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The application of monoclonal antibody methodology as a tool for serotyping leptospira isolates in Cuba

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SUMMARY

By using the monoclonal antibodies methodology we have classified Cuban serovars of leptospires isolated from blood culture of confirmed leptospirosis patients. The serogroups Pomona and Canicola were the most predominant found in our study. Pomona, Mozdok and Canicola serovars were the most prevalent types in these serogroups. Our study has essentially presented a validation of a monoclonal antibody method for the serotyping of Cuban leptospire isolates.

Key words: Leptospirosis, monoclonal antibodies, serotyping.

Present identification methods for leptospira isolates are not always concordant and when used single they may fail to show small but possible important differences between leptospire strains.

There is a limited number of serological methods available to classify new leptospire isolates. Conventional typing by the cross agglutination absorption test (CAAT) (gold standard test) is complicated, time consuming, subjective and insensitive.

For this reason the CAAT for the classification of leptospires has been complemented by various newer methods. The availability of monoclonal antibodies has increased the speed and specificity of the classification of leptospire serovars. Monoclonal antibodies can also be used for the diagnosis or evaluation of vaccine preparations and for the detection of antigenic mutants in culture.

In this study we are presenting the general results about the application of monoclonal antibody methodology as a tool for serotyping Cuban leptospiras isolates.

Leptospira strains: Sixteen Cuban strains of *Leptospira* spp. isolated from blood culture of confirmed leptospirosis patients were studied. All strains were cultured in Ellinghausen, Mc Cullough, Jonhson and Harris (EMJH) media at 30 ºC and checked for the presence of contaminating aerobic bacteria after 7-10 days growth.

Polyclonal rabbit immune sera: Polyclonal rabbit immune sera employed in serogrouping were kindly supplied by the Royal Tropical Institute, Amsterdam, The Netherlands. They were directed against the leptospira serogroups representing *Leptospira interrogans* complex (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Lousiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Sarmin, Sejroe, Shermani, Tarassovi).

Microscopic agglutination test (MAT): The conventional MAT was performed, to classify into serogroups the leptospira strains, as described by Dikken and Kmety in 1978.[1]

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Monoclonal antibodies-Monoclonal antibodies were obtained according to standard techniques as previously described by Terpstra et al.[2] The whole set of Pomona, Icterohaemorrhagiae and Canicola monoclonal antibodies were kindly offered by the Royal Tropical Institute, Amsterdam. Serial dilution of antibodies were made in phosphate buffered saline (PBS) pH 7.2 and 50 µL aliquots placed in each well of a microtitre plates to which was added an equal volume of a 7-10 days old culture of live leptospires in EMJH medium. After 2-4 h of incubation at 37 ºC, the agglutination titre was determined by dark field microscopy.

The results showed that ten (63 %), five (31 %) and one (6 %) of the strains belonged to Pomona, Canicola and Icterohaemorrhagiae serogroups, respectively.

Of the 10 leptospire isolates identified as Pomona serogroup, five were classified as Pomona serovar, and the other five as Mozdok serovar (table 1). The monoclonal antibodies designed F43C9 and F48C6 recognize Pomona like strains.[2]

The monoclonal antibodies F46C4, F46C1, F48C6, F48C1, F58C2, F58C1, F46C10, were used to identify the Mozdok serovar.

The Canicola group represented the 31 % of all the studied strains. The monoclonal antibodies classifying these strains into Canicola serovar were: F152C1, F152C2, F152C4, F152C8. All the titres obtained were high (table 2).

Only one strain (1110-95) belonged to the Icterohaemorrhagiae group Copenhageni serovar, which was typed with the monoclonal antibodies: F12C3 (1/120480), F20C3 (1/140960), F20C4 (1/ /81920) and F10C20 (1/140960).

The monoclonal antibodies based classification technique is easy to apply and the results can be completed in 2-4 h. The serovar specifity of monoclonal antibodies is very high in order to discriminate between two strains belonging to the same group.[2]

TABLE 1. Reciprocal agglutination titres by microscopic agglutination test to Pomona group using the monoclonal antibodies

Strain number	Monoclonal antibodies (titre)								
2 112-96[a]	F43C9 (80)					F48C6 (160)			
1 975-95[a]	F43C9 (160)					F48C6 (1280)			
2 112-95[a]	F43C9 (160)					F48C6 (1280)			
1 977-95[a]	F43C9 (160)					F48C6 (1280)			
2 011-95[a]	F43C9 (160)					F48C6 (2560)			
1 362-95[b]	F46C4 (20480)	F46C9 (10240)	F46C1 (1280)	F48C1 (2560)		F48C6 (20480)		F58C2 (40960)	F58C1 (40960)
1 794-95[b]	F46C4 (2560)	F46C9 (2560)	F46C10 (5120)			F58C1 (40)		F58C1 (730720)	F58C2 (66530)
1 922-95[b]	F46C2 (5120)	F46C5 (2560)	F46C9 (5120)	F46C10 (10240)		F48C6 (20480)	F58C6 (20480)	F58C1 (41960)	F58C2 (81920)
1 924-95[b]	F46C1 (665320)	F46C2 (81920)	F46C4 (2560)	F46C10 (10240)	F48C1 (10240)	F48C3 (10240)	F48C6 (20480)	F58C1 (10240)	F58C2 (5120)
1824-95[b]	F46C1 (40960)	F46C4 (20480)	F46C5 (20480)		F46C10 (20480)	F48C1 (2560)	F48C9 (20480)	F58C1 (163840)	F58C2 (81920)

a: Pomona group Pomona serovar, b: Pomona group Mozdok serovar

TABLE 2. Classification of Canicola group using monoclonal antibodies by microscopic agglutination test

Strain number	Monoclonal antibodies (titre)								
1 699-96[a]	F152C1	F152C2	F152C7	F152C10	F152C11	F152C13	F152C14	F152C17	F152C18
256-96[a]	(20480)	(10240)	(40960)	(20480)	(20480)	(2560)	(5120)	(5120)	(2560)
274-96 [a]	F152C1	F152C11	F152C4		F152C8		F152C10	F152C14	
	(20480)	(5120)	(2560)		(320)		(80)	(80)	
2 299-95[a]	F152C10 (640)								
48-96[a]	F152C1 (5120)		F152C2 (5120)		F152C7 (10240)		F152C8 (40)		F40C1 (20480)
45-96[a]	F152C1 (640)		F152C2 (320)		F152C14 (320)		F40C1 (20480)		

a: Canicola group Canicola serovar

Experiments to test serotypes variability was not found in our research. However, some authors had not been able to explain the reason for variability in titres when MAT using monoclonal antibodies had been made.[3,4]

In the Netherlands over the period 1987-1993, seven hundred and twenty eight (728) Leptospire isolates were identified to serovar level, which was possible mainly by using monoclonal antibodies, by cross agglutination absorption tests or by DNA based techniques.[5]

Dutch isolates belonging to 14 serovars were found: Autumnalis Bim, Ballum Arborea, Canicola Canicola and Dukuo, Grippotyphosa Grippotyphosa and Grippothyphosa like, Icterohaemorrhagiae Copenhageni and Icterohaemorrhagiae, Pomona Mozdok and Pomona, Sejroe Hardjo and Saxkoebing and Tarassovi Tarassovi. In some cases a comparison is made between leptospire isolates and the reference strain of serovar to which these isolates belong (histograms).[5]

Peripheral laboratories can type leptospires isolates when equipped with panels of monoclonal antibodies adjusted to the locally circulating strains.[6]

Clasificación de leptospiras aisladas en Cuba con la utilización de anticuerpos monoclonales

RESUMEN

Se clasificaron, mediante la metodología de los anticuerpos monoclonales, los serovares cubanos de *Leptospira* aislados en hemocultivos de pacientes con leptospirosis confirmada. Los serogrupos Pomona y Canicola fueron los predominantes. Los serovares Pomona, Mozdok y Canicola resultaron los más frecuentes dentro de esos serogrupos. Se presentó la validación del método de anticuerpos monoclonales para la tipificación serológica de las leptopiras aisladas en Cuba.

Palabras clave: Leptospirosis, anticuerpos monoclonales, tipificación, clasificación.

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