



Pre-clinical and clinical trials

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Mantova, Italy. November 2010

OVERVIEW:

- **Product composition: some more details**
- **Pre-clinical trials:**
 - ✓ Safety
 - ✓ Efficacy
- **Clinical trials**
 - ✓ Safety
 - ✓ Efficacy



Summary of Product Characteristics

Product name: RHINISENG®

- ⊗ Inactivated vaccine against **progressive and non-progressive** atrophic rhinitis.

Composition:

Active substances:

- ⊗ Inactivated *Bordetella bronchiseptica*
- ⊗ Recombinant Type D *P. multocida* toxin (PMTr, non-toxic derivative)

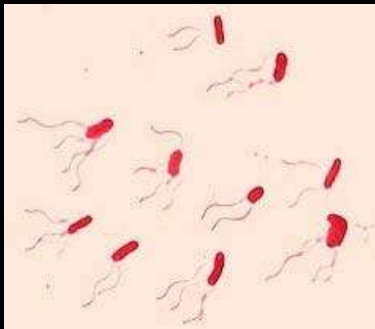
Adjuvant:

- ⊗ Aluminium Hydroxide gel
- ⊗ DEAE-dex
- ⊗ Ginseng

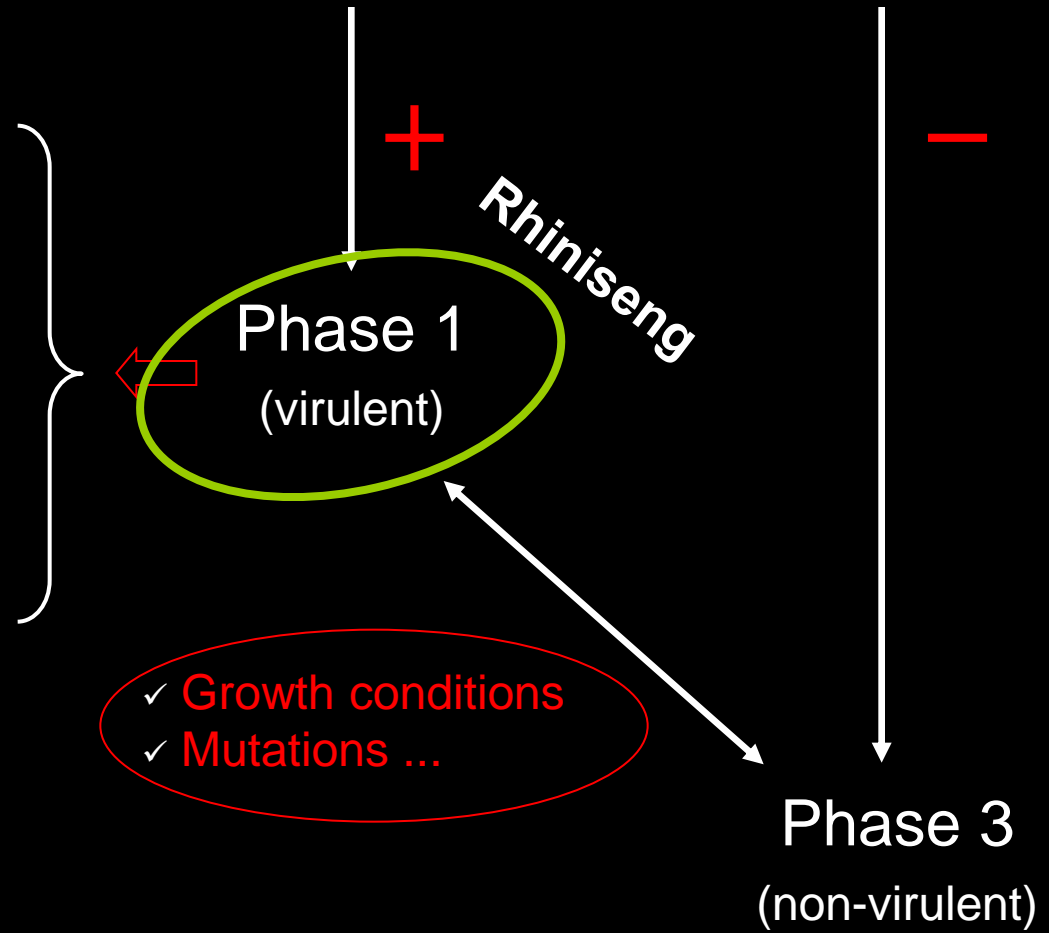
Bordetella bronchiseptica

Bvg (*Bordetella* virulence genes)

- ✓ DERMONECROTOXIN
- ✓ Fimbriae
- ✓ Adenilate ciclase
- ✓ Haemolysin
- ✓ Filamentous haemagglutinin
- ✓ Pertactin



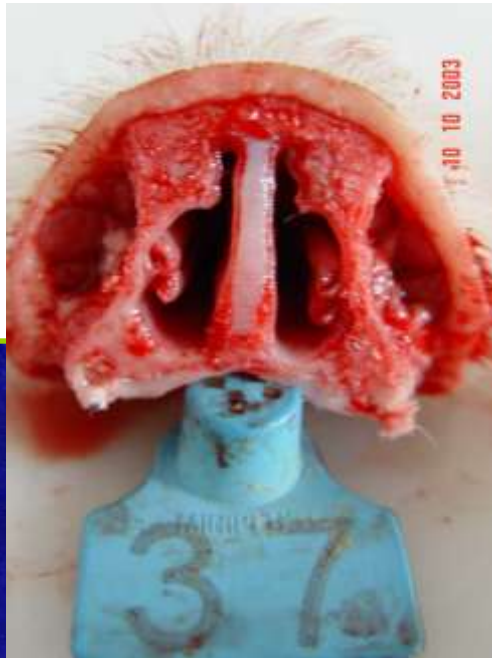
Leifson stain



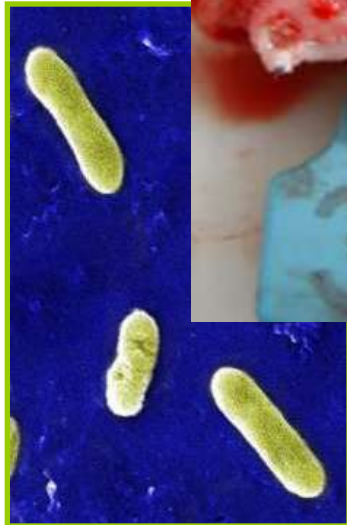
Bordetella bronchiseptica and NPAR

Challenge strain: **BP-21** = *B. bronchiseptica* 4609* **Phase I strain DNT+**

BP-21 infected



Control



BP-21 CAUSES AR BY ITSELF!

* Ackermann *et al.*, 1991; Register and Ackermann, 1997

Nasal Lesion Score (NLS)

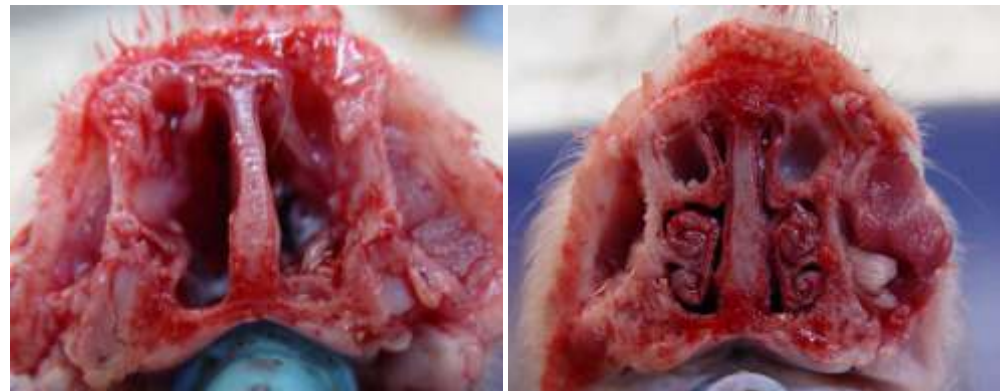
Total Nasal Lesion Score = maximum 18

Turbinate atrophy (0-4 for each turbinate) x 4 turbinates = maximum 16

- 0 No atrophy
- 1 Slight atrophy (less than half scroll is absent) *
- 2 Moderate atrophy (more than half scroll is absent) *
- 3 Severe atrophy (the turbinate bone is straightened) *
- 4 Very severe atrophy (complete or nearly complete disappearance of the turbinate) *

Septum deviation (0-2)

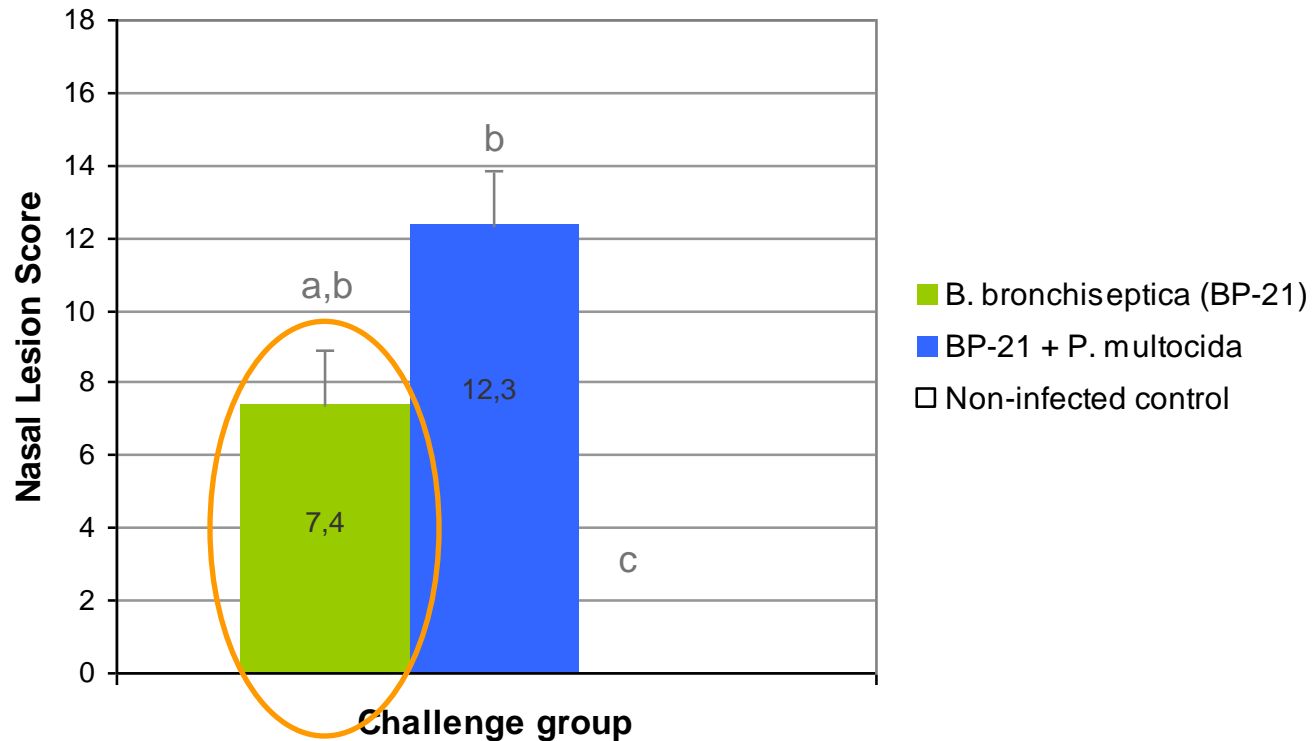
- 0 No deviation
- 1 Very slight deviation
- 2 Deviation of the septum



* Eu. Ph. AR vaccines Monograph, Magyar et al. 2002.

Bordetella bronchiseptica and NPAR

Challenge strain: **BP-21** = *B. bronchiseptica* 4609 phase I strain DNT+

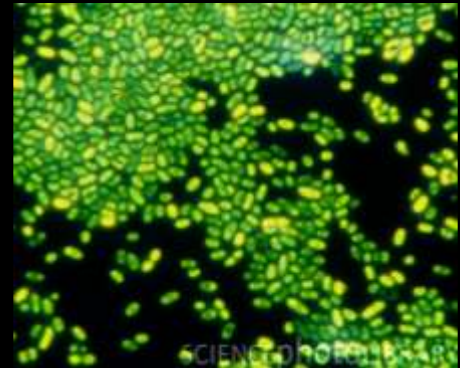


a, b, c Values with different superscripts are statistically different ($p < 0.05$; ANOVA and Mann-Whitney U test).

RHINISENG efficacy was tested against a strain causing NPAR

Pasteurella multocida

- **Virulence factors:**
 - Capsula (types A and D in pigs)
 - LPS (endotoxins)
- **Toxin (PMT)**
 - ✓ Dermonecrotic
 - ✓ Mitogenic
 - ✓ Increases osteoclastic activity
 - ✓ Decreases osteoblastic activity



PMT IS THE KEY FACTOR IN PAR PATHOGENESIS

PMT (*Pasteurella multocida* toxin)

- ToxA gene (codifying PMT) is in a prophage (*Siphoviridae*). *In vivo* the lytic cycle of the bacteriophage allows PMT release (Pullinger *et al.* 2004). Not secreted *in vitro*

- Monomeric protein
- Thermolabile exotoxin

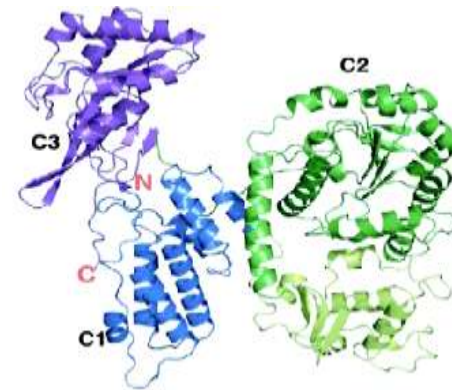


Figure from Kitadokoro *et al.* 2007

**RHINISENG CONTAINS A PMT NON-TOXIC DERIVATIVE THAT
KEEPS THE IMMUNOGENIC EPITOPES**

P. multocida and PAR

Challenge strain: Pm1990 = *P. multocida* type D NCTC 12178 **PMT+**

PAR was reproduced by experimental infection as follows:

Bb + Pm group:

B. bronchiseptica (BP-21) + Toxigenic *P. multocida* (Pm1990)

Pm group:

Toxigenic *P. multocida* (Pm1990)

A higher Pm titre was necessary to comply the Eu. Ph requirements than in group Bb + Pm

**RHINISENG EFFICACY WAS TESTED AGAINST A HIGHLY
PMT PRODUCING STRAIN CAUSING PAR**



P. multocida + *B. bronchiseptica* and PAR

The RHINISENG challenge against PAR could have been performed with a DNT⁻ strain of *B. bronchiseptica*:

Vet Microbiol. 2007 Dec 15;125(3-4):284-9. Epub 2007 Jun 6.

Expression of the dermonecrotic toxin by *Bordetella bronchiseptica* is not necessary for predisposing to infection with toxigenic *Pasteurella multocida*.

Brockmeier SL, Register KB.

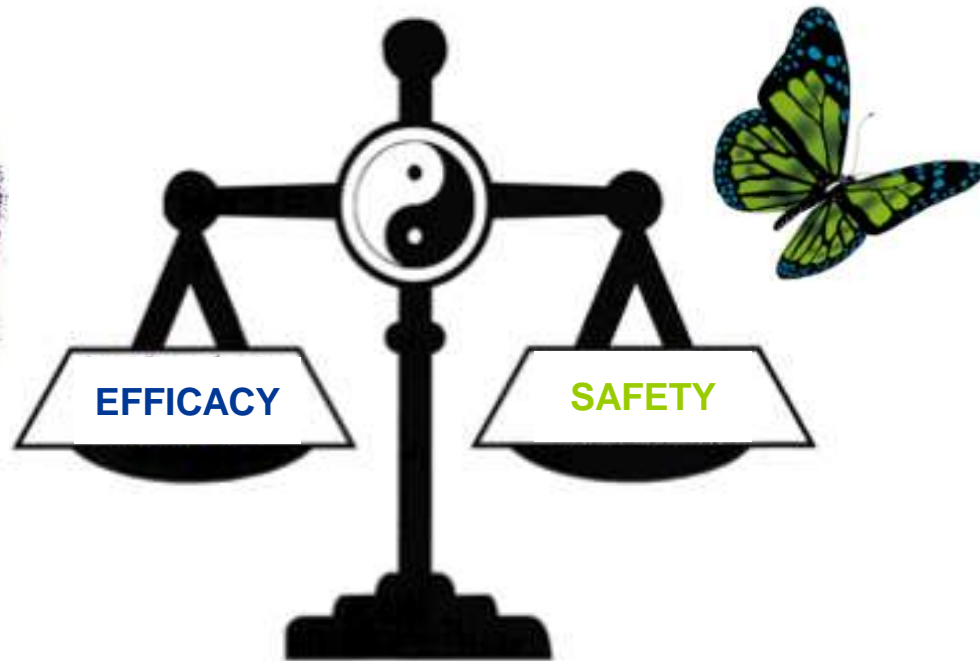
Respiratory Diseases of Livestock Research Unit, USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010, USA.
susan.brockmeier@ars.usda.gov

**RHINISENG EFFICACY WAS TESTED AGAINST A
VERY POTENT CHALLENGE**



Adjuvant selection

A challenging step in RHINISENG® development!



After testing a lot of different candidates, the best option:

Al(OH)₃ + DEAE-dex + Ginseng

Why this aqueous adjuvant combination?

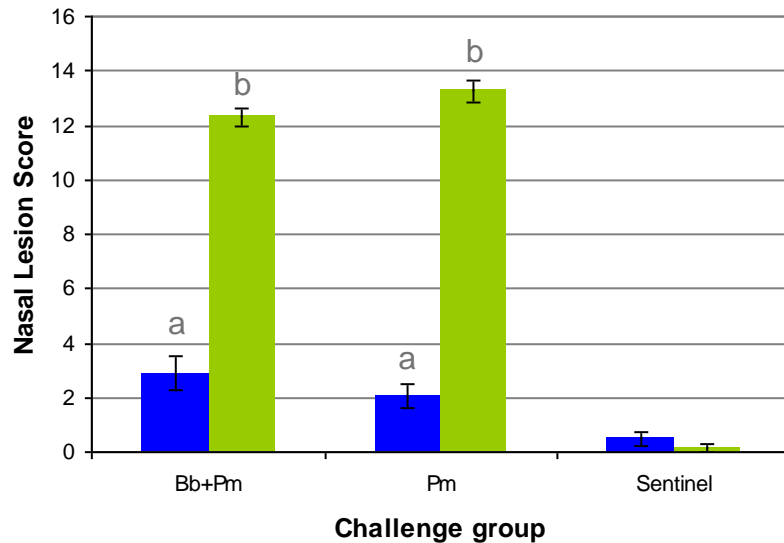
- **DEAE-dex** greatly potentiates seroconversion against PMT.
- **GINSENG** allows to reduce the concentration of DEAE-dex, thus reducing the body temperature increase caused by vaccination, while increasing the antibody levels against PMT.
- **Aluminium hydroxide** has an antigen depot effect.

PRE-CLINICAL TRIALS

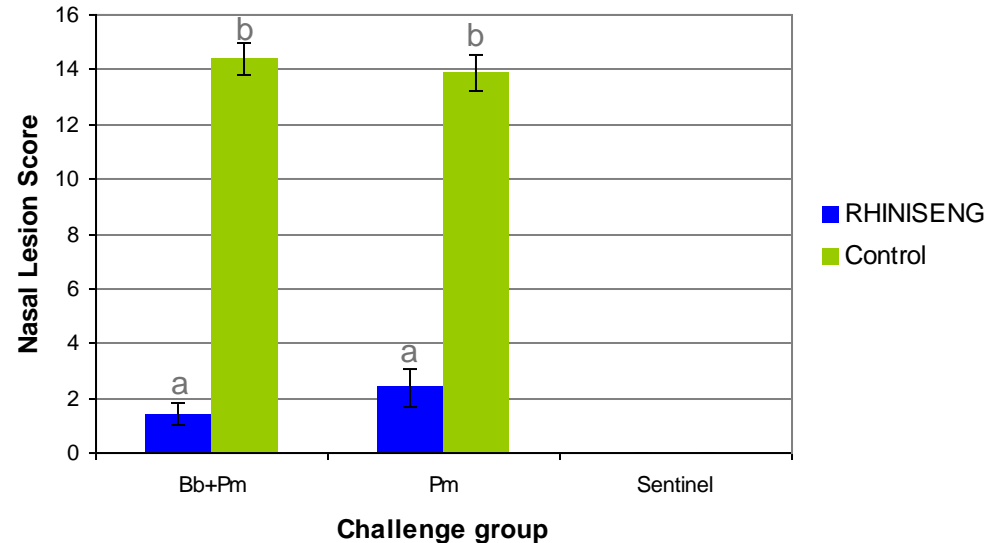
Pre-clinical trials: EFFICACY

Reduction of atrophic rhinitis lesions (NLS)

After the basic vaccination plan (vac + revac):



After boosting vaccination:

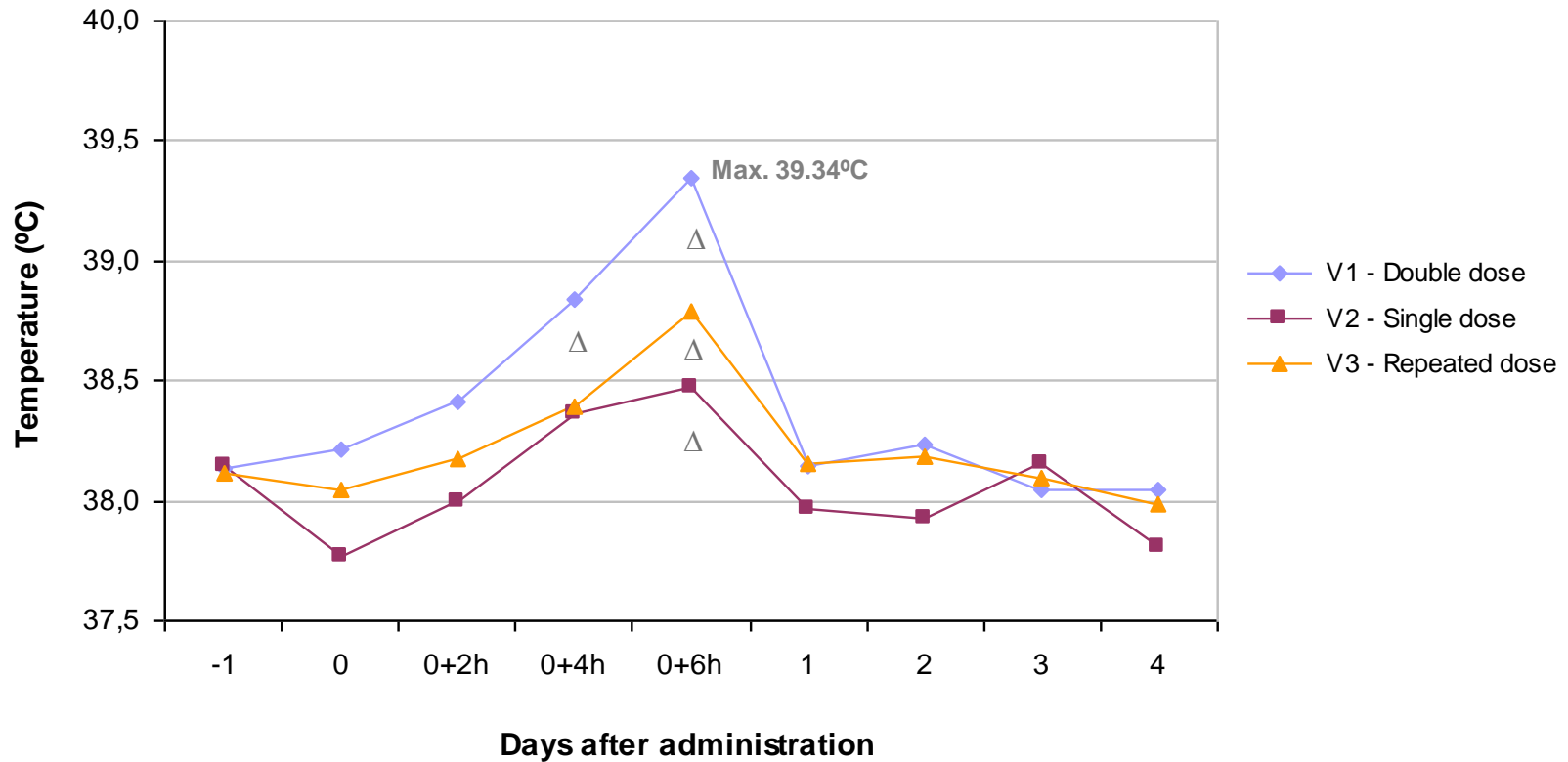
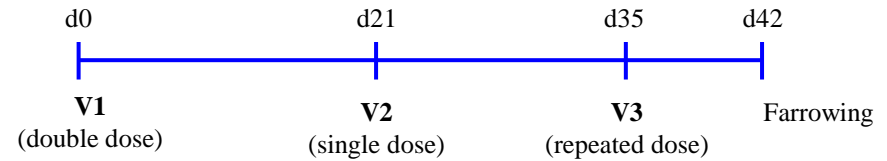


^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, t-test for independent samples).

(study complying E. Ph. Monograph guidelines)

Pre-clinical trials: SAFETY in gilts

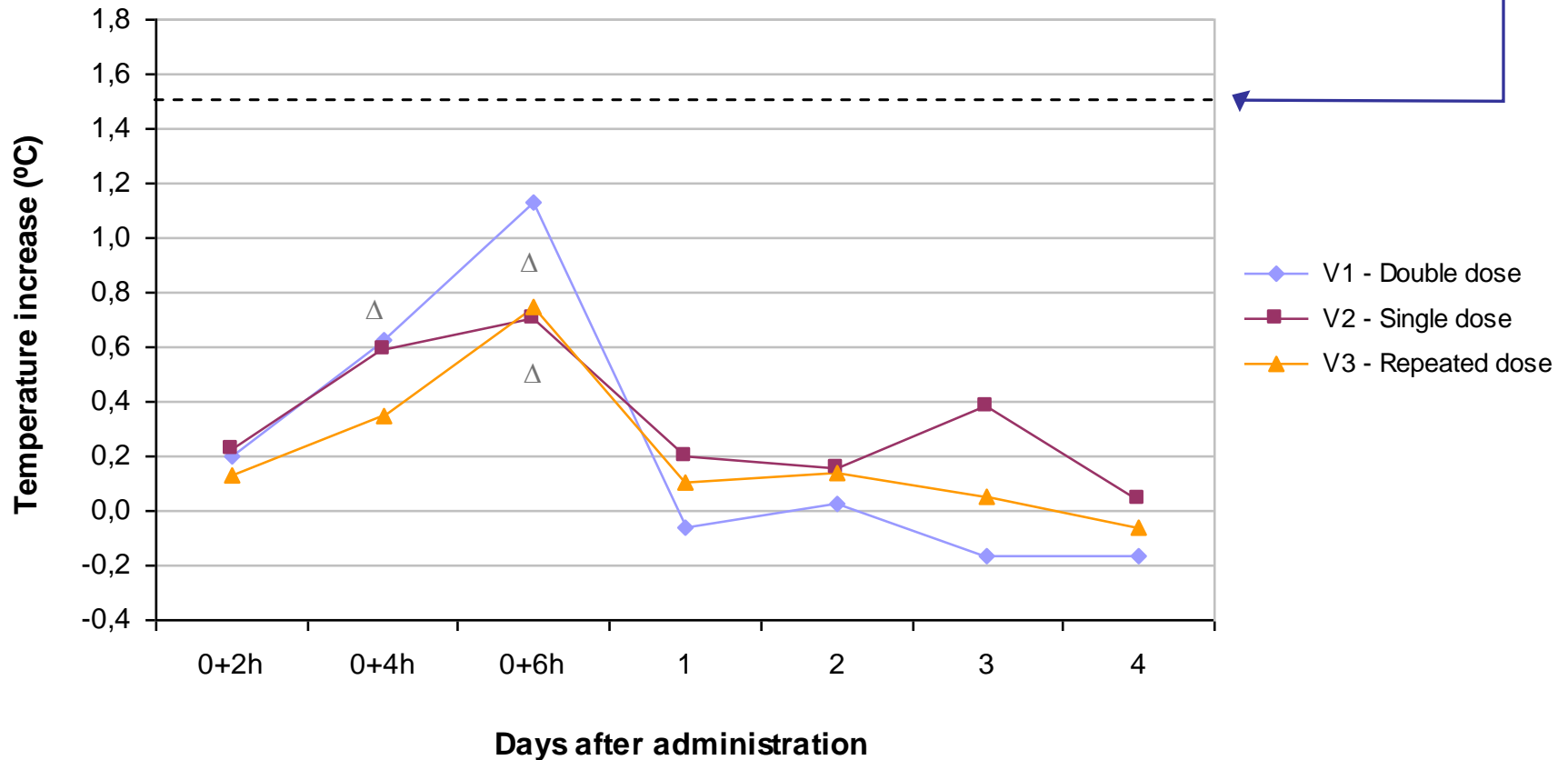
Body temperature



Δ Statistically significant increase (mixed model analysis of variance with an 'unstructured' covariance structure, $p < 0,05$)

Pre-clinical trials: SAFETY in gilts

Low temperature increases (complying with E.Ph. requirements)



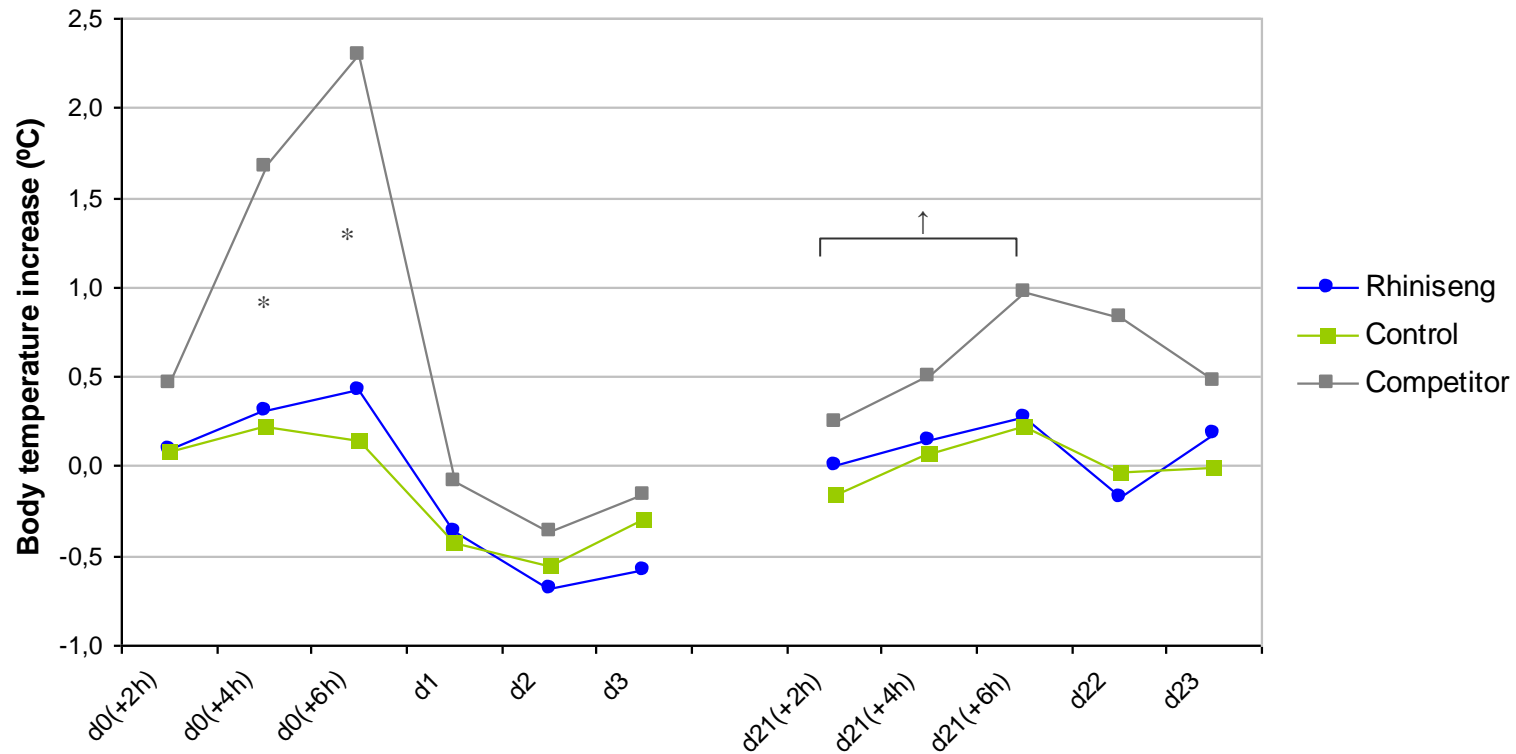
△ Statistically significant increase (mixed model analysis of variance with an 'unstructured' covariance structure, $p < 0,05$)

Pre-clinical trials: SAFETY in gilts

- No effects on **REPRODUCTIVE PERFORMANCE** were observed.
- **LOCAL REACTIONS:** a transient slight swelling of **less than 2-3 cm** detected **only by palpation**. The most common finding is **a spot of less than 1 cm** at the injection site.
- **HISTOLOGICAL REACTIONS: granulomatous inflammatory reaction** (associated mainly to $\text{Al}(\text{OH})_3$) [Valtulini et al., 2005]. WHO report no. 595 states that "development of a small granuloma is inevitable with vaccines adjuvanted with aluminium, and is to be considered necessary to the efficacy of the adjuvant".

RHINISENG vs COMPETITOR

RHINISENG induces a significant lower body temperature increase



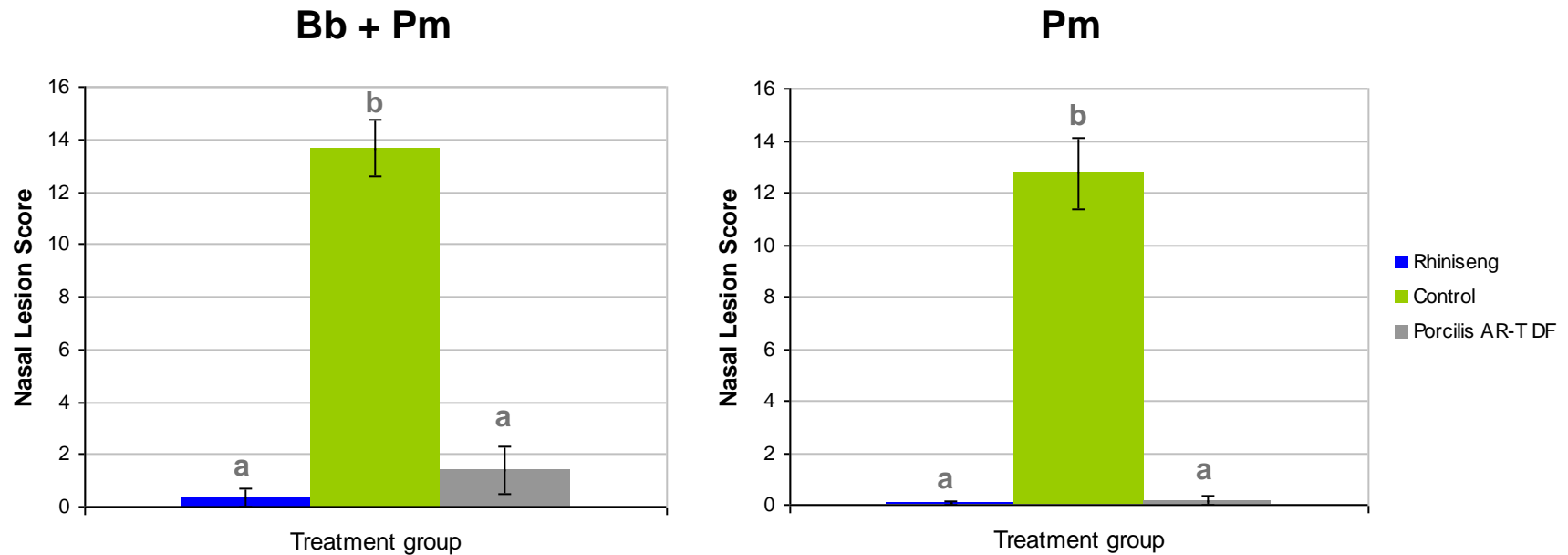
* Statistical differences between RHINISENG and Competitor vaccine ($p < 0,05$, mixed model ANOVA).

↑ Statistically significant temperature increase 6h after revaccination in Competitor vaccine ($p < 0,05$, mixed model ANOVA).

RHINISENG vs COMPETITOR

Reduction of atrophic rhinitis lesions

RHINISENG = COMPETITOR



a, b Different superscripts indicate statistical differences among treatment groups ($p < 0,05$, One-Way ANOVA and Mann-Whitney U test).

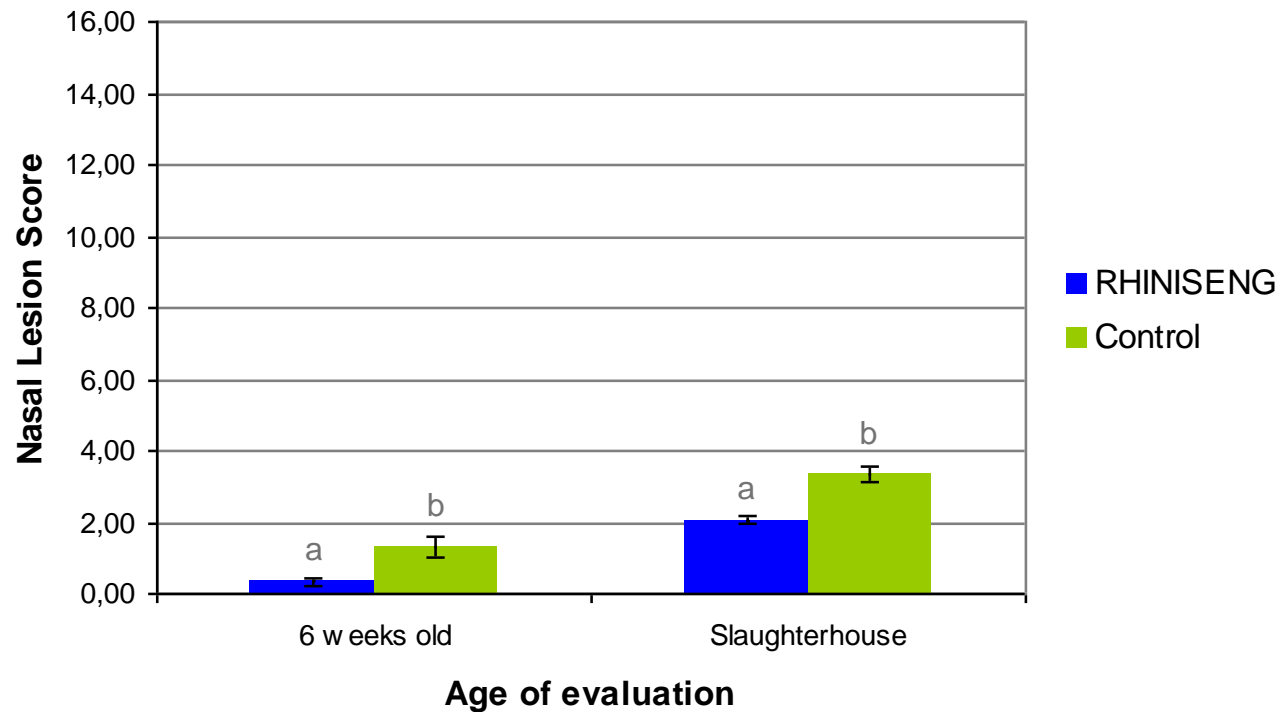
CLINICAL TRIALS

Clinical trials:

- Three different farms:
 - ✓ Presenting AR clinical signs
 - ✓ Seropositive to PMT and Bb
 - ✓ Isolation of toxigenic Pm and/or Bb
- Negative control group
- Full-blinded basis
- Vaccination + revaccination (1st)/ Boosting (2nd)
- Pigs (1st farrowing) monitored until slaughter age

Clinical trials: EFFICACY

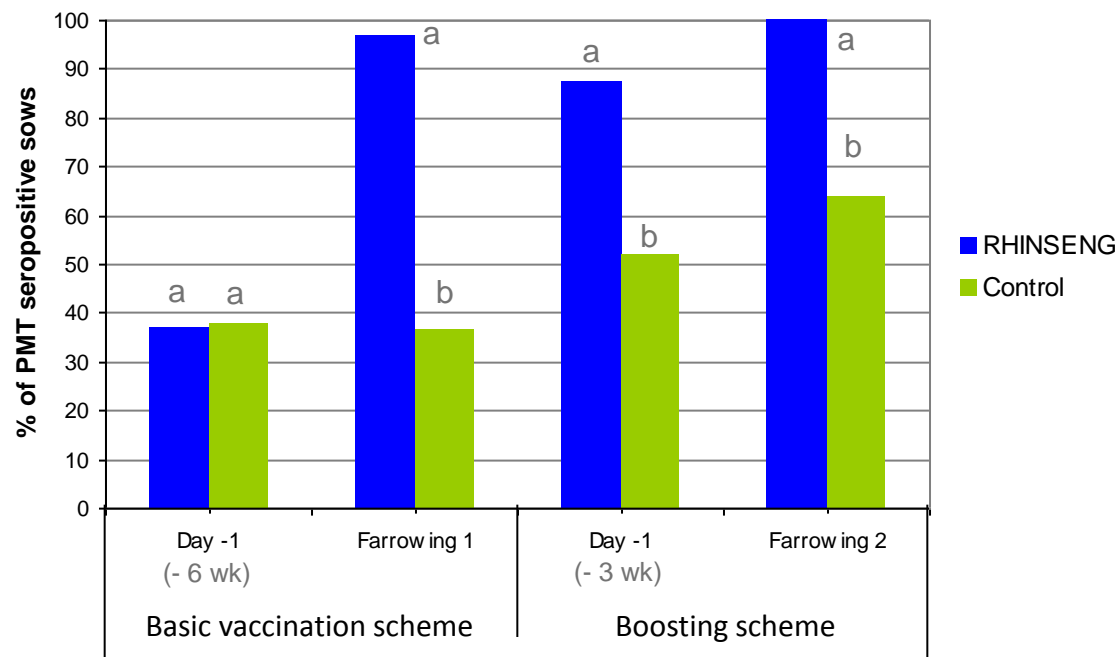
Reduction of atrophic rhinitis lesions



^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, multivariate ANOVA).

Clinical trials: EFFICACY

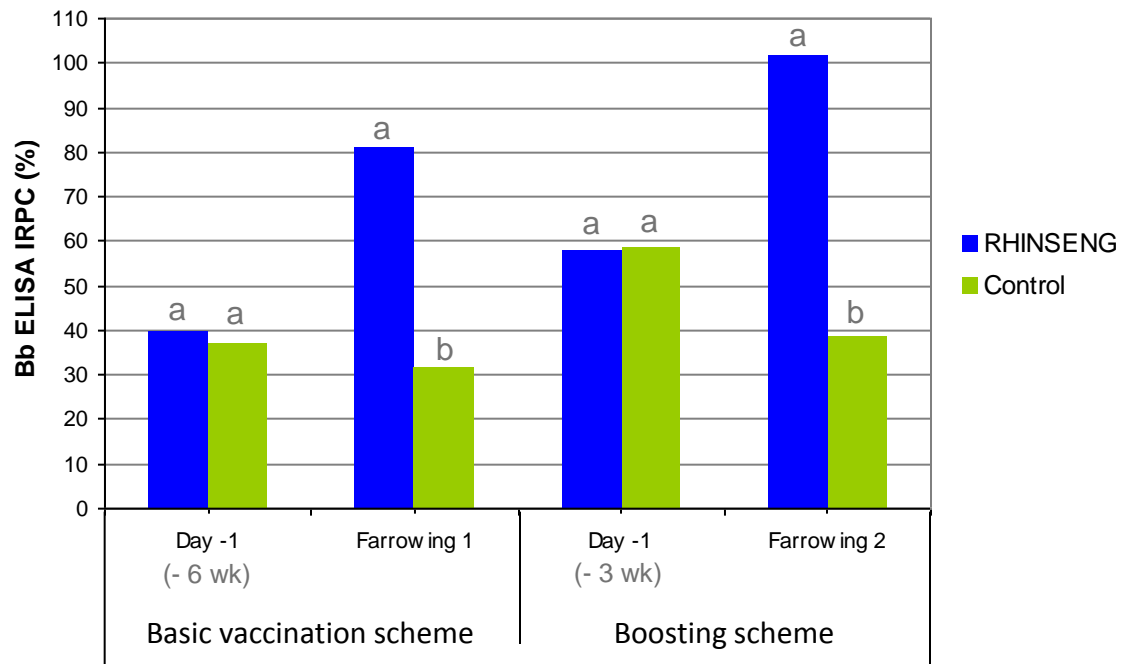
Serology against PMT (ELISA PMT Kit) in sows



^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, Fisher exact test).

Clinical trials: EFFICACY

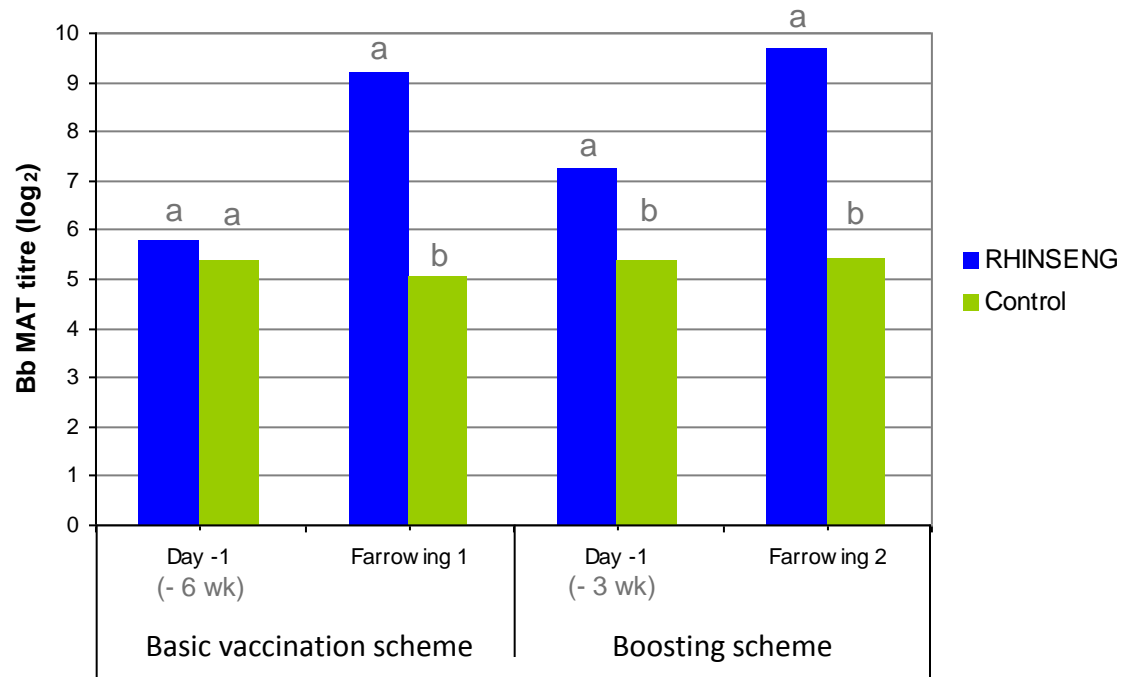
Serology against *B. bronchiseptica* (ELISA IgG1) in sows



^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, multivariate ANOVA).

Clinical trials: EFFICACY

Serology against *B. bronchiseptica* (MAT test) in sows

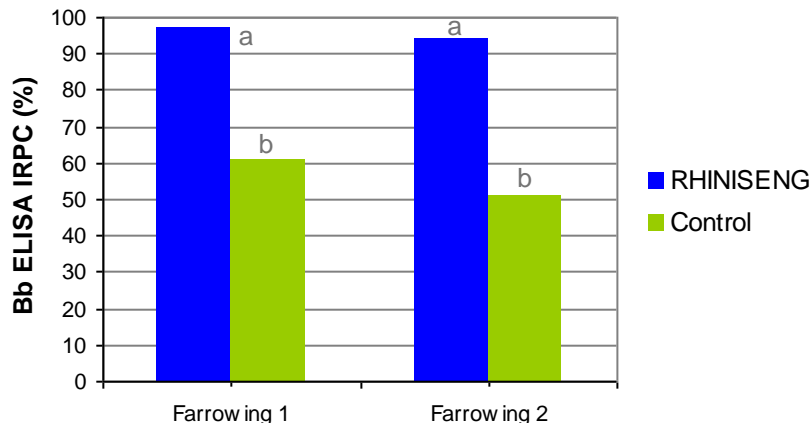


^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, Mann-Whitney U test).

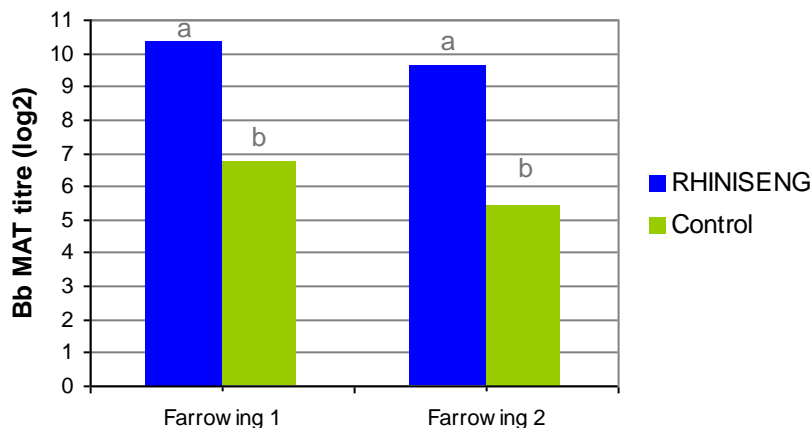
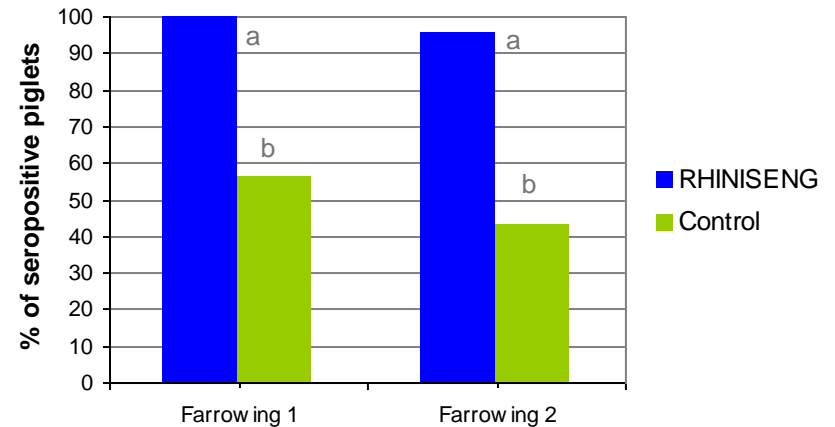
Clinical trials: EFFICACY

Serology in 5-7 day old piglets

B. bronchiseptica



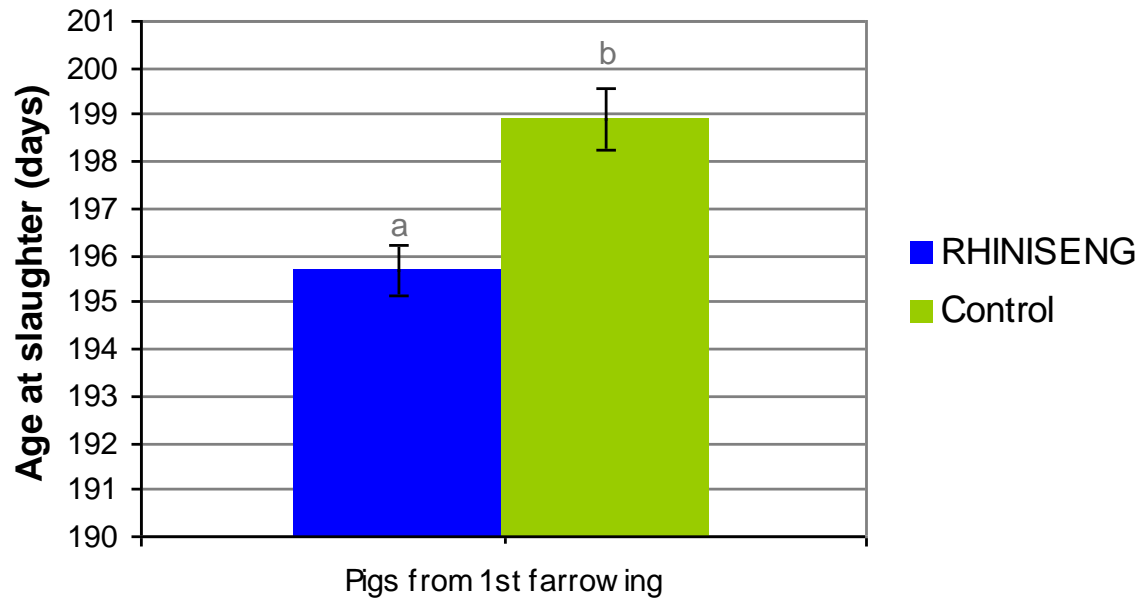
PMT



^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, multivariate ANVOVA, Mann-Whitney U test and Fisher exact test, respectively).

Clinical trials: EFFICACY

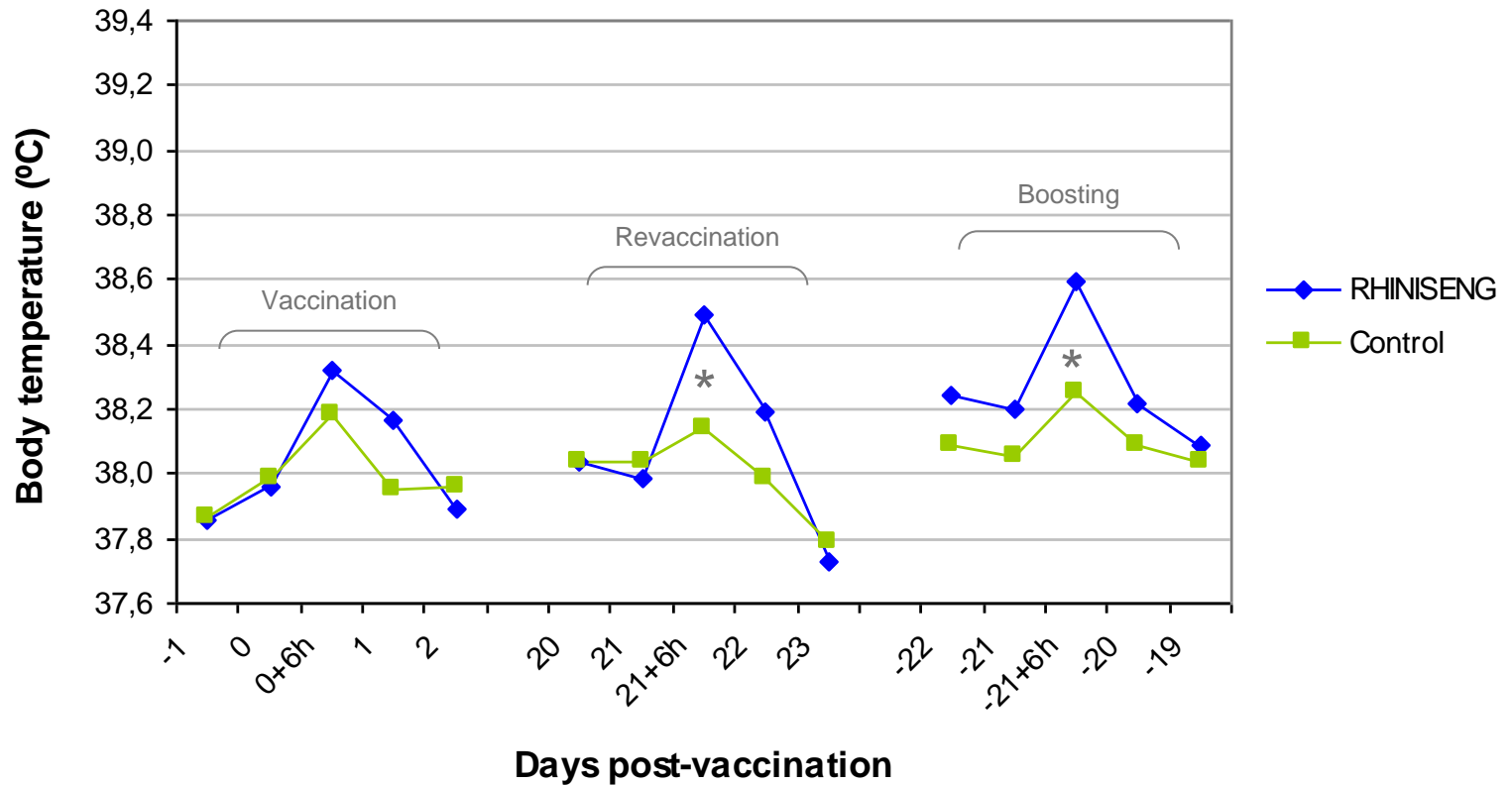
Age at slaughter (∇ 3 days): pigs from 1st farrowing



^{a,b} Different superscripts indicate statistical differences between groups ($p < 0,05$, multivariate ANOVA).

Clinical trials: SAFETY

RHINISENG® causes a very **low temperature increase** in gilts/sows under field conditions



* Statistical differences between RHINISENG and Control ($p < 0,05$, multivariate ANOVA).

Clinical trials: SAFETY

Reproductive performance 1st farrowing

Number of piglets born alive, stillborn and mummies = CONTROL

	N° piglets born alive					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	11.905	86	0.317	11.279	12.532	0.676
RHINISENG	12.078	98	0.292	11.502	12.654	

	N° stillborn piglets					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	1.248	86	0.202	0.850	1.647	0.622
RHINISENG	1.113	98	0.186	0.747	1.480	

	N° mummified piglets					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	0.148	86	0.066	0.019	0.278	0.656
RHINISENG	0.188	98	0.060	0.070	0.306	

No abortions were recorded

Clinical trials: SAFETY

Reproductive performance 2nd farrowing

Number of piglets born alive, stillborn and mummies = CONTROL

	N° piglets born alive					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	10.36	53	2.10	6	16	0.171
RHINISENG	11.25	65	2.51	3	17	

	N° stillborn piglets					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	0.57	53	1.08	0	5	0.730
RHINISENG	0.51	65	0.87	0	3	

	N° mummified piglets					
	Mean	n	Standard deviation	Minimum	Maximum	P value
Control	0.15	53	0.57	0	3	0.496
RHINISENG	0.15	65	0.57	0	3	

No abortions were recorded

Summarising...

- **RHINISENG IS EFFICACIOUS UNDER LABORATORY AND FIELD CONDITIONS WITH DEMONSTRATED BENEFICIAL EFFECTS UNTIL SLAUGHTER AGE**
- **RHINISENG IS SAFE FOR SOWS AND GILTS. RHINISENG DOES NOT ALTER THEIR REPRODUCTIVE PERFORMANCE.**

**THANK YOU VERY MUCH
FOR YOUR ATTENTION!!!**



Bibliographic references

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