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Short communication

Serum cross-reaction among virulence-associated trimeric autotransporters (VtaA) of *Haemophilus parasuis*

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ABSTRACT

Glässer's disease is a fibrinous polyserositis and polyarthritis of swine caused by the bacterium *Haemophilus parasuis*. Control by vaccination has been limited for years due to lack of cross-protection among strains. However, 6 trimeric autotransporters (VtaA) of the Nagasaki strain were shown to be antigenic and gave partial protection to a lethal challenge. The antigenic relationship among the VtaAs was examined by immunizing mice with individual VtaA showing that they cross-reacted by ELISA mainly with VtaA from the same group. When sera from protected and non-protected vaccinated piglets were examined no differences in VtaA cross-reactivity profiles were found. In addition, sera from commercial pigs immunized with a single VtaA (VtaA9) showed a wider range of VtaA cross-reaction, probably due to the previous colonization by *H. parasuis*. These results can help the development of new vaccine formulations against *H. parasuis* by allowing a rational VtaA selection.

1. Introduction

Glässer's disease is a systemic infection caused by virulent strains of *Haemophilus parasuis*, characterized by a polyserositis and common in young pigs. Disruption of piglet colonization by early weaning has resulted in immunologically naïve populations and in increased morbidity and mortality due to Glässer's disease (Kielstein and Rapp-Gabrielson, 1992). Antibodies are critical to control *H. parasuis* infection (Aragon et al., 2012; Martin de la Fuente et al., 2009; Olvera et al., 2009, 2010) and exposure to low doses of bacteria protects against a subsequent challenge (Nielsen, 1993; Oliveira et al., 2001). Bacterins have been used to control the disease (Miniats et al., 1991a,b; Riising, 1981; Smart and Miniats, 1989), but lack of cross-protection among serotypes has hampered complete control of the disease (Miniats et al., 1991a; Nielsen, 1993).

Like other *Pasteurellaceae*, *H. parasuis* has trimeric autotransporters (AT-2) named virulence associated trimeric autotransporters (VtaA) (Pina et al., 2009). AT-2 are characterized by a translocator domain that anchors the protein to the membrane and a functional passenger domain, which is divided into stalk, connector and head segments (Szczesny and Lupas, 2008). Based on the conserved sequence of the translocator domain,

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H. parasuis vtaA are divided in three groups (Pina et al., 2009). Six of the 13 VtaA of the Nagasaki strain (VtaA1, 5, 6, 8, 9 and 10; from groups 1 and 2) have been found to be antigenic (Olvera et al., 2010) and conferred partial protection against a lethal challenge with the highly virulent Nagasaki strain (Olvera et al., 2011b).

In the present study, individual and pooled VtaA were used to immunize mice and piglets. The cross-reaction of anti-VtaA sera among 15 VtaA of 2 different virulent strains was evaluated.

2. Materials and methods

2.1. Mouse sera

All procedures involving mice were approved by the Ethics Commission in Animal Experimentation of the Generalitat de Catalunya (Approved Protocol Number 5767). The 6 antigenic and partially protective VtaA were used individually to produce specific sera in mice (anti-VtaA1, 5, 6, 8, 9 and 10 sera). Groups of 4 CD1 mice were immunized with 15 μg of each VtaA using complete Freund adjuvant. After 3 weeks animals were re-immunized with 15 μg of the same VtaA and incomplete Freund adjuvant. Two weeks after the second immunization, animals were euthanized and the sera recovered.

2.2. Pig sera

All procedures involving pigs were approved by the Ethics Commission in Animal Experimentation of the Generalitat de Catalunya (Approved Protocol Number 5796). Two 3 week-old pigs naturally farrowed in a conventional farm and thus, colonized with *H. parasuis*, were vaccinated intramuscularly in both sides of the neck with 100 μg of VtaA9 and complete Freund adjuvant. Two weeks later, a second immunization was performed with 100 μg VtaA9 and incomplete Freund adjuvant. Two control animals were mock-immunized with PBS. Nasal cavities samples before vaccination were examined for *H. parasuis* colonization using a previously described PCR (Olvera et al., 2011a). IgG responses against the 13 individual VtaA of the Nagasaki strain were measured before and 14 days after vaccination.

In addition, sera from 6 protected and 6 non-protected animals from a previous study were used (Olvera et al., 2011b). In this study snatch-farrowed, colostrum-deprived (SF-CD) pig, free of *H. parasuis* before challenge were vaccinated with the 6 antigenic VtaA. Sera from 4 mock-immunized animals were used as negative controls.

2.3. Anti-VtaA IgG detection by ELISA

The complete passenger domain of the 13 VtaA of the virulent Nagasaki strain (VtaA1 to 13), together with 2 group 1 VtaA of the HP1319 strain (VtaA15 and 16) and the stalk, connector and head fragments of VtaA10 were produced as recombinant proteins as previously described (Olvera et al., 2011b). The recombinant VtaA were used to evaluate serum IgG anti-VtaA using an in-house ELISA as previously described (Olvera et al., 2011b). Sera from mice immunized with the same VtaA were tested in pools, while the sera from pigs were tested individually. ELISA positive thresholds were set as the mean value of negative controls plus three times its standard deviation. Pre-immune sera, mock-immunized sera and wells coated with Beta-barrel monomeric autotransporters 5 and 6 (Pina-Pedrero et al., 2012) were used as negative control.

3. Results

3.1. Cross-reactivity of mouse sera produced immunizing with individual VtaA

The mice sera against individual VtaA showed cross-reaction with VtaA from the same group (Table 1). Sera from animals immunized with VtaA from group 1 (VtaA1, 5, 6, 8 and 9) cross-reacted with the entire group 1 VtaA except VtaA2. On the other hand, sera produced against the group 2 VtaA10 reacted against the other group 2 VtaA, VtaA11, and the group 3 VtaA, VtaA13. Curiously, all of the sera obtained against group 1 VtaA except anti-VtaA5 sera reacted with group 2 VtaA10, while anti-VtaA10 sera did not react with any VtaA of group 1. None of the anti-group 1 VtaA sera reacted with group 2 VtaA11 and only anti-group 1 VtaA8 sera reacted with one of the group 3 VtaA, VtaA12. The cross-reactivity of the individual anti-VtaA sera with VtaA15 and 16 of strain HP1319 followed the same pattern as their close orthologs from the Nagasaki strain VtaA6 and 8 (Table 1). The VtaA15 and 16 passenger domains showed an amino acid identity with VtaA6 and VtaA8, of 79.3 and a 76.4%, respectively (Pina et al., 2009).

Table 1
Cross-reaction of mouse sera obtained after immunization with individual VtaA1, 5, 6, 8, 9 and 10. The 13 VtaA of the Nagasaki and VtaA15 and VtaA16 from the HP1319 strain were used individually as coating antigen for ELISA as indicated in the first rows. VtaA used to produce the mouse sera are indicated in the first column. Positive results are indicated by bold black numbers (threshold, 0.342), results below the threshold are indicated by grey numbers.

Anti-VtaA sera	VtaA														
	Nagasaki													HP1319	
	Group 1									Group 2		Group 3		Group 1	
	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16
1	**0.803**	0.099	**0.640**	**0.507**	**0.552**	**0.395**	**0.670**	**0.562**	**0.450**	**0.694**	0.126	0.226	0.246	**0.433**	**0.550**
5	**0.595**	0.110	**0.517**	**0.525**	**0.790**	**0.450**	**0.677**	**0.622**	**0.550**	0.330	0.069	0.103	0.138	**0.571**	**0.760**
6	**0.695**	0.137	**0.622**	**0.624**	**0.756**	**0.687**	**0.792**	**0.736**	**0.597**	**0.450**	0.151	0.180	0.272	**0.740**	**0.839**
8	**0.684**	0.129	**0.602**	**0.608**	**0.734**	**0.619**	**0.843**	**0.745**	**0.644**	**0.403**	0.121	**0.418**	0.172	**0.613**	**0.813**
9	**0.790**	0.209	**0.750**	**0.788**	**0.925**	**0.823**	**0.971**	**0.923**	**0.788**	**0.461**	0.159	0.128	0.196	**0.853**	**0.901**
10	0.095	0.074	0.055	0.057	0.079	0.064	0.116	0.070	0.075	**0.749**	**0.437**	0.310	**0.668**	0.075	0.074