SEROLOGICAL RESULTS FOLLOWING THE VACCINATION OF PRIMIPAROUS GILTS AGAINST ATROPHIC RHINITIS

Stefano Gozio¹, Fausto Cominotti², Paolo Pozzi²,
¹Intervet International, Boxmeer, The Netherlands; ²Intervet Italia, Peschiera Borромео (MI)

Key words
Swine, atrophic rhinitis, vaccination, serology, dermonecrotic toxin

SUMMARY
Ninety breeding gilts were divided into five groups: four equally-sized groups being vaccinated, each with a different commercial vaccine against Atrophic Rhinitis, and a smaller unvaccinated control group. Blood samples from all the animals were analysed for antibodies against toxins of *P. multocida* and *B. bronchiseptica*. The results demonstrated statistically significant differences between the groups.

INTRODUCTION
Atrophic Rhinitis (AR) is an infectious and contagious disease of pigs, the target organs being the turbinates and nasal septum of growing piglets. In Italy it is presumed to be endemic on 25-30% of farms (1); also, a sub-clinical form of the disease has been reported at slaughter (2); and positive results appear in routine laboratory reports (3), most likely reflecting diagnostic confirmations of clinical cases from the field. The causative agents of AR are the dermonecrotic toxin (DNT) produced by toxogenic strains of *Pasteurella multocida* (PmT+) and by the toxin of toxogenic *Bordetella bronchiseptica* (BbT+) (4), while various environmental and managemental factors also have a significant effect (4). The toxins released by BbT+ and PmT+ are the direct cause of the tissue damage, once they have diffused into the osseous tissue of the nasal turbinates and septum. In essence, BbT+ provokes hypoplasia with fibroblastic proliferation (reduction and substitution of osseous tissue with fibrous tissue), rarely osteolysis.

By contrast, DNT causes osteoclastic damage with subsequent substitution by fibrous tissue. It induces atrophy of the mucosa, osteolysis, proliferation of mesenchymal cells with substitution of osseous trabeculae, osteoblasts and osteoclasts (4).

BbT+ and DNT can induce AR independently, manifested in two distinct forms depending on the pathogen involved:
Non-progressive or regressive AR (NPAR), with reversible lesions, by BbT+ on its own.
Progressive AR (PAR) with irreversible lesions when only DNT is involved.

The sensitivity of the target organs to the two pathogens is also different: the sensitivity of the turbinates to BbT+ reduces from the third week of age onwards and infection is unusual in piglets older than 4-6 weeks of age. This is true of both the field situation and under experimental conditions in SPF animals (4). Following a pure NPAR infection, a partial regeneration of nasal turbinates is observed, sometimes almost complete. On the other hand, the sensitivity of the turbinates and septum to DNT extends from birth to 12-16 weeks of age, the lesions being irreversible.

As to the epidemiology of AR, the principal reservoirs of infection are the upper respiratory tract and the tonsils. The disease mainly spreads by horizontal transmission immediately after birth, from sows, an estimated 10-15% of which are chronic carriers (pharyngo-tonsillar) (4),
whose progeny is exposed to infection in the first week of life; in gilts the incidence is higher. Age confers no greater resistance to BbT+ nor, in particular, to DNT, passed, vertically, to the progeny in colostrum. Because of the prolonged sensitivity of piglets to DNT, the horizontal transmission of the disease between piglets must not be disregarded.

The impact of AR and, because of its irreversible nature, from PAR, is mainly derived from two factors:
- greater predisposition to deeper-seated respiratory disease, caused by the reduced effectiveness of the damaged or absent turbinates;
- reduced growth rate, by up to 22 % in the more severe cases (6). There is a direct correlation between anatomical damage, growth retardation and the quantity of DNT released by PmT+ (5).

**Graph 1: correlation between atrophy of nasal turbinates and growth (6, modified)**

One widely employed strategy for the control and/or prevention of PAR is vaccination. This depends substantially on the following pivotal points:
- infection only of dams with PmT+ does not induce antibodies against DNT which can be transmitted to their offspring via colostrum (4, 7);
- early infection of the piglet is reason for vaccinating sows and gilts, with regular pre-partum boosters, to ensure the passive transmission of protective vaccinal PAR antibodies via colostrum;
- there is a direct correlation between DNT-neutralizing antibody titres in young piglets and the integrity of their turbinates (4, 7). Protective titres are attainable by the specific vaccination of the dams with transmission via colostrum of specific antibodies directed against the DNT.

With respect to BbT+, high antibody titres reduce the prevalence and severity of nasal infection, and control the effects of *non-progressive* AR. They do not, however, control the *progressive* form of AR (4).

The objective of the present study was to explore the capacity to induce specific DNT-neutralizing antibodies of some AR vaccines marketed in Italy, while also measuring the induced antibody titres against BbT+. 
MATERIALS AND METHODS

On a closed breeding farm of 2,000 sows situated in the Po Valley in northern Italy, free of clinical signs attributable to AR, 90 prepuberal gilts were selected prior to insemination, and assigned to 5 groups: four groups of 20 gilts, each group to be vaccinated with a different commercially available vaccine, and one group of 10 gilts to act as unvaccinated controls.

Table 1 shows the composition of the vaccines and the vaccination schedules used (a primary 2-dose course and a pre-partum booster) according to the manufacturers’ instructions (10).

Table 1: vaccine composition and vaccination schemes

<table>
<thead>
<tr>
<th>Group</th>
<th>Stated composition of vaccine utilized</th>
<th>Primary course</th>
<th>Pre-partum booster</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Toxoid Pm D; Toxoid Bb</td>
<td>1+1 (3-week interval)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>B</td>
<td>Pm A; PM D; Toxoid Pm; Bb</td>
<td>1+1 (3-week interval)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>C</td>
<td>PmT+; Toxoid Pm T+; BbT+</td>
<td>1+1 (6-week interval)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>R</td>
<td>Toxoid Pm T+; BbT+</td>
<td>1+1 (6-week interval)</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>Not vaccinated</td>
<td>--------------</td>
<td>------</td>
</tr>
</tbody>
</table>

The animals were individually identified by earmark, and all were blood sampled at the time of the first dose (T0), 2 weeks after the second dose (T1) and 24 hours after farrowing (T2).

The T1 samples were collected on different dates, depending on the vaccination schedule of the product used. The timing of T2 was preferred to a pre-farrowing sample to avoid undue or excess stress on gilts in late gestation. It also allowed all the gilts’ titres to be measured at the same point in physiological terms.

The pigs were secured by a nose-twitch, and jugular blood samples obtained using vacutainers (without anti-coagulant), and a fresh needle for each pig. Serum was separated, frozen, and sent to the laboratory, where the samples were subjected to recognised micro-agglutination tests (MAT) for BbT+, and serum neutralization tests for DNT (11). The individual tests were performed on all sera during the same session, in order to eliminate the effect of variations in laboratory conditions and technique.

For both tests, the results are expressed as log₂ of the reciprocal of the greatest (positive) dilution. (The dilutions varied between 1:2 and 1:4096.) The following parameters were evaluated:
- the percentage of animals sero-converting following vaccination;
- the seroconversion index (SI);
- the specific antibody titres: SN-DNT and MAT-BbT+ induced by vaccination.

The results of the specific antibody titrations were statistically analyzed for MAT-BbT+ by the Dunnet test, and for SN-DNT by the Kruskal-Wallis & Bonferroni tests.

RESULTS AND DISCUSSION

The SN-DNT and MAT-BbT+ antibody titres were measured for all five groups at T0, T1 and T2. One sample was discarded because it yielded a value more than 2 standard deviations below the average for its group, probably due to laboratory error, and was considered to be a ‘false negative’. 

Seroconversion
Seroconversion was considered to have occurred when the SN-DNT and MAT-BbT+ titres of the T2 sample were at least double those of the T0 sample. The seroconversion results are illustrated in Graph 2.

Vaccine R induced the highest percentage seroconversion to both DNT (100%) and BbT+ (100%). In the control group, some seroconversion was demonstrated against BbT+ and, as illustrated later, there was a slight increase in the titre of MAT-BbT+ antibodies. It is assumed that some BbT+ infection must have been present. In the authors’ judgment, assuming all groups were subjected to the infection equally, the overall results, particularly for BbT+, remain valid.

Seroconversion index (SI)
Animal identification enabled antibody development to be studied in individuals as well. The SI is the average variation in titre between T2 and T0, within each group (SI >0 indicates a rise in titre). The SI value is calculated as follows (11): SI = (Titre at T2) - (Titre at T0)

The Standard Deviation (SD) of the SI was also evaluated for each group, breed on the SI of the individual animals. The results are presented in Table 2.

With respect to the DNT antigen, the R vaccine had the highest SI, demonstrating an increase by 8.2. The C vaccine showed a modest SI to DNT viz. 0.2, while for A, B and the Control the SI = 0.

For the BbT+ antigen, all vaccinated groups had a positive SI. The R vaccine showed the highest index with an increase of 8.5.

Graph 2: percentage of seroconversion towards antigens DNT and BbT+

Table 2: seroconversion index (SI), according to groups and antigen

<table>
<thead>
<tr>
<th>Group</th>
<th>SI DNT</th>
<th>SD</th>
<th>SI BbT+</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td>--</td>
<td>5</td>
<td>2.18</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>--</td>
<td>5.3</td>
<td>2.38</td>
</tr>
<tr>
<td>C</td>
<td>0.2</td>
<td>0.54</td>
<td>4.3</td>
<td>1.66</td>
</tr>
<tr>
<td>R</td>
<td>8.2</td>
<td>2.28</td>
<td>8.5</td>
<td>1.68</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>--</td>
<td>1.7</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Specific SN-DNT and MAT-BbT+ antibody titres

Table 3 illustrates the values for average SN-DNT and MAT-BbT+ antibody titres per group at T0, T1 and T2, with their standard deviations.

Table 3: mean antibody titres at each sampling and for each group

<table>
<thead>
<tr>
<th>Group</th>
<th>SN-DNT</th>
<th></th>
<th></th>
<th></th>
<th>MAT-BbT+</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>SD</td>
<td>T1</td>
<td>SD</td>
<td>T2</td>
<td>SD</td>
<td>T0</td>
<td>SD</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.6</td>
<td>1.12</td>
<td>8.4</td>
<td>1.32</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0.1</td>
<td>0.20</td>
<td>3.1</td>
<td>1.40</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>-</td>
<td>0.3</td>
<td>0.70</td>
<td>0.6</td>
<td>0.90</td>
<td>1.8</td>
<td>0.80</td>
</tr>
<tr>
<td>R</td>
<td>0.5</td>
<td>1.40</td>
<td>9.2</td>
<td>2.30</td>
<td>2.0</td>
<td>0.30</td>
<td>11.1*</td>
<td>1.10</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>2.6</td>
<td>1.10</td>
<td>2.4</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* higher (p < 0.05; significant) than in groups A, B, C and control

** higher (p < 0.001; highly significant) than in groups A, B, C and control

With respect to the MAT-BbT+ antibody titres, the comparison between groups was performed using the Dunnet test. The R group shows a significantly higher MAT-BbT+ antibody titre (p < 0.05) than the other vaccinated groups and the controls.

In the case of groups A, B and R, the T-test gives p < 0.001 with respect to the control group, while the same test on group C gives p < 0.01 with respect to the controls.

In considering the position with respect to SN-DNT, the vaccinated groups were first compared two-by-two regarding the T1 and T2 samples. In these comparative tests, the R group showed very significantly higher antibody titres (Kruskal-Wallis p < 0.001) than the other vaccinated groups and the controls.

All four comparative tests were then evaluated using the Bonferroni test, and the significance level for R (with regard to the other vaccinated groups and the controls) was established at p < 0.0125.
The development of the groups’ average antibody titres for SN-DNT and MAT-BbT+ are shown graphically in graphs 3 and 4.
ACKNOWLEDGEMENTS

The effort of all staff involved in the execution of the trial is highly appreciated. It is the main reason for the successful completion of the trial.

The results fulfilled the objectives of the trial by demonstrating that:
- on the whole, all the products tested had the capacity to induce MAT-BbT+ antibodies, with significant differences between them as to the size of the titre;
- with regard to SN-DNT, the differences between the products were statistically very significant, even though all products were claimed to contain an antigenic component based on the inactivated toxin of PmT+.

With respect to progressive Atrophic Rhinitis, piglets acquire protection solely by the passive transmission of antibodies from their dams via the colostrum; in this situation the anti-PmT+ protection transmissible to the piglets may be assumed to be better, when the SN-DNT titre of the dams is higher.

Based on these premises, vaccination can be said to be a useful tool for reducing and controlling the clinical and pathological effects, and thus the economic impact, of Atrophic Rhinitis. It is also evident that the high titre of antibodies against DNT is different between the tested vaccines.

References

3. IZS, (2005), relazione tecnica
10. L’informatore Farmaceutico (2006), 16 Edizione, OEMF, Masson Edit, Milano