EDQM Workshop
Bluetongue Vaccines
19 February 2013
Strasbourg, France

Session 2
Immunogenicity and batch potency testing of inactivated bluetongue vaccines
BTV MANUFACTURERS’ VIEWPOINT

Immunogenicity, batch potency test and safety

BLUETONGUE VACCINES Ovine

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>SEROTYPES</th>
<th>SOLD/USED</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMUN BLUETONGUE S1-8 ONE SHOT</td>
<td>Serotype 1, Serotype 8</td>
<td>France/Spain</td>
</tr>
<tr>
<td>PRIMUN LENGUA AZUL S1</td>
<td>Serotype 1</td>
<td>Spain</td>
</tr>
<tr>
<td>PRIMUN LENGUA AZUL S1-S8</td>
<td>Serotype 1, Serotype 8</td>
<td>--</td>
</tr>
</tbody>
</table>

Vaccine claim: PREVENTION OF VIRAEMLIA
BLUETONGUE VACCINES
OVINE

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>BTV8</th>
<th>BTV 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMUN BLUETONGUE S1-8 ONE SHOT</td>
<td>BTV-8/BEL2006/02 ≥ 1.59 log10*</td>
<td>BTV-1/ALG2006/01 E1 ≥ 1.48 log10*</td>
</tr>
</tbody>
</table>

* Logarithm of Neutralizing antibody titres obtained after one inoculation of one dose in sheep

<table>
<thead>
<tr>
<th>Challenge ASSAY</th>
<th>WHEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge: minimum potency</td>
<td>At release</td>
</tr>
<tr>
<td>Duration of immunity</td>
<td>At the end of self-life, 21 month</td>
</tr>
<tr>
<td>Effect of maternal antibodies</td>
<td>At release</td>
</tr>
</tbody>
</table>

According on new available data tightening of specification limits is being considered.

BLUETONGUE VACCINES
OVINE

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>BTV 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMUN LENGUA AZUL S1</td>
<td>BTV-1/ALG2006/01 E1 ≥ 1.10 log10*</td>
</tr>
</tbody>
</table>

* Logarithm of Neutralizing antibody titres obtained after one inoculation of one dose in sheep

<table>
<thead>
<tr>
<th>Challenge ASSAY</th>
<th>WHEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge: minimum potency</td>
<td>At release</td>
</tr>
<tr>
<td>Duration of immunity</td>
<td>At the end of self-life, 24 months</td>
</tr>
<tr>
<td>Effect of maternal antibodies</td>
<td>At release</td>
</tr>
</tbody>
</table>

According on new available data tightening of specification limits is being considered.
**BLUETONGUE VACCINES OVINE**

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>BTV8</th>
<th>BTV 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMUN LENGA AZUL S1-8</td>
<td>BTV-8/BEL2006/02 ≥ 1,26 log10*</td>
<td>BTV-1/ALG2006/01 E1 ≥ 1,10 log10*</td>
</tr>
</tbody>
</table>

* Logarithm of Neutralizing antibody titres obtained after one inoculation of one dose in sheep

<table>
<thead>
<tr>
<th>Challenge ASSAY</th>
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<tr>
<td>Challenge: minimum potency</td>
<td>At release</td>
</tr>
<tr>
<td>Duration of immunity</td>
<td>At the end of self-life, 24 month</td>
</tr>
<tr>
<td>Effect of maternal antibodies</td>
<td>At release</td>
</tr>
<tr>
<td></td>
<td>At 17 months</td>
</tr>
</tbody>
</table>

According on new available data tightening of specification limits is being considered

**CONTROLS DURING PRODUCTION**

- The finished product is formulated on the basis of the antigen content measured on the harvest before inactivation by means of a validated BTV titration (for BTV 8 and BTV1). The titre before inactivation is considered the most reliable parameter of the antigen quantification which measures all the viral particles.
- In addition VP7 determination by ELISA is performed. This assay can be applied on the inactivated antigen, but is not specific for each serotype of virus.
- Then, once formulated, the finished product is tested by the batch potency test.
**BATCH POTENCY TEST**

- The finished product is tested by the batch potency test.
- The batch potency is the IMMUNOGENICITY ASSAY (CHALLENGE)
- The test described under Potency is not carried out for routine testing of all batches of vaccine
- Where the test is not carried out, a suitable validated test is carried out, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency (correlation).

**IMMUNOGENICITY: challenge test**

The challenge model was established in LABORATORIO CENTRAL DE VETERINARIA-ALGETE (LCV).
After 21 days post vaccination at least 5 vaccinates and 2 controls shall each be challenged with virulent bluetongue virus and observed for 25-28 days.
From challenge day to 28 days after challenge, animals are monitored for the presence of BTV genome by RT-PCR-testing on days 3, 5, 7, 10, 13, 17, 20, 24 and 28 days after, in order to assess viremia.
ALTERNATIVE BATCH POTENCY TEST: seroneutralisation

- The test described under Potency is not carried out for routine test.
- The vaccine is injected into each of 5 seronegative animals, 2 other animals are left as controls. After a suitable interval (21d) blood is collected. The antibody titres against virus is determined by seroneutralisation on suitable cell cultures. The test is invalid if the control animals show antibodies against BTV.
- The vaccine complies with the test if the level of antibodies is not lower than that found for a batch of vaccine that has given satisfactory results in the test described under Potency.

ALTERNATIVE BATCH POTENCY TEST:
seroneutralisation

- Seroneutralization response in ovine

SN test has been validated in order to optimize the sensitivity and accuracy by using a large number of repetitions. Serum samples of vaccinated and control animals are tested in two-fold dilutions starting with an initial dilution of 1 in 2. Equal volumes of BTV virus are added to all sera, and the plate is incubated. Vero cells are added to all wells, and the plates are incubated at 37°C for 4 -7 days. Characteristic cytopathic effect (CPE) is observed. The number of virus-positive wells is confirmed. The neutralization titre of each sample is defined as the last dilution in which at least half of the monolayer was intact (TCID$_{50}$).
**ALTERNATIVE BATCH POTENCY TEST: seroneutralisation (1)**

- Similar assays are described in Pharmacopeia monographs for viral inactivated vaccines for veterinary use, for example Bovine viral diarrhoea vaccine.
- OIE, recommends a potency test of BTV vaccine which consists in vaccinating sheep and measuring neutralizing antibodies titres.
- Code of Federal Regulations 113.303 Bluetongue Vaccine. Establish a model of immunogenicity trial which sets requirements for protection of vaccinated animals against a challenge as well as neutralizing antibody levels to be achieved by these animals (95% of vaccinated animals must show SN titre > 1:4).

**ALTERNATIVE BATCH POTENCY TEST: seroneutralisation (2)**

- This model of alternative assay implies minimal suffering of the animals involved in the trial. Animals are not challenged, merely are vaccinated and bled. There is no need to sacrifice at the end of the trial.
- This model test directly measures an immune response in the target animal.
- The method is capable to ensure that each released batch will produce the desired immunological response: neutralising antibodies in the target animals. The presence of type-specific neutralising antibodies is indicative of protective immunity to BT.
- The alternative batch potency test has been validated.
ALTERNATIVE BATCH POTENCY TEST: seroneutralisation. Conclusions

- The method used for potency test consisting in the determination of neutralising antibodies in sera of 5 ovine inoculated with a single dose of vaccine presents an adequate dose-response relationship.

- Specifications for the batch release involve the obtention of SN titres higher than those obtained with the minimum potency batch.

- Compliance with this specification guarantee the effectiveness of the vaccine with a large safety margin because many animals have past the challenge with titres below these specifications.

- The method is sufficiently accurate in detecting sub-formulated batches.

Safety- laboratory trials bluetongue vaccines

- Parameters:
  - Temperature at before, after 4h hours and at 1, 2, 3 and 4 days after each vaccination.
  - Local reaction: daily
  - Clinical signs: daily
  - Pregnant sheep: prolificacy, abortions, perinatal mortality, lamb weight at birth, lamb weekly weight gain.
  - Lactating sheep: milk production, milk composition
SAFETY EVALUATION IN 3 month LAMBS
x2 doses
10 lambs plus 10 control

SAFETY EVALUATION OF BLUETONGUE IN PREGNANT SHEEP
x2 doses
10 sheep plus 10 control (last quarter, merino)

SAFETY EVALUATION IN LACTATING SHEEP
x2 doses
10 sheep (Assaf 4 month lactation) plus 10 control

Local reaction onset within the 2-6 days. Nodules of 1-3 cm, which reduce gradually from after a week, the remaining nodule was \( \approx 1 \) cm

NON EFFECTS IN GESTATION (prenatal or perinatal mortality rates)

NON EFFECTS IN MILK PRODUCTION or COMPOSITION

VLA A 063 S 09

Safety of PRIMUN BLUETONGUE 1-8 ONE SHOT- doble dose in lambs (batch maximum potency nº 09/001ª.
TEMPERATURE)

Comparison of temperatures between control and vaccinated group

<table>
<thead>
<tr>
<th>Day</th>
<th>Vaccinated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>0+4h</td>
<td>39.2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>41.2</td>
<td></td>
</tr>
</tbody>
</table>
VLA A 064 S 09 Safety of PRIMUN BLUETONGUE 1-8 ONE SHOT in gestating sheep (maximum potency batch)
Local reaction evolution after vaccination (double dose- 4 ml)

Parameter Control Vaccinated RSD
prolificacy 1.50 1.20 0.582 ns
Weight at birth 4.36 4.56 0.828 ns
Weight gain week 1 2.62 2.26 0.702 ns
Weight gain week 2 1.84 1.68 0.459 ns
Weight gain week 3 2.45 2.02 0.658 ns
Weight gain week 4 1.88 1.63 0.505 ns
abortion 0 0
Perinatal mortality 0 0
Safety of PRIMUN BLUETONGUE 1-8 ONE SHOT in lactating sheep (batch maximum potency)

The Pharmacovigilance system showed an increase since October 2008 in the Notification of SARs. The most common types of SARs in animals were reported in ovine managed in extensive, after revaccinations and were: nerve Symptoms, Wasting syndrome, abortions or death.

Companies were asked to conduct a safety trial in field conditions with vaccines used in the official campaign. The aim of this study was to evaluate the possible relationship between the administration of our PRIMUN BLUETONGUE S1-8 ONE SHOT vaccine and the emergence of these SARs.
SAFETY EVALUATION OF PRIMUN BLUETONGUE S1-8 ONE SHOOT in field Farms/ REGIONS VLAP004 S10, ADVERSE REACTION control SARs in previous vaccinations.

**Navarra**
- 6 farms (all with previous SARs)
- Controlled animals: pregnant females (30/GROUP)
- Ataxia, Motor alterations, salivation, prostration, respiratory distress, loss of herd instinct

**Aragón**
- 3/6 farms with previous SARs
- Controlled animals: young lambs (30/GROUP)

**Extremadura**
- 3/6 farms with previous SARs
- Controlled animals: pregnant females (30/GROUP)
- Same, plus abortion

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**Safety- field trial**

**PRIMUN bluetongue S1-8 one shot**

<table>
<thead>
<tr>
<th>Study day</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15</td>
<td>Sampling of blood and faeces</td>
</tr>
<tr>
<td>-15 a -10</td>
<td>coprological Analysis</td>
</tr>
<tr>
<td></td>
<td>Serology: BTV, Border, paratuberculosis, Visna-Maedi</td>
</tr>
<tr>
<td>-2</td>
<td>Corporal conditions evaluation</td>
</tr>
<tr>
<td>0</td>
<td>Temperature measurement.</td>
</tr>
<tr>
<td></td>
<td>Vaccination</td>
</tr>
<tr>
<td></td>
<td>General tolerance evaluation</td>
</tr>
<tr>
<td>+1</td>
<td>General tolerance evaluation</td>
</tr>
<tr>
<td>+2 a +7</td>
<td>General tolerance evaluation</td>
</tr>
<tr>
<td>+ 7</td>
<td>Temperature measurement.</td>
</tr>
<tr>
<td>+ 14</td>
<td>General tolerance evaluation</td>
</tr>
<tr>
<td>+ 30</td>
<td>Temperature measurement.</td>
</tr>
<tr>
<td></td>
<td>Tracking animals with reactions</td>
</tr>
<tr>
<td></td>
<td>General tolerance evaluation</td>
</tr>
<tr>
<td>6 months</td>
<td>Follow-up until 6 month after vaccination.</td>
</tr>
<tr>
<td></td>
<td>Record any adverse reaction</td>
</tr>
</tbody>
</table>
### Safety- field trial

**PRIMUN bluetongue S1-8 one shot**

<table>
<thead>
<tr>
<th>REGION / GROUPS</th>
<th>Re-vaccinated group A</th>
<th>First vaccinated Group B</th>
<th>Control A (revaccinated)</th>
<th>Control B (first-vaccinated)</th>
<th>Adverse reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Navarra (pregnant postpartum)</td>
<td>6439 (247)</td>
<td>680 (147)</td>
<td>105 (105)</td>
<td>75 (75)</td>
<td>0</td>
</tr>
<tr>
<td>Aragón (Young lambs)</td>
<td>-</td>
<td>1107 (175)</td>
<td>--</td>
<td>170 (170)</td>
<td>0</td>
</tr>
<tr>
<td>Extremadura (pregnant)</td>
<td>6205 (170)</td>
<td>18 (18)</td>
<td>155 (155)</td>
<td>15 (15)</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>12644 (417)</td>
<td>1805 (340)</td>
<td>260 (260)</td>
<td>260 (260)</td>
<td>0</td>
</tr>
</tbody>
</table>

### BLUETONGUE VACCINES

**OVINE**

Thank you very much for your attention
BLUEVAC VACCINES

Inactivated vaccines against bluetongue disease

EDQM, Strasbourg – 19 Feb 2013

BLUEVAC Monovalent vaccines

<table>
<thead>
<tr>
<th>BLUEVAC-1</th>
<th>BLUEVAC-4</th>
<th>BLUEVAC BTV8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype 1</td>
<td>Serotype 4</td>
<td>Serotype 8</td>
</tr>
<tr>
<td>Bovine, ovine</td>
<td>Bovine, ovine</td>
<td>Bovine, ovine</td>
</tr>
<tr>
<td>Spain, Portugal, France</td>
<td>Spain, Portugal</td>
<td>Spain, Germany (authorised in EU following a CP)</td>
</tr>
</tbody>
</table>
BLUEVAC Bivalent vaccines

<table>
<thead>
<tr>
<th>BLUEVAC 1+4</th>
<th>BLUEVAC 1+8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotypes 1 and 4</td>
<td>Serotypes 1 and 8</td>
</tr>
<tr>
<td>Bovine, ovine</td>
<td>Bovine, ovine</td>
</tr>
<tr>
<td>Spain</td>
<td>Spain</td>
</tr>
</tbody>
</table>

SAFETY

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BLUEVAC BTV8, 1 and 4
Safety - Ovine

Laboratory trials
- Administration of one dose
- One administration of an overdose (2x)
- Overdose in pregnant females (serotype 8)
- The repeated administration of one dose
- Examination of Reproductive function (females and males, serotypes 8 and 1)
- Examination of Immunological functions through immune response: SNT and antibodies anti VP7

Field trials
- Collaborative trial carried out by Animal German Health authorities and Friedrich-Loeﬄer Institute (357 sheep vaccinated with BLUEVAC BTV8)
- Trial in Spain, requested by the authorities, (16,408 animals vaccinated with BLUEVAC 1+8)

Pharmacovigilance.

BLUEVAC BTV8, 1 and 4
Safety - Bovine

Laboratory
- Administration of one dose
- One administration of an overdose (2x, serotype 8)
- The repeated administration of one dose
- Examination of Immunological functions: SNT and antibodies anti VP7

Field trials
- Collaborative trial carried out by Animal German Health authorities and Friedrich-Loeﬄer Institut (298 cows, adults and heifers).
- Pregnant females, serotype 1

Pharmacovigilance
BLUEVAC Safety conclusions

- An average increase in body temperature can occasionally be observed varying between 0.5 and 1.0 ºC. Transient fever was observed in rare cases.

- Painless local reactions were observed (up to 3.0 cm in sheep and 5 cm in cattle, disappearing within 14 days and 41 days respectively).

- Safe during pregnancy in ewes and cows. No negative impact in lactating ewes and cows.

- No negative impact on immune response.

EFFICACY/POTENCY

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BLUEVAC BTV 8, 4 and 1  
Efficacy - Ovine and Bovine

- Controlled laboratory trials with challenge
  - First studies in collaboration with Animal Health Authorities
  - CZV Level 3 biological containment infection facilities.
- Vaccinated groups/Non vaccinated control group
- Dose response (3 concentrations/1 or 2 doses)
- Immune response before challenge – SNT and antibodies antiVP7
- Clinical symptoms post challenge.
- Viremia by real time PCR.

### BLUEVAC BTV8 - Ovine SNT and anti-VP7 results

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine Titre CCID50/ml</th>
<th>No. of doses</th>
<th>SNT Day 0 before challenge</th>
<th>Anti-VP7 Day 0 before challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$10^{6.0}$</td>
<td>1</td>
<td>&lt;4</td>
<td>$2.6 \pm 1.9$</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>2</td>
<td>$4.6 \pm 2.8$</td>
<td>$41.2 \pm 59.9$</td>
</tr>
<tr>
<td>III</td>
<td>$10^{6.5}$</td>
<td>1</td>
<td>&lt;4</td>
<td>$7.6 \pm 7.8$</td>
</tr>
<tr>
<td>IV</td>
<td>$10^{6.5}$</td>
<td>1</td>
<td>&lt;4</td>
<td>$4.8 \pm 6.5$</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>2</td>
<td>$11.8 \pm 3.5$</td>
<td>$44.8 \pm 34.7$</td>
</tr>
<tr>
<td>VI</td>
<td>$10^{6.8}$</td>
<td>1</td>
<td>$0.8 \pm 1.7$</td>
<td>$2.2 \pm 0.83$</td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td>1</td>
<td>&lt;4</td>
<td>$2.8 \pm 3.34$</td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td>2</td>
<td>$9.0 \pm 5.4$</td>
<td>$86.4 \pm 38.5$</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>0</td>
<td>&lt;4</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>
### BLUEVAC BTV8 - Ovine
Global Clinical Score post challenge

![Graph showing Global Clinical Score post challenge](image)

### BLUEVAC BTV8 – Ovine
Protection against viraemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine Titre CCID50/ml</th>
<th>No. of doses</th>
<th>Protection (Negative PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$10^{6.0}$</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>II</td>
<td>$10^{6.5}$</td>
<td>2</td>
<td>80%</td>
</tr>
<tr>
<td>III</td>
<td>$10^{6.5}$</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>IV</td>
<td>$10^{6.5}$</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>V</td>
<td>$10^{6.5}$</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>VI</td>
<td>$10^{6.8}$</td>
<td>1</td>
<td>40%</td>
</tr>
<tr>
<td>VII</td>
<td>$10^{6.8}$</td>
<td>1</td>
<td>40%</td>
</tr>
<tr>
<td>VIII</td>
<td>$10^{6.8}$</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
**Duration of immunity**
**Design and methodology**

- All animals challenged at the end of the study
- Vaccinated group
- Non vaccinated control group

- Monitoring of immune response until the end of the study – SNT and antibodies antiVP7
- Clinical symptoms post challenge.
- Presence/absence of viraemia by real time PCR.

- **BLUEVAC BTV8 and BLUEVAC-1**
  - Ovine and bovine

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**BLUEVAC BTV8 - Ovine**
**Serum neutralisation response**

Legend:  
Antibody anti-VP7 response

DOI at 305 days after 1st dose
Temperature post challenge
**DOI at 305 days after 1st dose**

**Clinical score post challenge**

![Graph showing clinical score over days post challenge](image)

Legend: VG: Vaccinated group, CG: Control group.

**Viraemia data at 305 days after 1st dose by RT-qPCR**

<table>
<thead>
<tr>
<th>Days post challenge</th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 17</th>
<th>Day 21</th>
<th>Day 24</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vac</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Con</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Inco</td>
<td>Pos</td>
<td></td>
</tr>
</tbody>
</table>
To evaluate the effect of MDA antibodies on the humoral response in lambs after vaccination.

To assess the degree of interference of MDAs in the efficacy of the vaccine by experimental challenge.

Seropositive and seronegative groups

Immune response by SNT and ELISA
Presence/absence of viraemia by real time PCR.

No significant differences were observed between MDA positive and negative animals in terms of humoral response at the selected date of vaccination and after.

Low interference of MDAs was observed in the efficacy showed by vaccinated and challenged animals.
Batch potency test
BTV8, BTV1 and BTV4

- **Challenge BPT in sheep**
  - The test passes if:
    - Vaccination group protection is 100% (no presence of viral genome by RT-PCR).
    - Control unvaccinated group protection is 0% (presence of viral genome by RT-PCR).

- **Alternative potency test**
  - Challenge test in mice
  - Protection is given in terms of survival of vaccinated against controls (significant difference)

Thank you for your attention
Immunogenicity and batch potency test
Looking back or going forward?

BTV incursions into Europe

- Past outbreaks: BTV 1, 2, 4, 8, 9, 16
- Future outbreaks: BTV 3, 5, 6, 7, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24
Experiences from BTV8 episode 2006-2013

- 2006: Outbreak BTV8
- 2008: Year 1 of vaccination:
  - top priority speed of delivery (high political pressure)
- 2009: Year 2 of vaccination:
  - top priority inventory management and shelf life
  - low political and significantly increased regulatory pressure
- 2010 - 2012: Year 3 and following:
  - “business as usual”
  - high regulatory pressure
  - customer breach supply contracts for regulatory reasons
- 2013: BTV8 disease under control, market is gone but still regulatory issues

Technical challenges BTV8

- Information about vaccination policy (=vaccine demand) only 6 months before first supply:
  - roller bottles first production platform
  - scaling up to fermentor parallel to sudden increase in demand
- R&D studies according to monograph 0062 but squeezed in 21 months:
- Immunogenicity study:
  - Target species (sheep, cattle), minimum age
  - Challenge model? / challenge virus?
  - Interpretation of efficacy
    - Parameters: clinical protection, PCR, viraemia, transmission data?
    - Claim reduction or prevention?
**MSD immunogenicity and batch potency test**

**Immunogenicity**
- Challenge virus grown on mammalian cells
- Challenge virus by subcutaneous route
  - Claim sheep: prevention viraemia from 1 month of age
  - Claim cattle: reduction viraemia from 6 weeks of age

**Batch potency test**
- Antigen quantification
  - pre-inactivation antigenic mass by ELISA
- Batch potency test:
  - In chickens, antibody by virus neutralization test
  - Potency compared to reference batch
  - Correlation batch potency test with protection in challenge (cattle + sheep)

**Lessons learned**
- 21 months to market only possible by taking decisions on limited data and running parallel processes
- Challenge models differ and results are not always comparable
- Efficacy parameters (e.g. PCR) differ and are not always comparable
- Antigenic mass test (development + validation) not possible in this short time frame
EU: future is more challenging than the past

- Past outbreaks: BTV 1, 2, 4, 8, 9, 16
- Future ???: BTV 3, 5, 6, 7, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24

MSD recommendations for new serotypes

- Insufficient data / time to develop / validate:
  - Challenge model in cattle and sheep
  - Substrate challenge virus: insect cells, blood, cell culture
  - PCR only in-company but not inter-company: if PCR values are used to compare data: we need 1 single validated PCR by reference lab
  - Antigenic mass test
  - In vitro potency test

- Conclusion and recommendation:
  To allow the shortest possible time to develop a vaccine, DO NOT draft a monograph for new (=non-EU) serotypes
Thank you for inviting us
ZOETIS feedback regarding potential BTV monograph
Dr Catrina Stiring – EDQM 19th Feb 2013

What vaccines do we have?

• Four monovalant vaccines for BTV 1 and 8 registered centrally
  – Zulvac 1 Ovis, Zulvac 1 Bovis, Zulvac 8 Ovis and Zulvac 8 Bovis

• Two bivalent vaccines for BTV 1+8 registered centrally
  – Zulvac 1+8 Ovis and Zulvac 1+8 Bovis

• Two of these are now full licenses the others we expect to be converted to full MA’s within 2013

• A monovalent BTV 4 vaccine registered nationally for sheep in Spain
Development

• All the current BTV vaccines were developed under emergency licensing strategies with the primary focus being on having a safe vaccine that prevented viremia

• Potency test methods were developed over time

• Flexibility in licensing requirements facilitated rapid registration of these vaccines

DO WE NEED A MONOGRAPH?

• In principle we support the idea of a monograph for existing serotype vaccines as it provides clear guidance on regulatory expectations

• However
  – We need to ensure that any monograph would not cause issues in an emergency situation with new serotypes
  – Requirements need to remain flexible for the emergency situation with new serotypes
  – And flexible enough within a monograph to cover all existing products – if possible?
Safety Data

• Zoetis BTV vaccines all contain Aluminium/Saponin adjuvants and use BEI inactivation

• With the exception of local injection site reactions which are expected no major safety risks have been noted with this profile

• Testing is based on Sheep and Cattle with BTV 1, 4 and 8

SAFETY ASPECTS for a MONGRAPH

• Basic safety requirements as per current guidance are OK

• If safety during pregnancy is required it needs to be optional
  – Current data are based on trials in sheep at 2 phases of gestation and field data in pregnant cattle

• No significant differences expected between sheep and cattle or across serotype for inactivated vaccines
IMMUNOGENICITY

• All efficacy/immunogenicity testing done to date has been based on challenge studies

• The primary efficacy variable has been prevention of viremia using a validated RT-PCR test

• There are differences in clinical signs observed with different serotypes and between sheep and cattle with the same serotype so these are not good indicators of efficacy.

• Serology although indicative is not ideal either

IMMUNOGENICITY for a MONOGRAPH

• There are significant differences in challenge models between sheep and cattle and between serotypes

• Immunogenicity testing could be based on prevention of viremia only
  – 10 vaccinate and 5 controls
  – min 80% viremia in controls to validate challenge (we usually see 90-100% depending on the number of animals used)
  – Prevention of viremia required (? 100% using validated RT-PCR)

• We have seen 100% prevention of viremia with serotype 1, 4 and 8 using a validated RT-PCR method
BATCH POTENCY TESTS

• Currently using a mouse challenge model giving RP against a reference vaccine of proven efficacy against challenge

• Test method for the adjuvant is required for fully in vitro

• Looked at RT-PCR for antigen quantification prior to blending but does not work on finished product

• Antigen ELISAs need serotype specific antibodies and method that works in the presence of adjuvant (or extraction method)
  – We have not pursued these currently
  – A generic option for antigen quantification based ELISA tests could be acceptable in a monograph though.
2006: Start of BTV vaccines development (BTV-4)

2008: Development of BTV-1 and BTV-8 vaccines

Applications submitted for the authorisation under exceptional circumstances for emergency use (Concept Paper EMEA/CVMP/IWP/105008/2007)

Temporary authorisations granted:

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>SPECIES</th>
<th>COUNTRIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYVAZUL® 4</td>
<td>Sheep</td>
<td>Spain</td>
</tr>
<tr>
<td>SYVAZUL® 1</td>
<td>Cattle &amp; Sheep</td>
<td>Spain, France, Portugal, UK and Belgium</td>
</tr>
<tr>
<td>SYVAZUL® 8</td>
<td>Cattle &amp; Sheep</td>
<td>Spain, France</td>
</tr>
<tr>
<td>SYVAZUL® 1+8</td>
<td>Cattle &amp; Sheep</td>
<td>Spain</td>
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<tr>
<td>SYVAZUL® 1+8 One shot</td>
<td>Sheep</td>
<td>Spain</td>
</tr>
</tbody>
</table>
**BTV-4: Syvazul 4**


**Species and Dosage:** Sheep: 2 doses (2 x 2 ml, 3 weeks apart)

**Sold in:** Spain 15,942,000 doses (2006-2009)

Oil based adjuvant

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**BTV-1: Syvazul 1**

Temporary Authorisations:

- 2008: Spain and France
- 2009: UK and Belgium

**Species and Dosage:** Sheep: 2 doses (2 x 2 ml, 3 weeks apart)

Cattle: 2 doses (2 x 4 ml, 3 weeks apart)

**Sold in:**

- Spain 17,284,000 doses (2008-2010)
- France 11,812,500 doses (2008-2010)
- Portugal 6,720,000 doses (2008-2010)
- UK 20,900 doses (2008-2010)

≈ 36 M doses
BTV-1 and BTV-8: Syvazul 1+8

Temporary Authorisations:
2009: Spain

Species and Dosage:
Sheep: 2 doses (2 x 2 ml, 3 weeks apart)
Cattle: 2 doses (2 x 4 ml, 3 weeks apart)

Sold in: Spain 5,000,000 doses (2009)
- 3.4 M sheep
- 1.6 M cattle

Development of vaccines

Immunogenicity

Development of vaccines strongly encouraged by Spanish Authorities (MAGRAMA)

Challenge model set up by CVL of Algete (Spanish NRL for BT)
- BTV-4
- BTV-1
- BTV-8

Dosage: $2 \times 10^6$ TCID$_{50}$ per animal

Route:
- subcutaneous in sheep
- intravenous in cattle
Development of vaccines

**IMMUNOGENICITY**

**Efficacy criteria**
- Development of virus neutralising antibodies after vaccination
- Protection from challenge
  - Clinical symptoms and lesions
  - Viraemia

**Virus neutralising antibodies (VNA) after vaccination**

**Syva's experience**
- Minimum VNA protective titres
  - BTV-1
    - Sheep: 4
    - Cattle: 8
  - BTV-8
    - Sheep: 4
    - Cattle: 4

**Vaccination induces VNA response**
- High variability on individual titres
  - Same trial, same vaccine, variations in titres > 4-5 Log2 (32 to 1024)

**Protection against challenge achieved with low and high VNA titres**

**Low value of VNA as primary efficacy criteria**

...unless VN development = protection
Efficacy criteria

Protection from challenge

Clinical symptoms and lesions
Viraemia

Hyperthermia ($\geq 40.5^\circ$C)

- **The most consistent symptom:**
  100% BTV-1 challenged control sheep
  92% BTV-8 challenged control sheep

- **Duration:** from 1 to 7 days (more frequently 3 to 5)

- **From days 6 to 10 after challenge**

Mild clinical symptoms

100% of BTV-1 challenge trials
70% of BTV-8 challenge trials

General symptoms appear more frequently
Lethargy, anorexia and decreased activity

Specific symptoms/lesions, usually with low intensity
Mucosal hyperaemic lesions, conjunctivitis, nasal and ocular discharge

Frequency of presentation and intensity
BTV-1 > BTV-8

Death of animals: < 10% of challenged control sheep
**Efficacy criteria**

**Protection from challenge**

**Clinical symptoms and lesions**

- Viraemia

- Mild symptoms

- High variability

- No objective valuation

**Cattle: no clinical manifestation**

**Low value of clinical evaluation as primary efficacy criteria**

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**Efficacy criteria**

**Protection from challenge**

**Clinical symptoms and lesions**

**Viraemia**

100% control animals (sheep & cattle) developed viraemia after challenge with either virus (BTV-1 or BTV-8).

Detection of virus RNA on blood samples by RT-PCR or qRT-PCR

**Onset of viremia:**

<table>
<thead>
<tr>
<th>Day pc</th>
<th>% viremic sheep</th>
<th>% viremic cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BTV-1</td>
<td>BTV-8</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100</td>
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</tr>
</tbody>
</table>

**Duration:** up to 28 pcd
% Viraemic controls

Sheep

Cattle

Highest virus load on blood (qRT-PCR): 2nd week (d5-7 to 14)

Sheep

Cattle
Efficacy criteria

Protection from challenge
  Clinical symptoms and lesions
  Viremia

Constant feature in non-protected sheep and cattle challenged with either BTV-1 or BTV-8

Long lasting

Detection: reliable, easy and objective
  Validated method

Viremia (prevention or reduction) must be the primary efficacy criteria

Potency test

Syva's approaches:

1. VN Antibodies in target specie: sheep
2. Serology in experimental animals
3. Differential protein quantification on final product
Potency test

1.- VN Antibodies in target specie: sheep

It is possible to compare batches of vaccines, however:

High individual variation in responses
large groups to calculate GMT

Difficult to correlate with vaccine efficacy
Presence of VNA = protection

Low sensitivity
Do not detect changes of ≥25% antigen content

Needed a wide safety range in specifications: Danger of rejecting protective batches

Potency test

2.- Serology in experimental animals

ELISA detection and quantification serotype-specific response in rabbit, guinea pig and mouse.

Correlation response- antigenic content

Higher sensitivity: able to differentiate lower antigen content variations

Due to: technique and reagents
possible use of larger number of animals

Potency expressed in relation to a standard vaccine (successful in immunogenicity trial)

Difficulty: development of serotype differential assay
Potency test

3.- Differential protein quantification on final product

- EIA able to detect **virus protein serotype-specific**
- Quantification expressed in relation to an efficacious vaccine

**Advantage:** accurate comparison among vaccine batches

**Difficulties in development:**
- Finding specificity
- Set up
- Validation

Safety studies

**Specific safety studies carried out:**

<table>
<thead>
<tr>
<th>BTV-4</th>
<th>Laboratory studies:</th>
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<tbody>
<tr>
<td></td>
<td>Sheep of minimum age</td>
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<tr>
<td></td>
<td>Pregnant sheep</td>
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<table>
<thead>
<tr>
<th>BTV 1+8</th>
<th>Laboratory studies:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sheep of minimum age</td>
</tr>
<tr>
<td></td>
<td>Calves or minimum age</td>
</tr>
<tr>
<td></td>
<td>Pregnant sheep</td>
</tr>
<tr>
<td></td>
<td>Lactating sheep</td>
</tr>
</tbody>
</table>

**Field studies:**
- Pregnant cows
- Lactating cows

**Adverse reactions detected**
- Slight hyperthermia lasting about 24-48 hours
- Local reaction on 100% of lambs. Swelling of the injection site, with slight to moderate oedema that evolves to a nodule. Lesion could persist up to 7 weeks, the size of the nodule decreasing during this period.

**No effect on lactation or pregnancy**
¡Muchas Gracias!