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SHORT COMMUNICATION

Serotyping of porcine S*treptococcus suis* recovered from diseased pigs in the western region of Cuba

Ivette Espinosa[I], Michel Áreas[I], María Irian Percedo[I], Evelyn Lobo[I], Siomara Martínez[I], Marcelo Gottschalk[II]

[I]Department of Microbiology, National Centre for Animal and Plant Health (CENSA), San José de las Lajas, La Habana, Cuba. [II]Groupe de Recherche sur les Maladies Infectieuses du Porc and Centre de Recherche en Infectiologie Porcine, Faculte de Medecine Veterinaire, Université de Montreal, St-Hyacinthe, Qc, J2S 2M2, Canada.

ABSTRACT: *Streptococcus suis* is an important swine pathogen in most countries with intensive swine production. *S. suis* has also been isolated in humans and other animal species. Data regarding serotyping of *S. suis* isolates from diseased pigs in Cuba using a reference antiserum are not available. *S. suis* isolates from pigs of four farms located in the western region of Cuba were characterized by serotyping. Most of the isolates was collected from pigs with pneumonia, followed by arthritis cases. Serotypes 2, 4, 8, 15, 16 and 34 were found in isolates from diseased pigs during the period 2002-2008, while serotypes 1, ½, 2, 3, 9, 13, 23, 24 and 31were found from samples collected during 2009-2014. Serotype 2 recovered from systemic (arthritis) and localized (pneumonia) infections was the most frequent. This research sets the basis for more complete studies on the infection status of *S. suis* in Cuba.

Key words: swine, *Streptococcus suis,* serotype.

Serotipificación de *Streptococcus suis* porcinos recobrados de cerdos enfermos en la región occidental de Cuba

RESUMEN: *Streptococcus suis* es uno de los patógenos de mayor importancia que afecta la crianza porcina en los países que se basan en sistemas de producción intensiva. *S. suis* se ha aislado en humanos y en otras especies de animales. En Cuba no existen datos con respecto a los serotipos existentes de *S. suis*. En este trabajo se realizó la serotipificación de aislados de *S. suis* procedentes de cerdos de cuatro granjas localizadas en la región occidental de Cuba. La mayor cantidad de aislados se obtuvo de animales con neumonía, seguidos por casos de artritis. Los serotipos 2, 4, 8, 15, 16 y 34 se encontraron en muestras procedentes de animales enfermos en el periodo 2002-2008, mientras que los serotipos 1, ½, 2, 3, 9, 13, 23, 24, 31 se encontraron en muestras colectadas en el período 2009 -2014. El serotipo 2 fue el más frecuente y se recobró de la infección localizada (neumonía) y de la sistémica (artritis). Esta investigación establece las bases para los estudios sobre el estado de la infección por *S. suis* en Cuba.

Palabras clave: cerdos, *Streptococcus suis,* serotipo.

Streptococcal diseases are a worldwide problem for the swine industry. In most parts of the world, *S. suis* is the predominant agent of streptococcal infections, and it is associated with a variety of diseases including meningitis, septicemia, arthritis, endocarditis, and pneumonia (1). In addition to swine, *S. suis* has also been isolated from humans and other mammalian (wild boars, horses, dogs, cats) and avian species (2, 3). Thirty-five serotypes of *S. suis* have been characterized, and serotype 2 has been the most virulent type to swine and humans (4,5).

Serotype specificity of *S. suis* is determined by the capsular polysaccharide present on its surface (6). The *cps loci* of all the serotypes have been sequenced (7). Based on serotype-specific genes in the *cps locus*,

rapid, sensitive and specific PCR assays have been described (8, 9, 10, 11). A recent study at our laboratory identified two capsular types by using specific primers to *cps2* and *cps9* (12). However, due to the fact that the serotypes 1/2 and 2 share gene sequences, the reported PCR assays described to date are unable to distinguish between them (9). For that reason, it is necessary a serological assay to corroborate the detection of isolates belonging to serotype 2.The purpose of the present work was to provide data on the presence of different serotypes of *S. suis* recovered from diseased pigs in Cuba.

A collection of 41 *S. suis* isolates was investigated in this study. The samples were obtained from four farms located in different regions of Cuba. Isolation and identification of *S. suis* was performed at laboratories of the National Centre for Animal and Plant Health (CENSA) over a 12 year period (2002-2014). Isolates were recovered in pure culture or as predominant bacteria from lungs with gross abnormalities (n=34), from animals with abscess in joints (n=3) and spleen (n=1), from different organs of an aborted fetus (n=1), and vagina discharge (n =2). Origin, type of production, and the number of isolate of each year are shown in Table 1. Each isolate was chosen according to the following criteria: the isolates originated from different site of isolation as well as the same farm and date of collection were considered as one isolate and conserved when at least three out of 10 animals were positive.

Appropriate amounts of materials from infected organs or swabs were aseptically transferred to the surface of a Columbia agar plate (Oxoid, LTD, Basingstone, Hampshire, UK) supplemented with 5% bovine blood. The plates were incubated aerobically at 37°C and inspected for growth after 24 and 48h. Colonies 1-2 mm in diameter showing alpha-hemolysis were suspected of being potentially *S. suis*. If Gram staining showed Gram positive cocci in pairs or chains, isolates were biochemically typed using the API 20 STREP test kit (Bio Mérieux, L´etoile,France) and further confirmed as *S. suis* by a species-specific PCR assay (13).

For the coagglutination test used for serotyping, *S. suis* isolates were grown overnight in 5 ml of Todd Hewitt broth (Sigma-Aldrich, NichtDirektem, Sonnenlicht). The culture was centrifuged at 3000 rpm for 15 min and resuspended in saline solution (0.9%). The coagglutination test using rabbit hyperimmune sera and a reference panel of all 35 *S. suis* serotypes was performed as previously described (6, 14). Based on capsular typing, we confirmed that different serotypes of *S. suis* are circulating among swine herds located in different region of Cuba. From this small scale survey, fourteen serotypes (1, ½, 2, 3, 4, 8, 9, 13, 15, 16, 19, 23, 31, 34) were identified among the 41clinical isolates of *S. suis*. Results showed that serotype 2 (n=16) was the most frequently isolated, followed by serotype 9 (n=2), serotype 3 (n=3) and serotype 34 (n=2) (Table 2). These four serotypes together accounted for 60% of the isolates.

The 37 isolates of *S. suis* recovered from lungs were included in this study because they were isolated in pure culture from organs presenting compatible lesions. Respiratory diseases are one of the most important problems in intensive swine production in most countries (15), including Cuba. Important economic losses are observed due to reduced daily gain, prolonged time of fattening and enlarged costs of treatment (16). The interpretation of bacteriologic examination results of lung samples has been questioned, mainly due to the fact that, under some conditions, bacteria can be found in both healthy and diseased lungs with little or no clinical significance (17). However, reports about the isolation of this bacterium from pigs suffering from pneumonia are frequent, especially when isolated in pure cultures (18, 19).

TABLE 1. Distribution of *S. suis* obtained from different sources of isolation (n = 41)./ *Distribución de* ***S. suis*** *obtenidos de diferentes fuentes de aislamientos.*

Farm	Type of production	Year of isolation	Site of isolation					
			Lung	Fetus aborted	Vaginal discharge	Abscess	Spleen	Total
A	Farrow to wean	2002-2008	10					10
		2009-2012	12			2		14
B	Farrow to wean	2002-2008	5					5
		2009-2012	10					10
C	Farrow to finish	2011		1	1	1		1
D	Nursery	2014			1	1	1	1

TABLE 2. Distribution of serotypes (number of isolates) of *Streptococcus suis* isolated from diseased pigs in Cuba between 2002-2014./ *Distribución de serotipos (número de aislamientos) de* ***Streptococcus suis*** *aislados de cerdos enfermos en Cuba entre 2002-2014.*

TISSUE	SEROTYPES															
	1	1/2	2	3	4	8	9	13	15	16	19	23	24	31	34	Nontypeable
LUNG	1	1	12	3	1	1	2	1	1	1	1	1	1	1	2	7
ABSCESS			2													
FETUS ABORTED			1													
SPLEEN			1													
Total	1	1	16	3	1	1	2	1	1	1	1	1	1	1	2	7

The isolate recovered from abscess in joints, aborted fetus, and vaginal discharge from one farrow-to-finish farm was confirmed as one isolate belonging to the serotype 2; this serotype has been significantly associated with systemic infections worldwide (20). Serotype 2 was the only type found in each of the four farms. Serotype 3 associated with pneumonia has been also found highly prevalent in China, although it is rare in other countries, except in Canada (20, 21). Serotypes 4, 8, 13, 15, 16, 19, 23 and 31 comprised only one isolates. From them, serotypes 13, 19, 23 and 31 were detected during the period 2009-2011.

The distribution of different serotypes varies depending on the geographical origins of the strains. *S. suis* serotype 2 has been considered the most pathogenic and the most prevalent capsular type among diseased pigs in North America and China, followed by serotypes 3 and 1/2 (20, 23). The serotype 9 was also frequently isolated in the samples examined in this study. The serotype 9 is prevalent in several European countries (8, 9, 24).

A total of seven strains were nontypable and they were confirmed to belong to the *S. suis* species by using a previously described specific PCR test targeting the r16S DNA gene (13). Two strains reacted with more than one antiserum, for example with antiserum 13-23 and 15-16. Nontypeable strains are frequently isolated from diseased pigs in other countries. Recently, it has been reported that most of them are non encapsulated (20).

Previous studies reported the presence of genotype *cps2j* in Cuba by using PCR assays (12). However, the primers used in the PCR assay are unable to discriminate between serotype 2 and 1/2. To the best of our knowledge, this study is the first report of *S. suis* serotyping from pigs in Cuba using reference rabbit hyperimmune sera that confirms the presence of isolates of *S. suis* serotype 2 and other serotypes from diseased pigs with pneumonia and invasive diseases. Although the current use of molecular assays like PCR for the characterization of microorganisms is based on its advantages such as sensitivity and fast performing, serological confirmation is needed, at least for serotype 2.

Although *S. suis* serotype 2 is considered to be the most virulent type to swine and humans (25), it is well known that strains within serotype 2 may differ substantially in virulence. *S. suis* as a zoonotic pathogen has received only limited attention with respect to epidemiology in humans, except for the outbreak in Sichuan, People's Republic of China, in 2005 (26, 28, 29 and 30). Other serotypes like 5, 16, 14, and 21 have been identified in human isolates (27). No human *S. suis* cases attributable to *S. suis* strains have been reported so far in Cuba. But in this country, the swine production has significantly increased. It is speculated that factors associated with modern swine production, such as early weaning, high concentrations of animals, and corrosive gases, all of them led to the selection of highly virulent *S. suis* strains (31). Among others, causes for disease emergence in swine, particularly for zoonotic agents, may be the rapid expansion and intensification of swine industries in the developing countries without incorporating the stringent biosecurity measures and a veterinary oversight (32). Based on these observations and the results obtained in this study, and aware of the zoonotic risk, we recommend caution when dealing with *S. suis* infected pigs and the implementation of a biosecurity manual for the farm as a way to prevent all risk though a health education program addressing food safety, in addition to encourage the proper handling of pork.

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