



Title	COMPARATIVE STUDY OF LEPTOSPIRAL STRAINS ICTERO NO. I AND RGA BY RESTRICTION ENDONUCLEASE DNA ANALYSIS
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BRIEF COMMUNICATION

COMPARATIVE STUDY OF LEPTOSPIRAL STRAINS  
ICTERO NO. I AND RGA  
BY RESTRICTION ENDONUCLEASE DNA ANALYSIS

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DNA analysis

Strain Ictero No. I, the first isolate of *Leptospira* and belongs to serovar *icterohaemorrhagiae*, was isolated by INADA & IDO<sup>2)</sup> from a patient suffering from Weil's disease in Kyushu University Hospital, Fukuoka Japan in 1914. Strain FGA, the next isolate and the first isolate of *Leptospira* in Europe, was isolated by UHLENHUTH & FROMME<sup>8)</sup> in Germany from a patient with Weil's disease in 1915. Strain RGA is designated as the type strain of *Leptospira interrogans*,<sup>3)</sup> in spite of the existence of the older Japanese strain, Ictero No. I. Strain Ictero No. I has been said to be insufficiently documented simply because many of the early reports on strain Ictero No. I were made in Japanese. The present communication deals with restriction endonuclease DNA analysis of strain Ictero No. I, as a part of our comparative studies of strains Ictero No. I and RGA.

Strain Ictero No. I was received in 1985 from Professor KOBAYASHI, Department of Medicine, University of Ehime, Shigenobu, Ehime Prefecture. Two lines of strain RGA were used. One line of strain RGA was provided in 1969 by Dr. KITAOKA, then Leptospirosis Reference Laboratory, National Institute of Health, Tokyo, Japan, and maintained in our laboratory (RGA line Sapporo). Another line was provided in 1987 by Dr. TERPSTRA, the Royal Tropical Institute, Amsterdam, The Netherlands (RGA line Amsterdam). Leptospiral DNA was extracted essentially by the method described by TERPSTRA et al.<sup>6)</sup> Bacterial DNA (3  $\mu$ g) were completely digested with 20 units of restriction endonuclease (BglII, EcoRI, HindIII or XhoI; Nippon Gene, Toyama, Japan) using the temperature and buffer specified by the supplier. Digested DNA was

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precipitated by ethanol and redissolved in 10mM Tris-HCl, 1mM sodium EDTA, pH 7.5. Electrophoresis was carried out in a horizontal 0.7% agarose slab gel in a Tris-acetate buffer system (40mM Tris base, 20mM sodium acetate, 2mM EDTA, pH 8.3) at 5V/cm for 5–7 hr. Gels were stained for 15 min in 0.5  $\mu$ g/ml ethidium bromide, and the DNA bands were visualized at 254nm with an ultraviolet light transilluminator (Ultraviolet Products, San Gabriel, Ca 91778, USA). Gels were photographed on Polaroid type 667 film by means of a Polaroid MR-4 land camera.

Figure 1 shows DNA restriction endonuclease patterns of strains Ictero No. I and RGA (lines Sapporo and Amsterdam) digested using the restriction endonuclease EcoRI or HindIII. No differences could be shown between the restriction endonuclease patterns of strains Ictero No. I and RGA (lines Sapporo and Amsterdam). The findings were confirmed using other enzymes, BglII and XhoI (data not shown).

Leptospiral serovars belonging to different serogroups were shown to be different in their DNA restriction endonuclease patterns.<sup>4,5)</sup> The technique was reported to be sensitive enough to differentiate among the different leptospiral serovars.<sup>4,7)</sup> However, ROBINSON et al.<sup>5)</sup> reported that the field strains of serovar *hardjo*, *balcanica* or *tarassovi* showed the DNA restriction endonuclease patterns slightly different from those of the reference strains of each serovar. YAMAGUCHI (Master's thesis, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 1987) reported that each antigenic variant derived from *canicola* and *hebdomadis*, respectively, and serologically different from the parent was found to be similar to the parent by restriction endonuclease DNA analysis. It seems that more comprehensive studies are needed before the technique can be applied in the classification of *Leptospira*, particularly in differentiation of serovars belonging to the same serogroup.

In the present study, strains Ictero No. I and RGA were indistinguishable from each other by restriction endonuclease DNA analysis using BglII, EcoRI, HindIII and XhoI. The results may show that strain Ictero No. I is genetically close to strain RGA. It was recently found that strain Ictero No. I was not serologically identical with strain RGA (HATA, K., Master's thesis, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 1987). Based on the finding and some additional experiments, HATA et al. (submitted for publication) proposed that strain Ictero No. I should represent serovar *icterohaemorrhagiae* and be recognized as the type strain of the genus. This is consistent with the report of BABUDIERI & SMITH.<sup>1)</sup> It seems difficult, at this stage, to differentiate strains Ictero No. I and RGA by restriction endonuclease DNA analysis.

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Figure 1. Comparison of DNA restriction endonuclease patterns of strains Ictero No. I and RGA using EcoRI and HindIII. Lanes: a. Ictero No. I; b. RGA (line Sapporo); c. RGA (line Amsterdam). Lane d is bacteriophage lambda DNA digested with HindIII as size standard. Fragment sizes are in Kb from top to bottom 23.13, 9.42, 6.56, 4.35, 2.32 and 2.02.

