ORAL ADMINISTRATION AND EVALUATION IN PIGLETS OF TOBACCO SEEDS EXPRESSING ANTIGENIC PROTEINS AGAINST OEDEMA DISEASE

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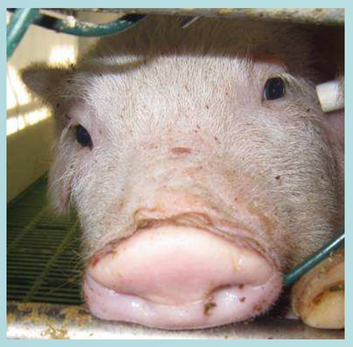
ABSTRACT

The vaccination is an important and cost-effective way to control animal and human infectious disease. The use of transgenic plants as delivery system for vaccine proteins is attractive for its simplicity and increases likelihood for local immune response at sites of infection. In particular in livestock, vaccination allows to reduce antibiotic treatments, as suggested by EC Regulation 1831/2003.

The aim of this study was to evaluate the transgenic tobacco seeds as edible vaccine in piglets. Tobacco seeds were previously transformed *via agroinfection* for the expression of antigenic proteins of *Escherichia coli* strains responsible of Pig Oedema disease: the F18 fimbriae adhesive and the VT2e-B subunit. 43 piglets, weaned at 20±2 days, were allocated in pens in the same environmental conditions and randomly divided in 4 experimental groups: Control Group (CG), Treatment 1 group (T1), Treatment 2 group (T2, Treatment 3 group (T3). Treatments were represented by different lines of tobacco seeds: F18 positive tobacco seeds (F18+), VT2e-B positive tobacco seeds (VT2e-B+) and wild type tobacco seeds (WT). Treatments were administered by oral route on days 0,1,2,14. T1 received 10 grams F18+ and 10 grams of VT2e-B+ containing about 6mg of F18 and 6mg of VT2e-B. T2 received 10 grams of VT2e-B+ containing about 6mg of VT2e-B. T3 received 25 grams of VT2e-B+ containing about 15mg of VT2e-B. CG received 20 grams of WT. Animals were fasted 3 hours before and 3 hours after the treatment administration. The amount of antigenic proteins was estimated by western blotting. On day 22 piglets were infected with $1*10^{10}$ CFU of O138 *Escherichia coli* strain, previously evaluated by polymerase chain reaction (PCR). In the pre-challenge phase faecal and blood samples were collected weekly to evaluate IgA and IgG amount by ELISA assays. After challenge faecal consistency and colour, body temperature, clinical signs related to Oedema disease (eyelids, epiphora, neurological and respiratory symptoms, vitality) were determined individually thought specific points scales daily for 15 days. Zootechnical performances and haematocrit percentage (HT) were evaluated during all the experimental period. T1 showed a significant higher level of IgA in faeces than the other experimental groups in pre-challenge period. After challenge average individual body weight, average daily gain and feed intake were higher in T1 and T2 than CG. No differences were observed in body temperature, faecal evaluation and HT. After challenge CG showed scores of symptoms related to Oedema disease worse than T1, T2 and T3 and treated piglets showed a rapider recovery. In conclusion we showed that oral administration of recombinant tobacco seeds expressing antigenic proteins against Oedema disease can induce the increase of mucosal antibodies and a protective effect against the challenge strain in piglets.

BACKGROUND

- **Oedema disease** (OD) is an enterotoxaemia that occurs in pigs during the weaning period and it is the result of an infection with certain serotypes of *Escherichia coli* (most frequently O138, O139, O141) F18+ able to produce verotoxins (VT2e). OD is responsible of important economic losses in pig livestock. The average morbidity is 30-40%, and the mortality among affected pigs is often as high as 90%, with typical lesions. VT2e is composed by A and B subunits. The receptor binding capacity of VT2e is associated with the B-subunits.
- Actually antibiotics are used to treat Oedema disease in pig livestock, no vaccines are available and vaccination continues to be an important and cost-effective way to control animal and human infectious diseases.
- The concept of using transgenic plants as delivery system for vaccine proteins is attractive for its simplicity and increases likelihood for local immune response at sites of infection.
- An efficient vaccination strategy may allow to reduce antibiotic treatments, as suggested by EC Regulation 1831/2003.
- Plant-derived vaccines present many potential advantages related to the management of intensive livestock.



OBJECTIVE

The aim of this study was to evaluate the immunological effects related to oral administration of tobacco seeds, expressing F18 fimbriae and VT2e-B subunit, as a model of oral vaccine against Oedema Disease in weaned piglets.

MATERIAL & METHODS

•TOBACCO SEEDS EXPRESSING ANTIGENIC PROTEINS

Genes codifing for F18 adhesive fimbriae and for the subunit B of the VT2e toxin from a wild type *Escherichia coli* strain were placed into two cassettes of expression (fig 1) under control of GLOB promoter (Reggi et al, 2005) according to methods described by Rossi et al. (2004).

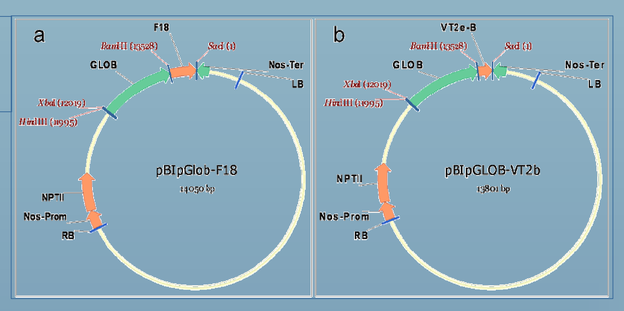


Fig.1: : Chimeric constructs used for *Agrobacterium tumefaciens* EHA105 transformations.. a: pBIpGLOB-VT2eB was 13800 pb; b: pBIpGLOB-F18 was 14049 pb.

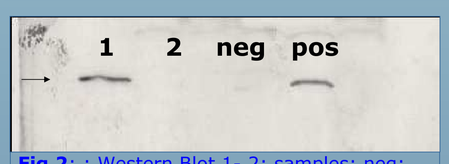


Fig.2: : Western Blot 1- 2: samples; neg: negative control (wild type); pos: positive control corresponding to VT2e-B produced in pET system.

Tobacco seeds, transformed *via Agrobacterium,* were evaluated for the seed-specific expression of antigenic proteins against Oedema disease by immunoblotting (fig.2).

• PIGLETS AND TREATMENTS

Treatments, represented by tobacco seeds expressing antigenic proteins, were administered on 0,1,2,14 days according to Joensuu et al. (2006) and Verdonck et al. (2007).

Two lines seeds derived from transgenic tobacco were used:

- Tobacco seeds expressing F18 fimbriae: **TSF18**
- Tobacco seeds expressing VT2e-B: **TSVT2EB**

Transgenic tobacco plants contained about **6mg/10 grams** of seeds of antigenic proteins.

Group	Number of piglets	Antigens	Tobacco seeds *(grams)*
T1	12	6mg F18+ 6mgVt2e-B	20
T2	9	6mg Vt2e-B	10
T3	10	15 mg Vt2e-B	25
control	12	no	20

Treatments were administered on **0,1,2,14** days.

•ANIMALS

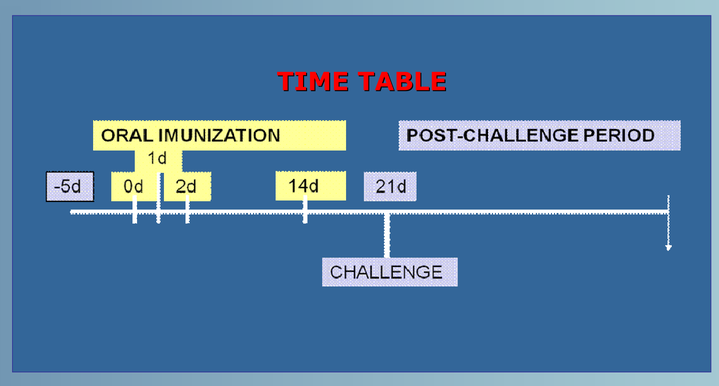
-43 piglets weaned at 20±2 days, were allocated in the same enviromental conditions

•MEASUREMENTS

-Zootecnical parameters (Average Daily Gain, Feed Intake, Body Weight) were registered in all the experimental period.

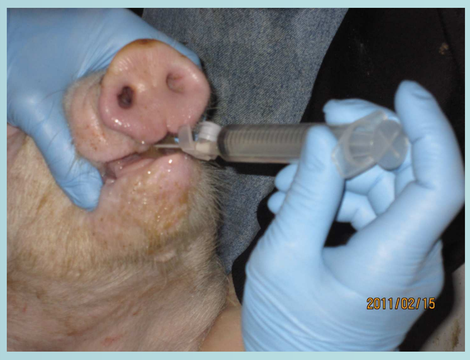
-IgA and IgG amount were evaluated in the pre-challenge period in faecal and serum samples (by ELISA).

-Clinical evaluations (symptoms related to Oedema disease) were scored daily after challenge.



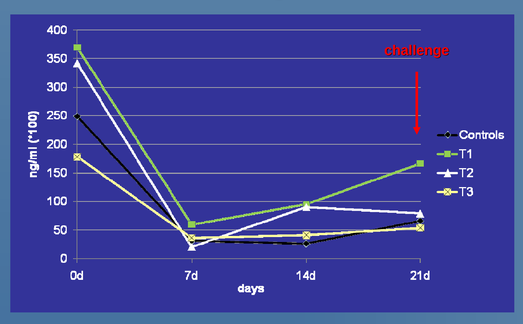
•CHALLENGE

5ml of bacterial medium with *1* 10^{10}* CFU of O138 *Escherichia coli*



RESULTS

•PRE-CHALLENGE IgA LEVELS IN THE FAECES



T1 group showed an higher level of IgA in the faeces.

•CLINICAL EVALUATION AFTER CHALLENGE

A general protective effect against the challenge strain was observed in all treated groups.

T1, T2 and T3 showed a significant lower total score (respiration, palpebral edema, epiphora, and vitality) than CG.

T1 showed also a faecal score lower than CG, T2 and T3.

No differences were observed between T2 and T3, and no dose dependent relations were detected.

Scores	Group	Days after challenge 1	2	3	4	5	6	7	8	9	total score (d1-9)
Respiration	control	0.6	0.4	0.6	0.7	0.4	0.4	0.7	0.5	0.5	**4.8**
	t1	0.3	0.2	0.3	0.2	0.1	0.2	0.3	0.3	0.0	**1.9**
	t2	0.3	0.1	0.3	0.3	0.3	0.1	0.4	0.4	0.1	**2.4**
	t3	0	0.1	0.1	0	0.1	0.3	0.4	0	0.0	**1.0**
Palpebral edema	control	0.6	1.1	1.0	1.0	0.9	1.0	0.6	0.8	1.0	**8.0**
	t1	0.2	0.2	0.4	0.1	0.6	0.4	0.0	0.0	0.0	**1.9**
	t2	1.0	0.6	0.9	0.3	0.0	0.6	0.0	0.3	0.7	**4.3**
	t3	0.8	0.5	0.5	0.3	0.1	0.3	0.1	0.1	0.0	**2.6**
Epiphora	control	0.2	0.4	0.9	1.0	0.5	0.5	0.4	0.8	0.4	**5.1**
	t1	0.3	0.3	0.2	0.2	0.2	0.3	0.1	0.2	0.6	**2.4**
	t2	0.1	0.4	0.6	0.3	0.0	0.3	0.1	0.1	0.3	**2.3**
	t3	0.0	0.3	0.5	0.0	0.0	0.3	0.1	0.0	0.0	**1.1**
Vitality	control	0.9	0.6	0.6	0.8	0.7	0.6	0.4	0.6	0.5	**5.7**
	t1	0.5	0.2	0.0	0.1	0.0	0.1	0.0	0.0	0.0	**0.9**
	t2	0.6	0.4	0.1	0.1	0.3	0.4	0.4	0.0	0.1	**2.6**
	t3	0.6	0.5	0.4	0.1	0.1	0.1	0.1	0.0	0.0	**1.9**
Faecal score	control	1.1	1.2	1.8	1.3	0.8	0.7	1.1	1.1	0.5	**9.5**
	t1	1.0	1.3	1.0	0.8	0.5	0.4	0.5	0.4	0.3	**6.1**
	t2	1.6	2.0	1.9	1.0	1.3	1.6	1.9	1.1	0.7	**13.0**
	t3	1.8	1.9	1.9	1.3	1.3	1.4	1.4	1.0	0.6	**12.4**

•ZOOTECHNICAL PERFORMANCES

After challenge T1 and T2 showed better performances (ADG and FI) than CG.

CONCLUSION

Oral immunization with tobacco seeds expressing F18+ Vt2e-B antigens induced the increase of mucosal antibodies (IgA) in the faeces and a protective effect against a subsequent VTEC F18+ *E. coli* challenge. Treated groups *vs* control showed a better clinical status. Transgenic tobacco seeds could be an efficient delivery system of antigens against Oedema disease for oral immunization of piglets. This trial showed that multicomponent treatment (TSF18 and TSVT2EB) received by T1 was the most effective.

Bibliografy: *Joensuu et al., Vaccine (2006); Mason at al., Vaccine (1998); Nagy and Fekete,IJMM (2005); Reggi et al. (2005);Rossi et al., Vet Res Comm (2003); Tiels et al., Vaccine (2008); Verdonck et al., Veterinary Immunologu and Immunopathology (2007); Vu Khac et al., BMC Vet Res.(2006).*

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