Ann. Parasitol. Hum. Comp., 1993, *68* : n° 1, 61-62.

Mémoire.

Key-words : Filaria. *Litomosoides.* Isoenzymes. Morphology. Specific diagnosis.

Mots-clés : Filaire. *Litomosoides.* Isoenzymes. Morphologie. Diagnose spécifique.

ISOENZYMATIC DIAGNOSIS OF *LITOMOSOIDES GALIZAI* AND *LITOMOSOIDES SIGMODONTIS* (1)

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SUMMARY

The morphological diagnosis of the two species, *Litomosoides sigmodontis* Chandler, 1931 and *L. galizai* Bain, Petit et Diagne, 1989 is confirmed by the isoenzymatic analysis. In *L. galizai,* the mannose-phosphate isomerase and the glucose-phosphate isomerase migrate much faster and therefore further than in *L. sigmodontis;* a polymorphism at MPI locus is shown in *L. sigmodontis* that does not exist in *L. galizai.*The « *L.* ? » line, derived from the meriones in which it and *L. sigmodontis* coexisted, is of the pure *sigmodontis* type, both in morphology and in isoenzymes.

RÉSUMÉ : **Diagnostic isoenzymatique de *Litomosoides galizai* et de *Litomosoides sigmodontis*.**

La discrimination morphologique des deux espèces *Litomosoides sigmodontis* Chandler, 1931 et *L. galizai* Bain, Petit et Diagne, 1989 est confirmée par l'analyse isoenzymatique. La mannose-phosphate isomérase et la glucose-phosphate isomérase (MPI) sont des loci diagnostics pour les deux espèces. *L. sigmodontis* a un polymorphisme génétique vis-à-vis de la MPI qui n'existe pas chez *L. galizai.* La lignée obtenue à partir d'un mérion où coexistaient les deux espèces est de type *sigmodontis* pur.

INTRODUCTION

The filaria *Litomosoides sigmodontis* Chandler, 1931, erroneously known as *L. carinii,* is a classical species in research, and *L. galizai* Bain, Petit et Diagne, 1989, has been recently adapted in the laboratories. Both species are parasites of American cricetids, respectively *Sigmodon hispidus* and *Oecomys trinitatis tapajinus.* They are differentiated by three well-defined morphological characters, e. g. the buccal capsule: thin walls for *L. galizai* vs irregularly thick walls for *L. sigmodontis,* the microfilaria: 76.8 ± 6.2 μm long vs 84.5 ± 2.9 μm, the sheath of the microfilaria: 107 ± 5.2 μm long and broad posteriorly vs 124.7 ± 2.3 μm and tapered (Bain *et al.,* 1989).

The two species may be maintained in *Meriones unguiculatus.* One of these jirds infected with *L. galizai* was subsequently inoculated with *L. sigmodontis;* the filarial line originating from this jird, and referred as « *L?* », was of the *sigmodontis* type.

An isoenzymatic analysis was performed to characterize *L. galizai* and *L. sigmodontis,* and to verify if the strain « *L?* » was hybrid or not.

MATERIALS AND METHODS

FILARIAE

Only female filariae were used for electrophoresis.

L. sigmodontis : 3 female filariae frozen at − 80° C. They were recovered from one *Mastomys coucha* received from Germany (Pr. Zahner's strain).

L. galizai : 8 females used fresh and 2 frozen females. They were recovered from 2 meriones inoculated in our laboratory.

L. ? : 26 females used fresh, 4 and 2 frozen females, respectively recovered from three meriones inoculated in our laboratory. The strain had been passaged for eight generations through jirds by inoculation of infective larvae obtained from the mite *Bdellonyssus bacoti.*

ELECTROPHORESIS TECHNIQUE

The female filariae were washed in distilled water and cut into 4 equal parts. Each part was crushed on a 2 × 3 mm rectangle of No. 3 Whatman paper and placed on starch gel. The gel was placed horizontally in a refrigerator at 4° C and an electric current of 130 to 140 volts applied for a minimum duration of 4 hours. The thickness of the gel permitted its division into 2 layers and thus the testing of 2 enzymes for each specimen provided. The technique is fully described by Gasnier, Cabaret and Moulia (1992).

The enzymes tested for were malate dehydrogenase (MDH, E. C. 1.1.1.37.), mannose-phosphate isomerase (MPI, E. C. 5.3.1.8.),

(1) This work was supported by grants from CEC (S1254 and TS2-0067-F).

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Accepté le : 22 septembre 1992.

lactate dehydrogenase (LDH, E. C. 1.1.1.27.). and glucose-phosphate isomerase (GPI, E. C. 5.3.1.9.).

RESULTS

LDH activity exists, but because there is no migration in any of the specimens tested, no conclusions can be made.

There is MDH activity and slight migration. This was the same in all the specimens examined.

Two reactions were seen with the GPI. In *L. galizai* the migration could be considered rapid, whereas in *L. sigmodontis* and *L.* ?, the GPI migrated slowly. The bands were at exactly the same height for *L. sigmodontis* and *L.* ? For each material, the locus did not indicate a genetic polymorphism *(Fig. 1)*.

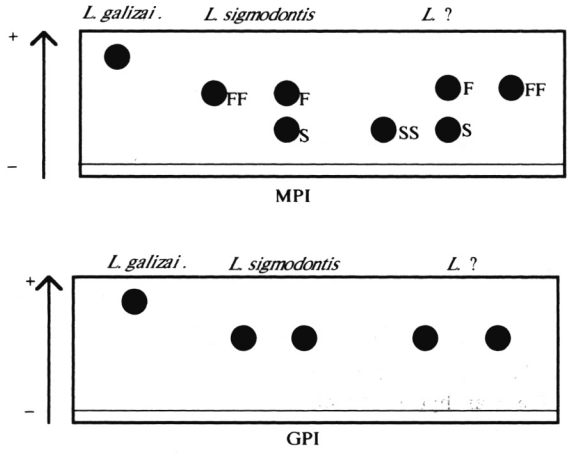

FIG. 1. — Electrophoretic patterns of *L. galizai, L. sigmodontis* and *L.* ? for the GPI and the MPI.

Two reactions were seen with the MPI. In *L. galizai* the migration was much faster than that of the other 2 materials; the locus appeared to the monomorphic. A genetic polymorphism appeared to exist in the MPI of *L. sigmodontis* and *L.* ? as heterozygotes (FS) were associated with the slow (SS) and fast (FF) homozygotes *(Fig. 1)*.

CONCLUSION

It has been suggested that the genus *Litomosoides* is undergoing a recent evolution in the neotropical cricetids (Bain *et al.*, 1989). The species are numerous and close and, therefore, it is interesting to compare the morphological taxonomy with the isoenzymatic diagnosis.

The two species studied here, *L. galizai* and *L. sigmodontis,* show two different isoenzymatic patterns. This result is in accordance with the morphological diagnosis and it confirms the discriminating validity of the characters shown by the buccal capsule, the microfilaria and the microfilarial sheath (Bain *et al.*, 1989).

The *L?* line, derived from a jird infected with *L. galizai* and subsequently with *L. sigmodontis,* is of the pure *sigmodontis* type, both in morphology and in isoenzymes, which indicates that no hybridisation took place.

Contrary to *L. galizai, L. sigmodontis,* appears to be polymorphic at one locus, the mannose-phosphate isomerase.

Acknowledgments. — We are grateful to Pr. H. ZAHNER, Institut für Parasitologie, Rudolf Bucheim Str,2 D-Giessen for specimens of *Litomosoides sigmodontis* and to J. F. Duc for his technical help.

RÉFÉRENCES

Bain O., Petit G., Diagne M. : Étude de quelques *Litomosoides* parasites de Rongeurs; conséquences taxonomiques. *Ann. Parasitol. Hum. Comp.*, 1989, *64*, 251-256.

Gasnier N., Cabaret J., Moulia C. : Allozyme variation between laboratory reared and wild populations of *Teladorsagia circumcincta. Int. J. Parasit.*, in press.