SHEEP IMMUNIZATION WITH LIVE Salmonella abortusovis VACCINE STRAINS

Rubino S.,[1] Leori G.,[2] Uzzau S.,[1] Santona A.,[1] Schiaffino A.,[1] Santona M.,[1] Solinas M.,[1] Satta M.P.[1] and P. Cappuccinelli[1]

[1]Istituto di Microbiologia e Virologia, Viale S. Pietro 43 B, 07100 Sassari, Italy and [2]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy.

S. abortusovis is a pathogen responsible of abortion in sheep. It cause a serious damage in economy based on sheep breeding, and an effective vaccine is still needed. Three observations indicate existence of a serviceable immunity: ewes infected with *S. abortusovis* usually abort once, it exists a cyclical evolution of abortive episodes inside a flock or a sheep-rearing regions, primo-infected ewes exhibit an enhanced resistance. For this purpose we have obtained several avirulent *S. abortusovis* strains using different strategies.

- a) Mutants with a double deletion in *crp-cya* genes respectively for cyclic AMP receptor protein or adenylate cyclase.
- b) A mutant with a deletion in *AroA* gene, therefore blocking the aromatic biosynthetic pathway.
- c) A plasmid cured strain.

Results

We have generated a *crp*-mutant in *S. abortusovis* transducting with P22 lysate *S. typhimurium* and subsequently with the same strategy *cya* mutation. The *crp-cya S. abortusovis* have been characterized phenotypically.

The *AroA* mutant has been obtained by two step transduction using as a first donors serC(*AroA*)::Tn10 *S. typhimurium* and as a second donor a AroAl48 deletion *S. typhimurium* mutant. The *AroA S. typhimurium* mutant obtained was auxotrophic as expected.

Displacement of *S. abortusovis* plasmid were obtained by incompatibility using plasmid pLL6. The three different vaccine candidates were avirulent in mice Balb/c by oral route and protective against the wild type strains.

Furthermore, sheep immunizations using Berrichon crossbred ewes, with no cultural or serological evidence of *S. abortusovis* were performed. Mutants were administred by subcutaneous (s.c.) route. Clinical parameters as well as infection levels in different organs were assessed. Five sheep and one ram were grouped together after synchronization of the estrus cycles of ewes by using vaginally applied fluorogestone sponges. The sheep were confirmed pregnant by ultrasound examination at 50 days of gestation.

Table 1. *Immunization of five sheep with different* S. abortusovis *mutants.*

	A	RF	DL	D
Wild type SS 44	3	0	1	1
AroA	1	2		2
Crp- cya	3	0	0	2
Plasmid cured	0	0	0	5

A: sheep aborted, RF: reabsorbition of fetus, DL: death lamb, D: delivered healthy lamb.

Tissues associated with abortion or foetus were examined for the presence of *S. abortusovis*.

Table 1 showed the results of the immunization experiments in sheep. The three mutants were protective and we propose then as candidates for a sheep vaccine. The degree of attenuation in sheep is currently under investigation.

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