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ORIGINAL ARTICLE

Characterization of *Actinobacillus pleuropneumoniae* field strains antigenically related to the 3-6-8-15 group from diseased pigs in Japan and Argentina

Ho To[a,*], Kaho Teshima[a], Shinya Nagai[a], Gustavo C. Zielinski[b,*], Tomohiro Koyama[a], Jina Lee[a], Fernando A. Bessone[b], Tetsuji Nagano[a], Atsushi Oshima[a], Nobuyuki Tsutsumi[a]

[a] *Nippon Institute for Biological Science, 9-2221-1 Shinmachi, Ome, Tokyo 198-0024, Japan*
[b] *Animal Health Group, Estacion Experimental Agropecuaria Marcos Juarez, INTA, CC n°21 (2580), Marcos Juarez, Province of Cordoba, Argentina*

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KEYWORDS
Actinobacillus pleuropneumoniae;
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Abstract The objectives of this study were to determine the serovar of a collection of *Actinobacillus pleuropneumoniae* strains within the 3-6-8-15 cross-reacting group and to analyze their phenotypic and genetic properties. Based on the serological tests, forty-seven field strains of *Actinobacillus pleuropneumoniae* isolated from lungs with pleuropneumonia lesions in Japan and Argentina were found to be serovars belonging to the 3-6-8-15 cross-reacting group. By using a capsule loci-based PCR, twenty-nine (96.7%) and one (3.3%) from Japan were identified as serovars 15 and 8, respectively, whereas seventeen (100%) from Argentina were identified as serovar 8. The findings suggested that serovars 8 and 15 were prevalent within the 3-6-8-15 cross-reacting group, in Argentina and Japan, respectively. Phenotypic analyses revealed that the protein patterns observed on SDS–PAGE and the lipopolysaccharide antigen detected by immunoblotting of the reference and field strains of serovars 8 and 15 were similar to each other. Genetic (*16S rDNA*, *apxIIA*, *apxIIIA*, *cps*, *cpx* genes, *apx* and *omlA* patterns) analyses revealed that the *apxIIA* and *apxIIIA* genes of the field strains of serovars 8 and 15 were similar to those of the reference strains of serovars 3, 4, 6, 8 and 15. The results obtained in the present study may be useful for the development of more effective vaccines against disease caused by *A. pleuropneumoniae* by including the homologous antigens to the most prevalent serovars in specific geographical areas.

* Corresponding authors.
E-mail addresses: toho@nibs.or.jp (H. To), zielinski.gustavo@inta.gob.ar (G.C. Zielinski).

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PALABRAS CLAVE
Actinobacillus pleuropneumoniae;
apx;
cps;
LPS;
Serotipos 8 y 15

Caracterización de cepas de *Actinobacillus pleuropneumoniae* antigénicamente relacionados al grupo 3-6-8-15 obtenidos de cerdos infectados naturalmente en Japón y Argentina

Resumen Los objetivos del presente estudio fueron determinar el serovar de una colección de cepas de *Actinobacillus pleuropneumoniae* pertenecientes al grupo 3, 6, 8, 15 de reacciones cruzadas y analizar sus propiedades fenotípicas y genéticas. En base a técnicas serológicas se determinó que cuarenta y siete cepas de *A. pleuropneumoniae* aisladas a partir de pulmones con lesiones de pleuroneumonía en Japón y Argentina pertenecen al grupo 3, 6, 8, 15. Mediante el uso de PCR basado en *locus* capsulares, veintinueve (96.7%) y una (3.3%) de los aislados japoneses fueron identificados como serovar 15 y 8 respectivamente, mientras que diecisiete (100%) de los aislados argentinos resultaron pertenecer al serotipo 8. Este hallazgo sugirió que los serovares 8 y 15 fueron los prevalentes dentro del grupo 3, 6, 8, 15 en Japón y Argentina, respectivamente. El análisis fenotípico reveló que los perfiles proteicos determinados por SDS-PAGE, y de antígenos lipopolisacáridos estudiados por *inmunoblot*, de las cepas de referencia y de campo de los serovares 8 y 15 fueron similares entre sí. El análisis genético (*16S rDNA, apxIIA, apxIIA, cps*, genes *cpx, apx* y los perfiles *omlA)* reveló que los genes *apxIIA y apxIIIA* de las cepas de campo de los serovares 8 y 15 fueron similares a sus homólogos de las cepas de referencia de los serovares 3, 4, 6, 8 y 15. Los resultados obtenidos en el presente estudio pueden ser útiles para el desarrollo de vacunas más efectivas contra la enfermedad causada por *A. pleuropneumoniae*, al posibilitar incluir antígenos homólogos a los serovares prevalentes en las áreas geográficas de interés.

Introduction

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, an economically important bacterial disease of swine[7]. The virulence of *A. pleuropneumoniae* has been linked with exotoxins, capsular polysaccharides (CPS), lipopolysaccharides (LPS) and membrane proteins[5,7]. To date, 15 serovars have been recognized mainly on the basis of the antigenic properties of CPS and the O-polysaccharide (O-PS)[3,5,24,25], and another one, serovar 16, was proposed based on serology alone[26].

It has been shown that the characterization of the *A. pleuropneumoniae* serovar involved is useful for understanding the epidemiology of an outbreak, for preparing vaccines for the control of the disease and for serological monitoring of infected herds[7]. Cross-reactions between some serovars (1, 9 and 11; 4 and 7; and 3, 6, 8 and 15) and variable results between individual batches of test sera are usually observed in conventional serotyping tests[7–9,13,14]. To overcome these limitations, PCR assays based on the capsule loci have been developed for serotyping of *A. pleuropneumoniae* strains[4,31,32].

Currently, numerous researchers have identified serovar 8 or 15 within the 3-6-8-15 cross-reacting group[3,5,9,12,16,17,21,23,31]. An understanding of the basic characteristics of strains of serovar 8 or 15 may be critical for the design of a vaccine against the infection by serovars belonging to this cross-reacting group. The aims of this work were to determine the serovars of *A. pleuropneumoniae* strains belonging to the 3-6-8-15 cross-reacting group isolated from pneumonic lesions of naturally infected dead or diseased pigs in Japan and Argentina. The serovar-identified strains were then characterized phenotypically and genetically by different techniques and compared to reference strains.

Materials and methods

Bacterial strains and culture conditions

The bacterial strains used in this study included nine reference strains of *A. pleuropneumoniae* (4074, serovar 1; CCM5870, 2; S1421, 3; M62, 4; K17, 5a; Femo, 6; 405 and CCM3803, serovar 8 and HS 143, 15), seventeen strains randomly chosen from 35 strains isolated from pneumonic naturally infected dead or diseased pigs in Argentina and 30 strains from pigs suffering from acute pleuropneumonia in Japan. The source of the field strains of *A. pleuropneumoniae* is shown in Table 1. The initial analyses revealed that these field strains possess the following toxin-related genes: *apxIBD*, *apxIICA*, *apxIIICA*, *apxIIIBD* and *apxIVA*. The characteristics of some Argentine and Japanese strains were reported in a Master's thesis by Fernando Bessone at the University of Buenos Aires, Argentina in 2012[2] and at the 4th Asian Pig Veterinary Society Congress in Tsukuba, Japan, in 2009, respectively. In addition to the reference strains, four field strains, each from Japan and Argentina, were randomly chosen for the phenotypic and genetic analyses.

Actinobacillus pleuropneumoniae strains were cultured in chocolate agar (BD, Becton, Dickinson Co., Detroit, MI, USA) or in heart infusion medium (BBL, Cockeysville, MD, USA) supplemented with 0.3% yeast extract (dried yeast

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