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LABORATORY STUDY

DNA relatedness and serotyping of *Leptospira* strains

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Abstract: *Background:* In a previous study (5), the constructed phylogenetic tree for leptospires belonging to 12 different serovars common in Central Europe made the prediction of serovar from knowing the genotype and vice versa possible.
Objective: The study was aimed at investigation of the usefulness of such procedure to distinguish in between at present to us available and worldwide accepted reference strains of pathogenic *Leptospira* serovars.
Material and methods: One hundred and seventy seven *Leptospira* strains representing different serovars were tested. DNA fingerprints of these strains were performed, digitally captured and as described earlier of those phylogenetic tree using different fingerptinting software was constructed using UPGMA clustering method with band matching by the Dice coefficient (5).
Results: At this tree, 145 of 177 *Leptospira* strains tested each took a unique position, and the remaining 32 strains were distributed at 15 different positions (each of 14 positions taken by two different strains and one position taken by four strains).
Conclusion: The constructed phylogenetic tree likely to be very useful in prediction of *Leptospira* serovar in most cases of an infection so the saving time and being helpful in serovar identification of the pathogenic agent *(Fig. 1, Ref. 9)*. Full Text in PDF *www.elis.sk*.
Key words: *Leptospira* serovars, RAPD, phylogenetic tree, DNA fingerprinting, genotyping.

For diagnostic and epidemiological purposes in cases of infections caused by leptospires in man or animals, rapid identification of the serovar of leptospires involved is often necessary (1, 2). DNA fingerprinting was found to be useful in distinguishing of limited number of *Leptospira* strains (3, 4). In a former study, depending on RAPD-fingerprinting of leptospires encountered in Central Europe, the constructed phylogenetic tree was found to be a useful and reliable method to differentiate between strains of leptospires belonging to distinct serovars/serogroups and it also made the prediction of the serotype from knowing the genotype and vice versa possible (5).

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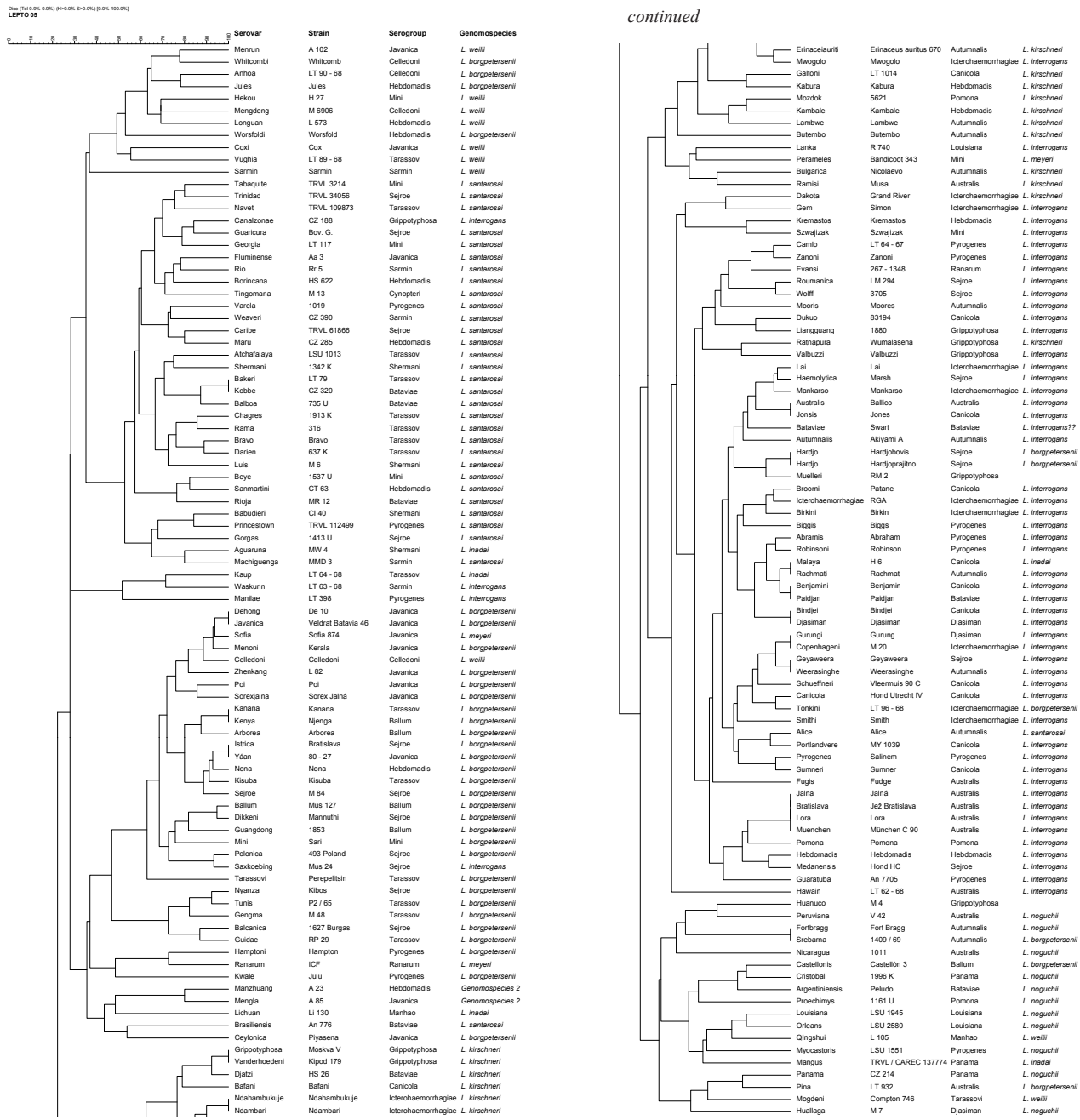
Therefore it was of interest to examine the usefulness of such procedure to distinguish in between at present to us available and as reference strains of *Leptospira* serovars worldwide in use.

Materials and methods

Leptospira strains (Fig. 1). By the Subcommittee on the Taxonomy of *Leptospira* recognized reference strains representing 177 pathogenic *Leptospira* serovars belonging to 23 serological groups (6) were used. The strains were grown in Korthof´s or Ellighausen-McCullough-Johnson-Harris liquid media (Difco laboratories, Detroit, MI, USA), namely serogroup Sejroe – 18 serovars, Tarassovi – 16, Javanica – 13, Pyrogenes – 13, Icterohaemorrhagiae – 12, Hebdomadis – 12, Canicola – 12, Australis – 11, Autumnalis – 11, Bataviae – 8, Grippotyphosa – 8, Mini – 7, Ballum, – 5, Sarmin – 5, Celledoni – 4, Shermani – 4, Pomona – 3, Djasiman – 3, Panama – 3, Louisiana – 3, Cynopteri – 2, Manhao – 2, Ranarum – 2). The reference strains belong to 9 genomospecies (*weilii, borgpetersenii, santarosai, interrogans, inadai, meyeri, kirschneri, noguchii* and genomospecies 2 (7).

DNA- exraction by DNeasy® Tissue Kit (QIAGEN Gmbh, Hilden, Germany) was performed. Amount of DNA measured by Dyna Quant 200 Fluorometer (Hoefer, Inc., San Francisco, CA, USA). RAPD testing using the primer O5 5´-AGGGGTCTTG-3´ synthesized by VBC- Biotech Service GmbH (Vienna, Austria), Ready-To-Go™ Analysis Beads [Amersham Biosciences / GE Healthcare)]. All amplification reactions were performed in a PerkinElmer Cycler 9600 (Norwalk, USA) under the amplifica-



continued

Fig. 1. DNA relatedness among reference strains recognized by the Subcommittee on the Taxonomy of *Leptospira* which represent 177 serovars of leptospires. Figure is shown on the website www.elis.sk

tion conditions recommended by Williams et al.(1990) (8) PCR-products were resolved by electrophoresis (100V/75 min. Bio-Rad castgel) using 2.5 % agarose (Ultra pure probe Agarose, Bio-Rad Laboratories, Inc., Hercules, CA, USA), stained with ethidium bromide and photographed; the digitally captured profiles were analysed using the Gel Doc™ 2000 system in combination with Quantity One® Software (Bio-Rad). To achieve reproducible DNA fingerprints, the GeneRuler™ 100bp DNA ladder Plus, #SM0323 (Fermentas UAB, Vilnius, Lithuania) was applied to the outer lanes and, normally, also to three intermediate lanes of each agarose gel.

Phylogenetic analysis of the DNA-fingerprints was performed with the Bio-Rad Fingerprinting™ II software package. The phylogenetic tree was constructed by the complete linkage using the UPGMA clustering method with band matching by the Dice coefficient.

All steps were performed as described earlier (5, 9).

Results

The constructed phylogenetic tree of this study allowed to distinguish between the serovars of leptospires tested, except 14

pairs of these, namely Bakeri and Kobbe, Dehong and Javanica, Kanama and Kenya, Istrica and Yáan, Grippotyphosa and Vanderhoedeni, Ndahambukuje and Ndambari, Australis and Jonsis, Hardjoprajitno and Hardjobovis, Malaya and Rachmati, Benjamini and Paidjan, Bindjei and Djasiman, Gurungi and Copenhageni, and Weerasinghe, Fortbragg and Srebarna Bratislava, and the tetrad (group of four) Jalna, Bratislava, Lora, Muenchen which took the same position at the tree. Nine pairs of the 14, each of them belong to different serogroups, while the remaining 5 pairs each of their member contains serovar of the same serogroup.

Nevertheless, some but not all *Leptospira* serovars of certains serogroups are sufficiently related to one another in order to form more or less apparent clusters. For example: serovars Istrica, Sejroe, Dikkeni, Polonica, Saxkoebing (serogroup Sejroe), serovars Atchafalaya, Bakeri, Chagres, Rama, Bravo, Darien (serogroup Tarassovi), serovars Dehong, Javanica, Sofia, Menoni, Zhenkang, Poi, Sorexjalna *(serogroup Javanica*) or serovars Jalna, Bratislava, Lora and Muenchen (serogroup Australis). It is noteworthy that some mentioned clusters encompass also serovars of other serogroups (serogroups Sejroe, Tarassovi).

Discussion

As described earlier (5), based on comparison of digitally captured and analysed RAPD- fingerprints of each of the 177 serovars of leptospires of this study using different computer programs the presented phylogenetic tree was constructed.

Of these worldwide as reference strains of leptospires in use, 145 serovars, each took a unique position at the mentioned tree and remained distinguishable. The correspondence between the position at the tree (genotype) and serotype of each of mentioned serovars of leptospires make in this cases the estimation of serotype from the genotype and vice versa possible.

These observations enhance and shorten the time of identification of isolates of leptospires involved in outbreaks what is of significant epidemiological and public health value.

In contrast to the distinguishable serovars of leptospires tested, the remaining 32 strains were distributed at 15 different positions, 14 of these each contain two serovars (the members of 9 pairs belong to different serogroups); in the case of five pairs each strain is member of the same groups) and position 15 occupied by four serovars belonging to the same serogroup.

Nevertheless, the constructed tree of this study is likely to be a useful tool in aiding rapid identification of each of the majority of *Leptospira* tested and to be helpful to estimate the member of undistinguishable pair or tetrad of these and so limiting further investigations necessary to find out which serovar involved in infection.

The constructed phylogenetic tree in this study does at least identify each of the majority of the reference serovars tested and to divide the remaining ones in pairs and in one tetrad. Additional genotyping of the later mentioned, at the phylogenetic tree not separated serovars is needed.

No correlation between the distribution of serovar and genomotype was found in agreement with results obtained by DNA – DNA hybridization performed some years ago (9). In spite of that, the constructed phylogenetic tree will be a very useful tool in prediction of at least the *Leptospira* serovar in most cases of leptospiral infection and so save the time and be very helpful for serological diagnostics.

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