SHEEP IMMUNIZATION WITH LIVE Salmonella abortusovis VACCINE STRAINS

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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 transducing 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 AroA48 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 Berichon crossbred ewes, with no cultural or serological evidence of S. abortusovis were performed. Mutants were administrated 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.

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<thead>
<tr>
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<th>A</th>
<th>RF</th>
<th>DL</th>
<th>D</th>
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</thead>
<tbody>
<tr>
<td>Wild type SS 44</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AroA</td>
<td>1</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Crp- cya</td>
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<td>0</td>
<td>0</td>
<td>2</td>
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<td>Plasmid cured</td>
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<td>0</td>
<td>5</td>
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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 them as candidates for a sheep vaccine. The degree of attenuation in sheep is currently under investigation.

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