Expression of vaccine antigens to edema disease in tobacco seeds and evaluation of immunogenicity on mouse model

L. Rossi1, A. Di Giancamillo1, C. Domeneghini1, S. Reggi2, A. Baldi3, V. Sala3 and C. Fogher4.

1Università degli Studi di Milano, Dept. of Veterinary Sciences and Technology for Food Safety, Milan, I-20134; 2Pantechnico, Vicomoscano-CR1-26460; 3Università degli Studi di Milano, D.P.A., Milan, I-20133; 4Botanic and Genetic Inst, U.C.S.C., Piacenza, I-29100.

ABSTRACT
Plant-derived vaccines present many potential advantages related to the management and intensive livestock. They could be administered without restraint of the animals, with low stress and without labour costs related to multiple injections of traditional vaccines. The aim of this study was the construction and subsequent evaluation in mouse model of transgenic tobacco seeds as edible vaccines for swine Edema disease. We focalized our attention on verotoxigenic Escherichia coli strains (O118, O139, O141), responsible of Edema disease, that occurs in pigs approximately one week after weaning and is characterized by edema in various sites and by damages to vascular endothelium. The adhesion of bacterial strains is related to different fimbriae and Shiga-like toxins (VT2e), that play an important role in the pathogenesis. Structural parts of F18 fimbriae and B-subunit of VT2e genes were inserted in expression vectors, under control of 35S promoter to obtain stable lines of transgenic plants and transformed in tobacco by agroinfiltration. We obtained two stable lines of transformed tobacco expressing the proteins in the seed: one included F18 gene (F18+) and another one included B-subunit of VT2e gene (VT2e+B+). Tobacco lines were characterized by molecular and immunological analysis and the expression of F18 and VT2e-B proteins. The amount of transgenic proteins was estimated at around 20% of seeds. Mice were divided randomly in two groups Control (CG) and Treatment (TG), with 7 mice each. Treatment diet, prepared as pellet to avoid different feed intakes in animals, contained 10% of tobacco seeds from F18+ and 10% of tobacco seeds from VT2e-b+. CG received a diet containing 20% of non-transgenic tobacco seeds. Treatments were administered on days 21, 35, 14, 19, 20. TG received an increment of Iga Ig at day 28, while CG at the same period decreased. The histomorphological data of the small intestine showed that TG crypts of the duodenum were significantly deeper than those of the CG. Immunostaining of the intestine showed that administration of transgenic tobacco seeds promotes a significant increase in the Iga-positive plasma cells production of the tonica propria if compared to control group. In conclusion our findings suggest that tobacco seeds might be a potential source of oral vaccines.

INTRODUCTION

Edema disease (ED) 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). ED 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 (Fig.1).

• Shiga-like toxins (VT2e) has been used to reproduce the clinical signs and pathological lesions of Edema disease.
• F18 fimbriae is responsible of adhesion of E.coli serotypes, related to Edema Disease. The fimbrial antigenic factor F18 is likely to be responsible for the local immunity.

MATERIALS & METHODS

- ISOLATION OF F18 AND VT2e B-SUBUNIT GENES
- Genomic DNA was extracted from O139 E.coli strains, isolated from different organs of swines dead for Edema Disease.
- F18 and B-subunit of VT2e genes were isolated by PCR analyses (Fig.2,3) oligonucleotide primers included sites for specific endonucleases (BanH-I; SacI-3) to facilitate direct subcloning of the fragments.

- TRANSFORMATION
- VT2e-B mild f18 genes were cloned into binary plasmid under control of Glob, seed specific promoter.
- The chimeric constructs (fig.4) were introduced in Agrobacterium tumefaciens EHA105 strains by electroporation.
- Leaf disks were infected with recombinant Agrobacterium (figs. 5,6,7) and plants or seeds were evaluated trough PCR, Northern Blotting, Western Blotting, agglutination on slides.

- MICROS INVOLVED AND TREATMENTS
- 14 female Balb c mice (4 weeks old) were allotsted in cages with 7 mice (Treatment groups, TG) and 7 mice (Control group, CG).
- Mice, fasted for 12 hours, were fed one of four treatments on days 0, 5, 8, 14, 19, 23.
- Treatment diet, prepared as pellet to avoid different feed intakes in animals, contained 10% of tobacco seeds from F18+ and 10% of tobacco seeds from VT2e-B+.
- CG received a diet containing 20% of non-transgenic tobacco seeds.

- ANALYSES AND MEASUREMENTS
- Iga and IgG amounts were evaluated in fecal samples collected on days 1-5, 14, 19-23.
- Intestinal immune samples were evaluated through histomorphological and immunohistochemical analyses.

- Conclusion
In conclusion our findings suggest that tobacco seeds, transformed for the expression of VT2e-B and F18, might be a potential source of oral vaccines to protect animals for Edema Disease. They could be administered to mice without restraint of the animals, with low stress and without labour costs related to multiple injections of traditional vaccines.

RESULTS

- EVALUATION OF Escherichia coli STRAINS
- E.coli strains, analyzed by PCR, presented f18 and VT2e-B genes (fig.2,3).

- EFFICIENCY OF TRANSFORMATION
- About 90% of tobacco plant presented f18 and VT2e-B genes.
- Northern blot analysis, carried out with a specific RNA probes on total RNA extracted from seeds of transformed plants, showed about 85% of positive samples for F18 fimbriae and about 45% of positive samples for VT2e B-subunit.
- All samples, positive for the f18 mRNA and for VT2e B subunit mRNA, were positive for the protein expression.
- The amount of transgenic proteins was estimated around 10kg/g of seeds.

- TO vs T1
- We obtained stable lines of transformed tobacco expressing F18 and VT2e-B subunit.
- The second generation of seeds was able to maintain seed accumulation of transgenic proteins (fig.8).

- IMMUNOLOGICAL EVALUATION
- TG showed an increment of f18 IgA at day 26, while CG at the same period decreased.
- The histomorphological data of the small intestine showed that TG crypts of the duodenum were significantly deeper than those of the CG (63.48μm vs 59.17μm; P<0.001).
- Immunostaining of the intestine (fig.9) showed that administration of transgenic tobacco seeds promotes a significant increase in the Iga-positive cells production of the tonica propria if compared to control group (Iga ileum 6.22±2.93%; P<0.001).

- Conclusions
In conclusion our findings suggest that tobacco seeds, transformed for the expression of VT2e-B and F18, might be a potential source of oral vaccines to protect animals for Edema Disease. They could be administered to mice without restraint of the animals, with low stress and without labour costs related to multiple injections of traditional vaccines.