Restriction endonuclease analysis of the DNA of local strains of *Leptospira interrogans* of Pomona serogroup

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**Summary:** Leptospirosis is a bacterial disease caused by Leptospira interrogans. The species *interrogans* contains more than 200 serovars which are often difficult to classify by ordinary serological procedures.

While *interrogans* occurs throughout the world, its serovars have a regional distribution. In Argentina, the serovars of greatest epidemiological importance belong to the Pomona serogroup.

Analysis of chromosomal DNA by restriction endonuclease was first applied to the taxonomy of the genus *Leptospira* in 1981. The present authors applied this technique to five isolates of different geographical origin, obtained from various animal species, and classified in the authors’ laboratory as belonging to the Pomona serogroup, using the serovars pomona and kennewicki as reference strains.

These isolates had a similar restriction profile when the enzymes ‘Eco RI’ and ‘Hha I + Bgl II’ were used; they were similar to serovar kennewicki and distinct from serovar pomona. These results may be of major epidemiological importance, especially for effective prophylaxis.

**KEYWORDS:** Leptospira interrogans - Molecular biology - Serovars - Taxonomy - Vaccine strains.

**INTRODUCTION**

Leptospirosis is an infectious disease capable of attacking human beings and animals, caused by *Leptospira interrogans*.

This species contains more than 200 serovars usually subdivided into 25 serogroups. *L. interrogans* occurs throughout the world, but the strains responsible differ slightly from one place to another because of a regional distribution of serovars (9).

In Argentina, serological surveys show that the prevalent serogroups are Sejroe and Pomona, while isolates obtained from clinical cases mostly belong to Pomona serogroup (3).

Conventional serological classification is based on titres obtained in the micro-agglutination test, before and after absorption by diluted hyperimmune serum against

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homologous and heterologous strains, and against reference and unknown strains. The results are easy to interpret to the level of serogroup, but it is more difficult to go to the serovar level.

Many other techniques have been used for taxonomic classification of *L. interrogans*, such as lipase analysis (1, 5), determination of base composition and DNA homology (2, 6), and immunodiffusion analysis of antigen prepared from the axial filament (4). In 1967, Kmetý (7) proposed a variation of the original serological method, but there has been difficulty in standardising the reagents.

The development of techniques in molecular biology has provided another approach to classifying leptospires. Terpstra *et al.* (10) produced and employed monoclonal antibodies for classifying serovars of the Sejroe serogroup.

In 1981, Marshall *et al.* (8) described restriction endonuclease analysis (REA) of the chromosomal DNA of *L. interrogans* for taxonomic purposes, and subsequently the technique was modified slightly by Thiermann *et al.* (11, 12), who demonstrated a correlation between the results obtained and epidemiological data.

This report describes the use of REA to compare reference strains with five Argentinian isolates of the Pomona serogroup, together with a discussion of their relationship to epidemiological findings.

**MATERIALS AND METHODS**

**Reference strains**

Serovars *pomona* and *kennewicki* of *L. interrogans* were obtained from the Centers for Disease Control, Atlanta, Georgia, USA.

**Local strains**

These were obtained by the Leptospirosis Unit of the Institute of Bacteriology of CICV-INTA at Castelar (Argentina) from distinct outbreaks of the disease and from different species: Longchamps strain (of human origin), Cañuelas I and San Alfredo I (of porcine origin) and Fulton (of bovine origin).

Strain Corrientes 266 of bovine origin was isolated at the EEA-INTA establishment at Mercedes-Corrientes.

All the local strains were classified as members of the Pomona serogroup by cross-absorption tests conducted at the authors' laboratory.

**Purification of leptospiral DNA**

The technique described by Thiermann *et al.* (11) was used.

**Digestion with restriction enzymes**

About 3 µg portions of DNA were digested under the conditions specified by the suppliers of restriction endonuclease, using the enzymes 'Eco RI', 'Hha I' and 'Bgl II' from BRL (Bethesda Research Laboratory, USA).
**FIG. 1**

Electrophoretic profile of chromosomal DNA from *L. interrogans* serovars *pomona* and *kennewicki* and from local isolates, digested with enzymes 'Eco RI' and 'HhaI + Bgl II'
Electrophoresis of digested DNA

This was performed in 0.7% agar gel with TBE buffer, as described by Thiermann et al. (11). The result was read by exposing the gel to ultraviolet light under transillumination after staining with ethidium bromide.

RESULTS

The results obtained are depicted in Figures 1 and 2. After digestion with 'Eco RI' and double digestion with 'Hha I + Bgl II', it was clear that the local strains, regardless of their origin, had identical electrophoretic profiles, similar to that of serovar kennewicki and differing from the reference strain of serovar pomona. After digestion with 'Hha I' alone, there were also differences between the isolates and the reference strain of serovar pomona.

DISCUSSION

The results proved the identity of the restriction profiles (obtained with 'Eco RI' and 'Hha I + Bgl II') of local strains, regardless of their species origin and geographical source. These profiles were very similar to the profile of serovar kennewicki, and different from that of serovar pomona. These findings agree with those of Thiermann et al., who examined isolates from USA, Canada and New Zealand, classified serologically as members of the Pomona serogroup (11).

The slight differences between local strains observed when 'Hha I' was used are difficult to interpret and require further study, but it is clear that the profile differs from that of serovar pomona.

The epidemiological features observed are a relatively high endemic occurrence in areas where routine vaccination has been practised, with outbreaks of acute infection (verified by isolating the causal agent) in pig and cattle herds vaccinated against leptospirosis.

The results obtained here, coupled with the epidemiological findings, demonstrate the need to revise the selection of vaccine strains. The use of strains of epidemiological importance in a given region in vaccine preparation would probably improve vaccine efficacy.

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FIG. 2
Electrophoretic profile of chromosomal DNA from *L. interrogans* serovar *pomona* and from local isolates, digested with enzymes ‘Hha I’ and ‘Bgl II’
REFERENCES


