Food Technology, Quality and Safety" and XVI International Symposium "Feed Technology", Novi Sad (28th to 30th October 2014)

TOBACCO SEEDS AS EDIBLE VACCINE IN PIG LIVESTOCK

Angela Lombardi[1], Francesca Saccone[1], Raffaella Rebucci[1], Luciano Pinotti[1], Luciana Rossi[1]

[1] Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy

INTRODUCTION

Plants have been recognized as an expression system for the production of edible vaccine because of the possibility of introducing antigenic proteins into their genome. In livestock, transformed plants for the expression of immunogenic proteins could be administered, orally, in feed to induce mucosal immune response in the gastrointestinal tract. the oral delivery of plant-made vaccines is attractive since the low costs, the heat stability, the avoidance of the injections and for the production of specific antibodies in the mucosa, where the major pathogens gain access to the body. Furthermore seeds provide a stable environment for transporting edible vaccine into the gut. In pig industry, verocytotoxic Escherichia coli (VTEC) strains are responsible for severe enterotoxaemia in the weaning period and novel strategies are required to control E. coli infections.

Currently, no vaccines are available and an outbreak of the disease requires antibiotic medication, which have disadvantages like the increasing of antimicrobial resistance and the environment impact; accordingly, the development of an effective oral vaccination strategy is of interest.

Tobacco presents many advantages including high transformation efficiency and easy cell culture protocols. Seeds do not contain significant levels of nicotine (less than 2 µg/Kg) and, once included in diets for weaned piglets, they showed a good palatability (Rossi et al., 2007).

OBJECTIVE

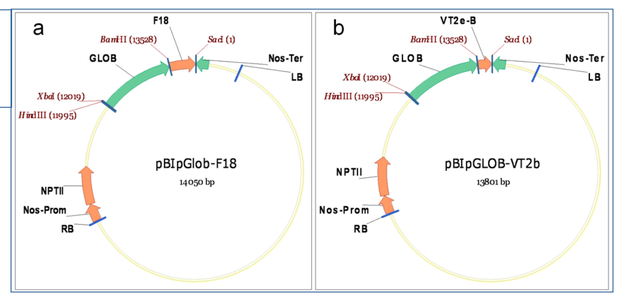
The first aim of this study was to produce different lines of transgenic tobacco plants expressing in the seeds antigenic proteins from VTEC strains, represented by the F18 fimbriae adhesive and the VT2e-B toxin, as model of edible vaccines.

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.



Tobacco leaf disks were transformed via Agrobacterium tumefaciens EHA105 with chimeric constructs containing structural parts of the major subunit FedA of the F18 adhesive fimbriae, VT2e B-subunit genes under control of a seed specific GLOB promoter (DDBJ no. AX006477). We showed that the foreign genes, incorporated in the tobacco genome, were specifically expressed, with the correct folding, in tobacco seeds.

• 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

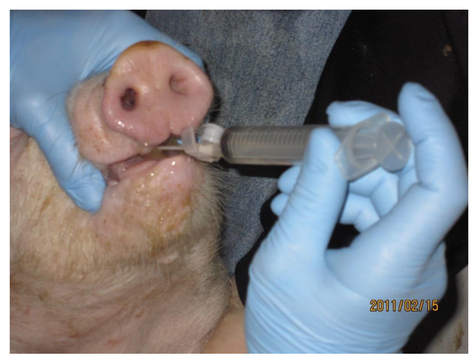
•ANIMALS

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

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

•CHALLENGE

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

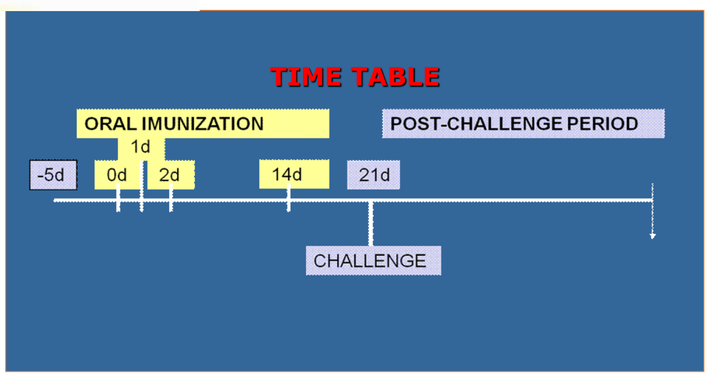


•MEASUREMENTS

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

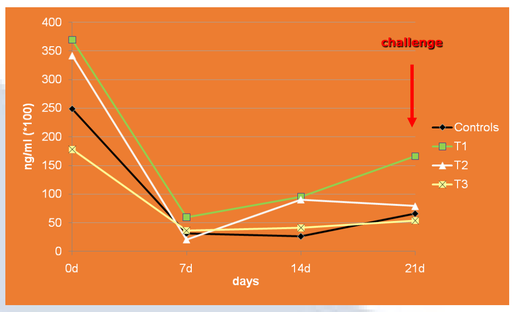
-IgA and IgG amount were evaluated in the pre-challenge period in faecal and serum samples (by ELISA).

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



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.

		Days after challenge									total score
Scores	Group	1	2	3	4	5	6	7	8	9	(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

Firstly we showed that the foreign genes, incorporated in the tobacco genome, were specifically expressed, with the correct folding, in tobacco seeds. Moreover, the oral administration of recombinant tobacco seeds expressing the antigenic proteins against VTEC strains can induce a protective effect against challenger strain in piglets. In particular, the use of the oral bivalent vaccine, including F18 positive seeds and subunit B of VT2e positive seeds, appeared more effective in preventing the negative impact of the challenge on animal health and productivity.

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).*

Acknowledgements: *funding by Regione Lombardia ID 4053*