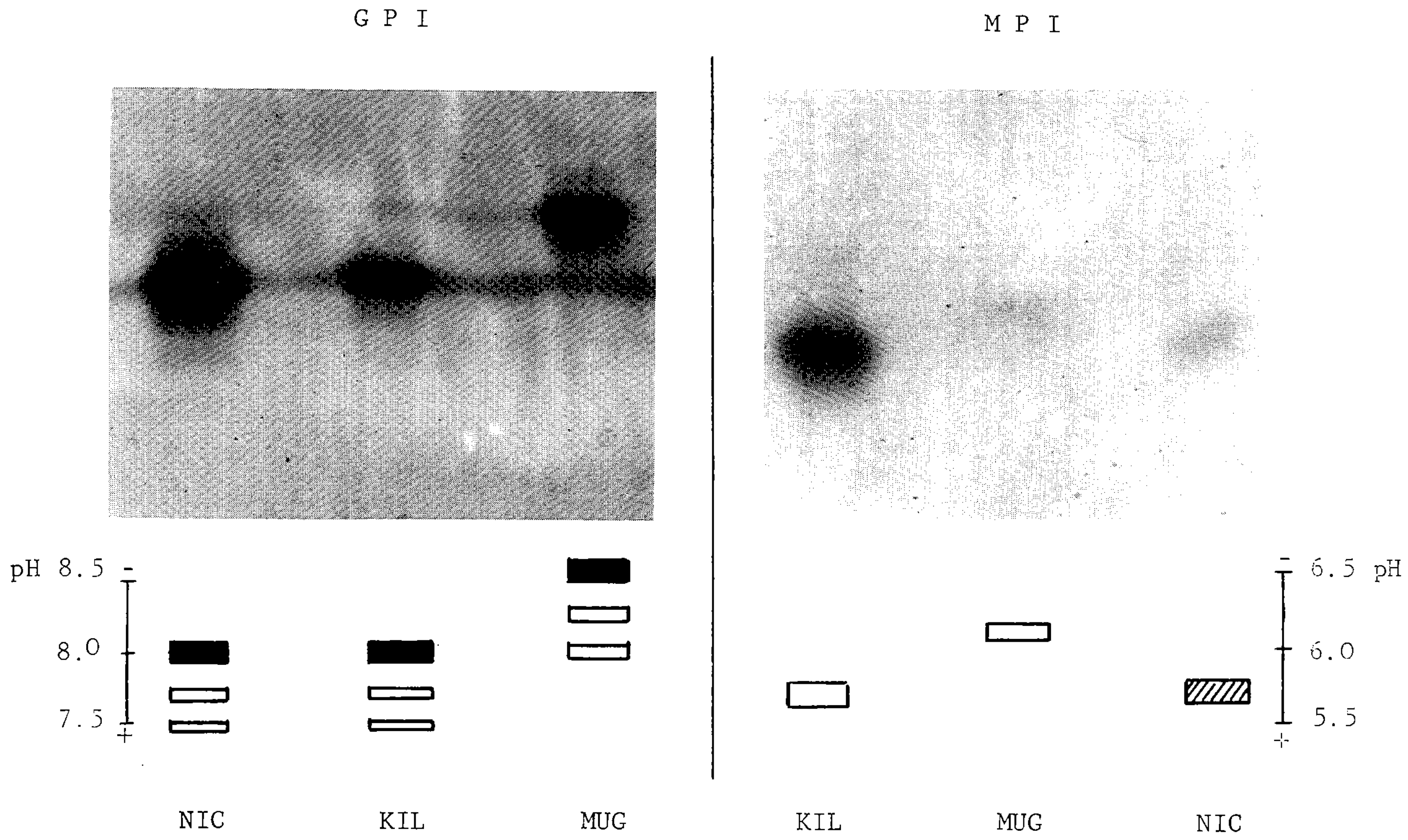

Fig. 1: Zymogram of the two different species (NIC and KIL are *R. zambeziensis*, MUG is *R. appendiculatus*).

iso-electric point. There is no difference between the NIC and KIL strain of *R. zambeziensis* for MPI and GPI. Fig. 1 gives the zymograms for the two species. The other zymograms show a non-species specific variation.

Discussion

Hunt and Hilburn (1) identified species of ticks (Ixodidae) with different isoenzyme systems. The intention of this study was to find an isoenzymatic key for identification of two ambiguous species of the Rhipicephalus genus. The MUG-strain of *R. appendiculatus* is a reference strain which is adapted to laboratory conditions. For this reason other *R. appendiculatus* specimens from the field were also checked (unpublished data). *R. zambeziensis sp.* is a closely related species of *R. appendiculatus sp.*, therefore only a few enzymes show a species genetic variation. Because of the major difficulty to distinguish these related species, isoenzyme analysis is a useful tool in the verification or identification.

Acknowledgements — *We wish to thank J.B. Walker of the Veterinary Institute, Onderstepoort, South Africa for providing ticks, M. Jochems and L. Van Peer for technical assistance.*

Caractérisation d'espèces de *Rhipicephalus appendiculatus* **et** *Rhipicephalus zambeziensis (Ixodidae: Acarina)* **par les isoenzymes.**

Résumé — Deux espèces *R. appendiculatus* et *R. Zambeziensis* ont été comparées du point de vue isoenzymatique. Puisque une identification morphologique est difficile, les glucose-phosphate-isomérase et mannose-phosphate-isomérase isoenzymes peuvent être utilisés pour identifier les deux espèces décrites récemment. Les cinq autres enzymes ne montrent pas des zymogrammes d'espèces spécifiques.

Soortbepaling van *Rhipicephalus appendiculatus* **en** *Rhipicephalus zambeziensis* **(ixodidae: Acarina) door enzymelektroforese.**

Samenvatting — Twee species *R. appendiculatus* en *R. zambeziensis* worden isoenzymatisch vergeleken voor 7 enzymen. Omwille van de moeilijke morfologische identificatie werden glucose-fosfaat-isomerase en mannose-fosfaat-isomerase isoenzymen gebruikt voor identificatie van de twee recent beschreven species. De andere 5 enzymen vertoonden geen species specifiek patroon.

Received for publication on June 18, 1987.

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