Safety and Efficacy Evaluation of the Oral Rabies Vaccine SAG2 in Indian Stray Dogs

J. Barrat¹, E. Picard¹, H.K. Pradhan², B. Pattnaik², S.S. Patil², P.R. Vanamayya², Rich Sood², Ripudaman Singh³, J.P. Gurbuxani³, F.X. Meslin⁴, A. Régnault⁵ and F. Cliquet¹

¹ AFSSA – LERRPAS, Malzeville, France
² HSADL, Indian Veterinary Research Institute, Bhopal, India
³ Petswill, Ludhiana Punjab, India
⁴ WHO, Geneva, Switzerland
⁵ Virbac, Carros, France
Several partners in the trial

- Coordinated:
  - Between WHO (Geneva) and VIRBAC (France)

- Executed and conducted:
  - By WHO Collaborating Centre for Research and Management in Zoonoses Control of Malzeville (France)
  - At High Security Animal Disease Laboratory, Bhopal (India)

- Other collaborator:
  - Petswill, 3 Kesar Complex, Malhar Road, Gurudev Nagar, Ludhiana Punjab (India)
Rabies in India

- Reported from all states of India. More than 20,000 deaths per year
- Approximately 95% of human rabies cases are due to stray dogs bites (jackals 1.7%, cats 0.8%, foxes 3%, …)
- Inadequate laboratory diagnostic facilities
- Estimated dog population : 27 millions ; dog/human ratio = 1/40
- Majority of dogs are stray, unowned and unprotected
Objectives of the trial

To evaluate in Indian stray dogs both the safety and the efficacy of a lyophilised SAG2 (DBL2) bait

- **Safety / innocuity:**
  - To assess the absence of pathogenicity of SAG2 for dogs even immunodepressed;
  - To assess the absence of salivary excretion of the vaccinal virus

- **Efficacy:**
  - To assess the protection induced by the vaccine in dogs

**Final objective:**
To associate parenteral vaccination of owned dogs and oral vaccination of stray dogs in a rabies control project

Lyophilised DBL2 bait (SAG2, Virbac, France)
Selected from SAD Bern strain and two successive mutations of the Arginin 333 codon
Animals used in the study (1)

- Dogs aged 6-12 months, all originating from Bhopal. Till challenge, all animals were kept in a traditional animal facility

- Identification with collar & numbered tag

- Dewormed and vaccinated against canine dispemper, Rubarth hepatitis, parvovirosis and leptospirosis
# Initial experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Dogs</th>
<th>Vaccine</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>5</td>
<td>Rabidog® bait</td>
<td>Oral</td>
<td>Single bait</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral single</td>
<td>5 (immuno-depressed)</td>
<td>Rabidog® bait</td>
<td>Oral</td>
<td>Single bait</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Efficacy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>11</td>
<td>Rabidog® bait</td>
<td>Oral</td>
<td>Single bait</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Comparison of the bait acceptance, efficacy and safety of lyophilised SAG2 bait

Schematic representation of the experimental protocol

Days

-28 0 1 3 5 14 28 56 109

preparation vaccination rabies challenge

in stray dogs

Rabies neutralising antibody test

Cell culture and RT-PCR tests on saliva samples

End of the trial euthanasia

SEARG meeting Windhoek, 22-26 January 2006
Groups I ("normal" vaccinees of safety trial) and IV (vaccinees of efficacy trial) have no treatment between D0 (vaccination) and D109 (challenge).

The control groups III and V have been treated identically till D109.

Salivary excretion, health and serological response have been monitored on 12 dogs (with 8 controls)
Vaccination and challenge

- **Vaccination**
  - Titre of lyophilized Rabidog® vaccine bait: $10^{8.50}$ TCID$_{50}$/dose

- **Rabies challenge**
  - Intramuscular challenge with the supernatant of homogenised sub-maxillary salivary glands of naturally rabid dogs titrating $10^{6.5}$ MICLD$_{50}$/ml
  - Strain: Street rabies virus, Tunisian origin, passaged once in dogs
  - Injection of 100 MICLD$_{50}$ of virus per animal at D109 (more severe test than 28 days post-baiting)

- **Clinical observation**
  - Daily observation all along the experiment and any unusual sign recorded.
  - 110 days post-challenge, euthanasia of all surviving dogs (D219)
Results
Bait acceptance and selection of dogs

<table>
<thead>
<tr>
<th>group</th>
<th>number of dogs</th>
<th>VNA titre &gt; 0.5 IU/ml at D0</th>
<th>Bait uptake</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vaccinated group</td>
<td>5</td>
<td>* 1/5</td>
<td>5/5</td>
<td>&lt;3 (2/5)</td>
</tr>
<tr>
<td>vaccinated immunodepressed group</td>
<td>5</td>
<td>0/5</td>
<td>5/5</td>
<td>&lt;3 (5/5)</td>
</tr>
<tr>
<td>control group</td>
<td>5</td>
<td>* 2/5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vaccinated group</td>
<td>11</td>
<td>* 2/11</td>
<td>11/11</td>
<td>&lt;2 (11/11)</td>
</tr>
<tr>
<td>control group</td>
<td>5</td>
<td>0/5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- all vaccinated dogs had eaten totally their bait in less than 20 min (70% < 3 min)
- * 5 dogs have been rejected (virus neutralizing activity > 0.5 IU/ml at D0)
## Safety trial

<table>
<thead>
<tr>
<th>group</th>
<th>number of dogs</th>
<th>dead</th>
<th>clinical signs</th>
<th>Salivary excretion (CC and PCR) D0 before, after baiting D1, D3, D5</th>
<th>Rabies diagnosis (FAT) at D219</th>
</tr>
</thead>
<tbody>
<tr>
<td>vaccinated groups I and IV</td>
<td>4 + 8</td>
<td>0/12</td>
<td>no</td>
<td>Neg (0/12)</td>
<td>Neg (0/12)</td>
</tr>
<tr>
<td>vaccinated immunodepressed group II</td>
<td>5</td>
<td>0/5</td>
<td>no</td>
<td>Neg (0/5)</td>
<td>Neg (0/5)</td>
</tr>
<tr>
<td>control groups III and V</td>
<td>3 + 5</td>
<td>0/8</td>
<td>no</td>
<td>Neg (0/8)</td>
<td>Neg (0/8)</td>
</tr>
</tbody>
</table>

- Clinical observation: all animals remained healthy all along the 219 days observation period
- No salivary excretion by using cells culture inoculation and no specific RNA detection using RT-PCR
Efficacy trial

Rabies virus neutralising antibodies (VNA)

- 8 controls
- 13 vaccinees
- 7 positive vaccinees
- 6 negative vaccinees

Threshold 0.5 IU/ml

Vaccinated dogs (groups 1 and 4): blood samples have been taken at D 14, 28, 56

SEARG meeting Windhoek, 22-26 January 2006
## Survival after challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of dogs</th>
<th>Number of positive for VNA</th>
<th>Number of dead</th>
<th>Mortality delay (days)</th>
<th>Clinical phase (days)</th>
<th>Rabies diagnosis (FAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated group</td>
<td>IV 9</td>
<td>5/9</td>
<td>0/9</td>
<td>-</td>
<td>-</td>
<td>Neg (9/9)</td>
</tr>
<tr>
<td>Control group</td>
<td>V 5</td>
<td>0/5</td>
<td>5/5</td>
<td>25 - 85</td>
<td>6 - 8</td>
<td>Pos (5/5)</td>
</tr>
</tbody>
</table>

FAT: performed on brain and salivary glands on D219, i.e. 110 days post-challenge

- 9/9 vaccinated dogs resisted a virulent challenge that killed 5/5 controls
Conclusions of safety trial

- No salivary excretion of infective particle or virus RNA
- No vaccinal rabies
- Absence of adverse symptoms even in imuno depressed dogs
- Absence of replication of SAG2 strain in brain and salivary glands of all vaccinated dogs

The innocuity of SAG2 vaccine is then established in dogs
Conclusions of efficacy trial

- Bait acceptability: 100% in less than 20 min

- Protection:
  - Serological testing shows that 50% of vaccinated dogs can be considered protected
  - Resistance to a virulent challenge that killed unvaccinated controls assesses the protection induced by SAG2 vaccine

- The efficacy of SAG2 vaccine is then established
The protective effect of SAG2 vaccine had been already demonstrated on laboratory and field dogs and on other species.

Efficacy established on:
- jackals in Zimbabwe (Bingham et al., 1999)
- captive artic foxes (Follman et al., 2004)
- ...

Safety and efficacy shown in Tunisian dogs and South African puppies (Schumacher et al., 1999).

Establishment of the safety:
- on Tunisian animals (Hammami et al., 1999):
  - Dogs (adult and young)
  - Domestic cats
  - Local animal species susceptible to consume vaccine baits: jackal, jerboa, merion, gerbil
Final conclusion

- SAG2 confirmed to be:
  - Safe for people and dogs because
    - neither salivary excretion
    - nor adverse clinical signs
    - or replication of virus were observed in vaccinated dogs
  - Efficient for vaccination
    - as half of vaccinated dogs seroconverted and
    - as all vaccinees resisted a virulent challenge

☞ SAG2 vaccine may be used in the field to orally vaccinate Indian stray dogs for rabies control
To finish: some pictures…
"Outside" quarantine facility of HSADL, before challenge
Bait acceptance

- All tested dogs had totally eaten the baits:
  - 16/21 in less than 3 minutes
  - 21/21 in less than 20 minutes
Acknowledgments

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