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Short Communication

Leptospira Serovars Circulating in Eastern Region of Cuba, from 2007 to 2012

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Abstract

Identification of *Leptospira* serovars is necessary for epidemiological surveillance. In Cuba, few papers have been published on this topic. However, Pomona, Canicola, Icterohaemorrhagiae and Ballum serogroups have been found in patients with leptospirosis. The goal of this research is to apply the hybridoma antibodies technique to typing *Leptospira* serovars from human cases living in the Eastern region of Cuba, during 2007 to 2012. Arborea, Canicola, Pomona, Icterohaemorrhagiae serovars were the most prevalent encountered in this investigation. The distribution of *Leptospira* serovars in two Cuban provinces from the Eastern region (2007-2012)is quite similar to other studies developed before in this country.

INTRODUCTION

Leptospirosis is a worldwide zoonosis, caused by *Leptospira interrogans* sensulato. Serovars antigenically related classify *Leptospira* in serogroups, which have clinical and epidemiological importance. Identification of serovars is necessary for epidemiological surveillance since some of them have preferential animal reservoirs or are associated with certain clinical forms of the disease. The serovar concept is still applicable in many situations but is not always fully satisfactory, as it may delineate artificially closely related strains on one side while failing to distinguish between antigenically similar strains that have different ecological niches on the other hand side. Isolation and identification of *Leptospira* strain is difficult and time consuming due to the slow growth rate, for that *Leptospira* is considered as fastidious a bacteria. Actually, more than 250 pathogenic serovars have been arranged in 26 serogroups [1].

Pending the development of a classification system based on the molecular typing methods, the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Leptospira* recommended that new field isolates should still be typed by the recognized standard method, the cross-agglutinin absorption test (CAAT), and given serovar status which represent the basic taxon. CAAT is satisfactory but the test procedures are too complicated and time consuming to be of value when rapid results are need. Factor sera allow rapid typing but they are only available in limited quantities and their quality varies. Using the hybridoma technique antibodies of known specificity can be reproduced in unlimited amounts. The availability of monoclonal antibodies has increased the speed and specificity of the system for classification of *Leptospira* serovars. Monoclonal antibodies can also be used for the diagnosis or evaluation of vaccine preparations and for detection of antigenic mutants in culture [2].

In America serovars isolation from human has been limited. In the Caribbean region there are some reviews to describe the changes in epidemiology of leptospirosis and serotyping of *Leptospira* [3-6]. However in Cuba, few papers have been published. Limitations on isolation from human cases can be determined by the lack of indication of this procedure from clinical samples obtained during the acute phase of the disease. During 1994-2007, by using polyclonal rabbit immune sera and monoclonal antibodies (mAbs) by the microaglutination technique (MAT) allowed to detect low titers of Pomona, Canicola, Icterohaemorrhagiae and Ballum serogroup/serovars in *Leptospira* isolates from Cuban confirmed patients. The aim of the current study is to identify the main serogroups and serovars of *Leptospira* in Cuban isolated from patients of two Eastern provinces during 2007-2012.

METHODS

Leptospira strains

79 Cuban strains of *Leptospira* spp. isolated from blood culture of confirmed leptospirosis cases from 2007 to 2012 were studied. All strains were cultured in Ellinghausen, McCullough, Jonhson and Harris (EMJH) media at 30ºC and observed for the presence of contaminating aerobic bacteria after 7 or 10 days growth [7].

Microscopic agglutination test (MAT)

The conventional MAT was performed, to classify into serogroups the *leptospira* strains, as described by Dikken and Kmety [8].

Polyclonal rabbit immune sera

Polyclonal rabbit immune sera employed in serogrouping were directed against the *Leptospira* serogroups representing *L. interrogans* sensulato complex (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Louisiana, Manhao, Mini, Panama, Pomona, Pyrogenes, Sarmin, Sejroe, Shermani, Tarassovi) [8].

Monoclonal antibodies

The whole set of Pomona (F46C1-5, F46C4-3, F48C1-4, F48C6-5, F43C9-5, F58C2-3), Icterohaemorrhagiae (F70C14, F70C24, F70C7-11, F82C1-3, F52C1-4, F12C3-11) Canicola (F152C7-4, F152C18-4, F152C11-3) and Ballum (F74C1-7, F74C4-4, F74C7-4) mAbs were used. 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 plate to which was added an equal volume of a 7 to 10 days old culture of live *Leptospira* in EMJH medium. After 2 - 4 h of incubation at 37ºC, the agglutination titre was determined by dark field microscopy [9].

PCR-LipL32

Isolation of chromosomal DNA of all non typable Cuban isolates was studied by PCR based on the pathogen-specific *lipL*32 genes previously described [10].

RESULTS

In the current study the 88.6% of isolates (70/79), corresponding 21 to Las Tunas and 58 to Holguin provinces were typed. Among the typed isolates by Polyclonal rabbit immunesera were found 21(30%) belonging to Ballum serogroup, 19 as Canicola (27.1%), 14 as Pomona (20%), 8 as Icterohaemorrhagiae (11.4%), 6 as Hebdomadis (8,6%), and only one (1.4%) as Louisiana and Pyrogenes,respectively (Figure 1).The 11.4% (9/79) were non typable serologically.

All the non typable as pathogenic isolates by polyclonal rabbit immune sera were confirmed by PCR based on the pathogen-specific *lipL*32 gene [10].

In the 90.5% (18/21) of all *Leptospira* serogroups the titres obtained were high; particularly to Ballum, Pomona and Icterohamorrhagiae serogroups were ≥ 12 800, to Canicola ≥ 25 600, and to Hebdomadis ≥ 3200 – 6400. However, titres to Louisiana and Pyrogenes sero groups were low (1600 -3200). In addition, the Cuban isolates showed several cross reactions. The most frequent were observed between Icterohaemorrhagiae and Canicola sero groups (with titres ranging from 100 to3200), Canicola and Tarassovi–Autumnalissero groups (with titers of 200), Canicola and Australis serogroups (with titre of 400), Canicola and Pomona serogroups (with 400 and 800) and Canicola and Hebdomadis serogroups (with a titre of 800). Also, Pomona isolates crossed with the Tarassovi polyclonal sera with a titre of 100, Icterohaemorrhagiaein 200, Autumnalisin 800, and Ballum in 3200. Similarly, the titres of the isolates of the Ballum serogroup showed cross reactions with Icterohaemorrhagiae –Australis (titre 400), and Canicola (titre 200). Isolates corresponding to Hebdomadis crossed with Sejroe (400) and those that were identified as Louisiana with Djasiman (800).

In the present investigation, the most prevalent serogroups of *Leptospira* identified in two Cuban provinces from the Eastern region during 2007-2012 were Ballum, Canicola, Pomona and Icterohaemorrhagiae. The numerical distribution of serovars for each detected serogroup was the following: 15 Arborea, 2 Ballum and 2 Kenya/Peru belonging to Ballum; 18 Canicola belonging to Canicola; 10 Pomona and 4 Mozdok belonging Pomona; 7 Icterohaemorrhagiae, and one Sarmini/Cuica belonging toIcterohaemorrhagiae (Table 1).

The monoclonal antibodies designed as F74C1-7, F74C4-4, F74C7-4 recognized allBallum like strains, which were 15 (79%) responding to Arborea-Quandong serovar, 2 (10.5%) to Kenya-Peru serovar and 2 (10.5%) as Ballum serovar. Two strains were non typable by mAbs. In the case of monoclonal antibodies designed F46C4, F46C1, F48C6, F48C1, F58C2, F58C1, F46C10which recognize Pomona like strains4 (33.3%) were as Mozdok serovar and 9 (66.7%) as Pomona serovar. One strain was not recognized by these mAbs. Fusions of monoclonal antibodies F152C7-4, F152C18-4, F152C11-3 were used to classify 18 isolatesas Canicola serovar, all with high titres. One strain was not recognized by these mAbs. A unique isolate belonging to the Icterohaemorrhagiae sero group was classified as Sarmini SA/ CUICA SA serovar. On the other side, seven isolates were classified as Icterohaemorrhagiae serovar, by the mAbsF70C14, F70C24, F70C7-11, F82C1-3, F52C1-4, and F12C3-11.

In the case of the four strainsn on typable by mAbs (Table 1) all were DNA amplified by PCR LipL32, demonstrating its belonging to the pathogenic Leptospira.

Eight isolates belonging to serogroups Pyrogenes (1), Hebdomadis (6), and Louisiana (1) could not be classified into serovars, due to un availability of the mAbs panel (Table 1).

DISCUSSION

Identification of serovars is necessary for epidemiological surveillance since some serovars are known to have preferential animal reservoirs or are associated with certain clinical forms of leptospirosis. The first attempt to introduce some order into the growing of serologically different strains of *Leptospira* was undertaken by Wolff and Broom in 1954 [1].

The hybridoma technique antibodies are easy to apply and results can be completed in 2 - 4 h. The serovar specificity of monoclonal antibodies is very high and can discriminate between two strains belonging to the same serogroups [11].

The first report about serogrouping of *leptospira* strains from Holguin province was carried out in 1996, where the Ballum, Canicola and Pomona serogroups predominated [11]. In 2002, 204 strains of *L. interrogans* pathogenic complex, isolated from different Cuban regions identified the serogroups Ballum, Pomona, Canicola, Pyrogenes, Autumnalis and Bataviae as the most prevalent. This investigation also reports that mice, pigs and dogs were the main reservoirs [12]. In addition, 18 strains of

Table 1: Typed human isolates by monoclonal antibodies technique, from Las Tunas and Holguin. Cuba (2007 to 2012).

Provincea (total of isolates)	Serogroup (+/total by serogroup)	Serovar (+/serogroup)
Las Tunas	Ballum (5/21)	Arborea (2/5) Ballum (1/5) No typed by mAbs (2/5)
	Icterohaemorrhagiae (4/8)	Icterohaemorrhagiae (3/4) Sarmini/Cuica (1/4)
	Pomona (4/14)	Pomona (3/4) No typed by mAbs (1/4)
	Canicola (3/19)	Canicola (3/3)
	Louisiana (1/1)	-
Holguín	Ballum (16/21)	Arborea (13/16) Kenya/Perú (2/16) Ballum (1/16)
	Canicola (16/19)	Canicola (15/16) No typed by mAbs (1/16)
	Pomona (10/14)	Pomona (6/10) Mozdok (4/10)
	Hebdomadis (6/6)	-
	Icterohaemorrhagiae (4/8)	Icterohaemorrhagiae (4/4)
	Pyrogenes (1/1)	-

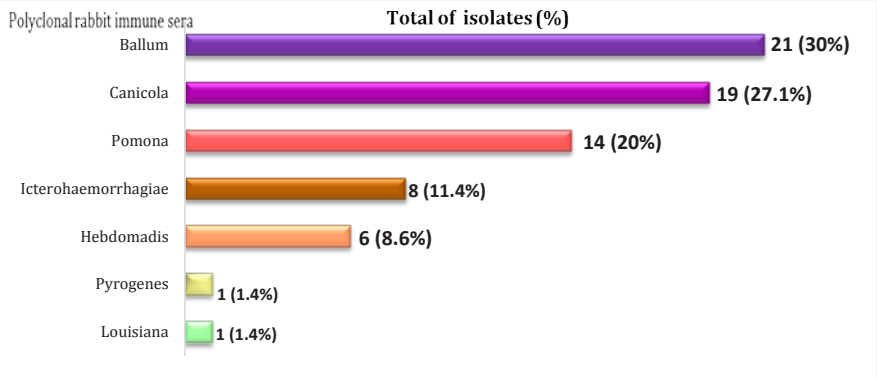

Figure 1 Typed human isolates by Polyclonal rabbit immune sera, from Las Tunas and Holguin. Cuba (2007 to 2012).

Leptospira, isolated from three provinces of Cuba, were studied by both serological and genetic methods [13].

A report from 2006-2008 demonstrated as the predominant serogroups, Pomona (11) 42.3%, Canicola (6) 23.1%, Icterohamorrhagiae (5) 19.2 %, Ballum (2) 7.7%, Hebdomadis and Lousiana (1) 3.8%. Sixteen of these isolates were classified into serotypes by monoclonal antibodies as Pomona Pomona, Pomona Mozdok and Canicola Canicola [14].

The reason why nine strains were not be classified by polyclonal rabbit immune sera could be the existence of mutations causing the loss of surface antigens useful for serogroup typing as consequence of successive subcultures [8]. Four isolates could not be typable by mAbs due to the unavailability of whole set of mAbs in the reference laboratory of Cuba.

A study aimed to analyze the changes in epidemiological features of leptospirosis cases from the hospital of Pointe à Pitre in Guadeloupe in 2003–2004 compared to reliable data in 1994–2001, demonstrated that Leptospirosis incidence increased fourfold during 2002–2004, a period with two El Niño events and Ballum serogroup rose dramatically (36% of incidence) competing with the Icterohaemorrhagiae serogroup (62%) [3].

From 1997-2005, was carried a study to determine the frequency of human leptospirosis in the sera of suspected clinical cases sent by 14 Caribbean countries for diagnosis to a regional laboratory. In this investigation, using the MAT on 100 sera tested, 98 (98%) were seropositive, of which the serogroup Icterohaemorrhagiae was most prevalent with the detection of serovars Copenhageni (70%), Icterohaemorrhagiae (67%), and Mankarso (29%) [4].

Pratt & Rajeev, in 2018; reported a review provided *Leptospira* seroprevalence data from 16 Caribbean islands (Barbados, Trinidad, Grenada, Puerto Rico, Saint Croix, St. Kitts and Nevis, Jamaica, Antigua, Carriacou, Dominica, Guadalupe, Martinique, Monserrat, St. Lucia, St. Maarten, and St. Vincent) in a variety of animal species. In this paper the differences found in seroprevalence could be to variable and small samples sizes.

These authors insist that serovar panels used for MAT were not consistent between studies. In addition this study indicates that the *Leptospira* exposure in a given geographic location may change with time and climatic and environmental conditions [5].

Peters et al., published a review about Leptospirosis in the Caribbean, from 1979-2013, demonstrating the prevalence rates and serovars vary greatly among the countries [6].

A longitudinal study in the Netherlands during 1925–2008 revealed that the main infecting serogroup in autochthonous cases of leptospirosis was Icterohaemorrhagiae (1.588 identifications; 71.2%), followed by serogroups Canicola (87 identifications; 3.9%), and Pomona (45 identifications; 2.0%), respectively. All infections caused by serogroup Canicola occurred during the first 50 years of the study. Serogroup Icterohaemorrhagiae appeared to be the major cause of fatal leptospirosis, followed by serogroup Canicola [15].

The distribution of *Leptospira* serovars in Holguin and Las Tunas provinces, Cuba, during 2007-2012 is quite similar to other previous Cuban reports and in some Caribbean countries. Interestingly, the current study represents the first report for humansof the Icterohaemorrhagiae Sarmini/Cuicaserovar. It also highlights the need to conduct continual surveillance in tropical countries where the climate supports the survival of *Leptospira* in the environment.

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