Endocine™ formulated nasal influenza H1N1 vaccine induces broad specific antibody responses and confers protection in ferrets

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MAIN FINDINGS

- **Nasal administration of inactivated influenza antigens formulated with Endocine™ conferred complete protection against virus replication in the lungs after viral challenge.**
- **Nasal immunization induced high haemagglutination inhibition (HI) and virus neutralization (VN) antibody titers.**
- **Substantial HI and VN antibody titers were demonstrated after a single nasal immunization.**
- **Cross reactive HI and VN antibodies against distant viruses of swine origin were induced.**
- **Vaccines with split antigen performed best with respect to both immune responses and protection against disease.**
- **All nasal vaccines performed significantly better than the parenteral control vaccine.**

Objective

Objective to evaluate the protective effect of nasal influenza vaccines formulated with Endocine™ and to compare with a commercial injected vaccine.

- Measure viral load in lungs and turbinates after challenge with a homologous wildtype H1N1 virus.
- Assess humoral responses, HI and VN antibodies against homologous and distant H1N1 viruses.

Material and Methods

- Ferrets: influenza naïve, ~12 months old, n= 6 per group
- Nasal administration: 3x (nose drops), 3 wks apart, inactivated influenza A/H1N1/California/7/2009 split antigen at 5, 15 and 30µg HA/0.2 ml and whole inactivated virus (WIV) at 15µg HA/0.2 ml formulated with Endocine™
- Parenteral administration: 2x (s.c), 3 wks apart, Fluarix® season 2010/2011 (including A/H1N1/California/7/2009)
- Intratracheal challenge: 10^6 TCID50 A/Netherlands/602/2009 (wt-pH1N1), 4 wks post last immunization

Legend

Group		Treatment	Route	Doses
■	1	Saline	i.n.	3
■	2	TIV	s.c.	2
■	3	Split Ag 5µg + Endocine™	i.n.	3
■	4	Split Ag 15µg + Endocine™	i.n.	3
■	5	Split Ag 30µg + Endocine™	i.n.	3
■	6	Whole Vir 15µg + Endocine™	i.n.	3

RESULTS

Table 1

Efficacy of Endocine™ formulated 2009 H1N1 vaccines in ferrets demonstrated by clinical, virological and gross-pathology parameters.

		Group 1	2	3	4	5	6
Clinical score	Survival	6/6	5/6	6/6	6/6	6/6	6/6
	Fever [˚C]	1.7±0.6 (6/6)	1.1±0.4 (6/6)	1.3±0.3(6/6)	1.2±0.6(4/5*)	1.1±0.6(6/6)	1.3±0.2(6/6)
	Body weight loss [%]	18.0±4.6 (6/6)	11.5±2.1 (6/6)	-2.2±2.6 (1/6)	1.7±1.5 (4/6)	2.7±3.3 (4/6)	4.7±3.1 (6/6)
Virology	Lung virus load [log10TCID50 /g]	5.7±0.5 (6/6)	5.5±0.9 (6/6)	≤1.5 (0/6)	≤1.4 (0/6)	≤1.3 (0/6)	≤1.3 (0/6)
	Turbinates virus load [log10TCID50 /g]	7.2±2.4 (6/6)	6.9±1.5 (6/6)	≤1.9 (0/6)	≤1.7 (0/6)	≤1.7 (0/6)	4.1±2.7 (3/6)
	Virus shedding in nasal swabs [log10TCID50/g]	2.6 (5/6)	1.2 (4/6)	0.058 (1/6)	0.0 (0/6)	0.0 (0/6)	1.4 (3/6)
	Virus shedding in throat swabs [log10TCID50/g]	10 (6/6)	10 (6/6)	0.0 (1/6)	0.14 (1/6)	0.0 (1/6)	4.2 (5/6)
Gross pathology	Affected lung tissue [%]	50±25 (6/6)	37±21 (6/6)	8±4 (5/6)	7±5 (4/6)	7±5 (4/6)	8±4 (5/6)
	Relative lung weight	1.5±0.5	1.3±0.1	0.8±0.1	0.8±0.1	0.8±0.2	0.9±0.1

** body temperature of 1 animal in group 4 was not available due to malfunction of the recorder*

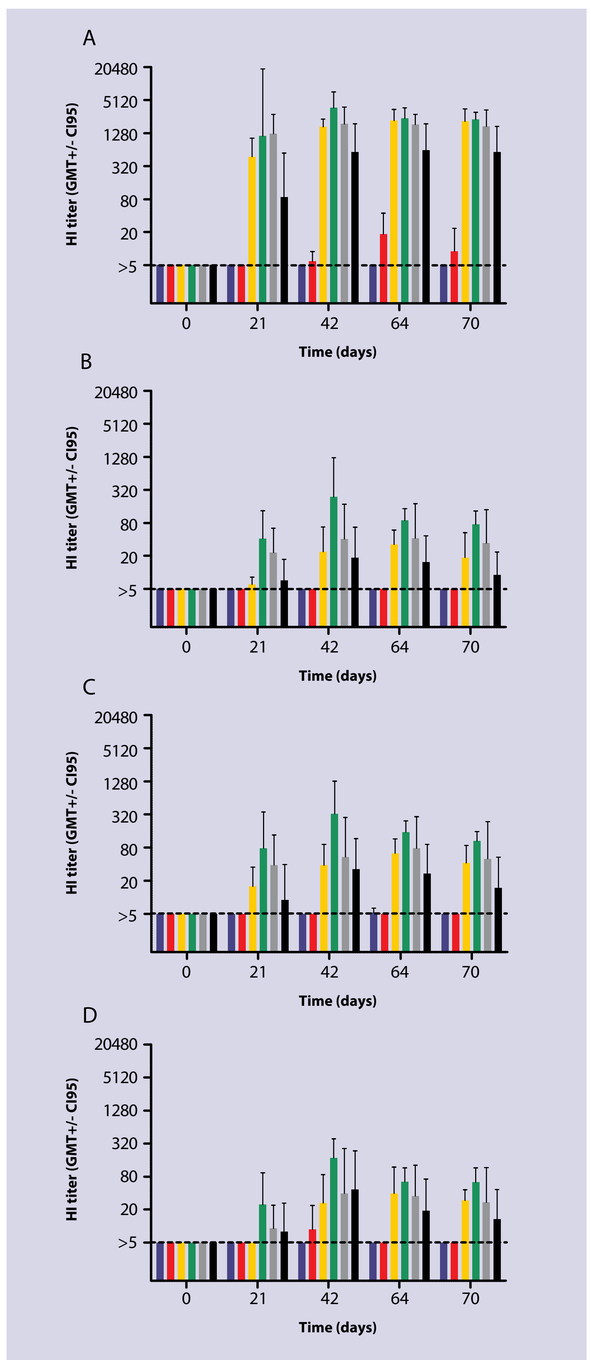

Figure 1. *Development of HI antibody titers against H1N1 A/Ned/602/09 (A), A/Swine/Ned/25/80 (B), A/Swine/Italy/14432/76 (C) and A/New Jersey/08/76 (D). Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. Blue; group 1 (control, i.n. saline), red; group 2 (s.c. TIV), yellow; group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), green; group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), grey; group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and black; group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA). Bars represent geometric mean of 6 animals per group with 95% CI (GMT +/- CI95).*

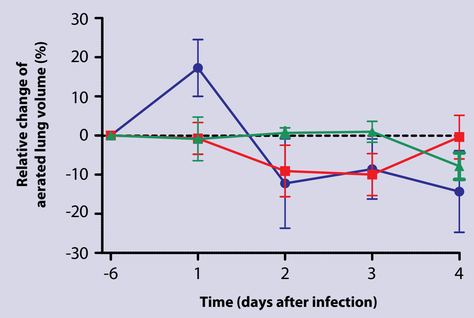

Figure 3. *Changes in aerated lung volume after infection with H1N1 A/Netherlands/602/2009. Blue; group 1 (control, i.n. saline), red; group 2 (s.c.. TIV), green; group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA). The aerated lung volume was calculated using lower and upper thresholds in substance densities of -870 to -430 Hounsfield units (HU) for the analysis of 3D-reconstructions of the lung. The percentage change of aerated lung volume was calculated using the individual base line aerated lung volumes of day -6 against the aerated lung volumes of the different days after infection. These data are expressed as mean ± SEM. Animals were intratracheally challenged with 106 TCID50 H1N1 A/The Netherlands/602/2009 on day 0.*

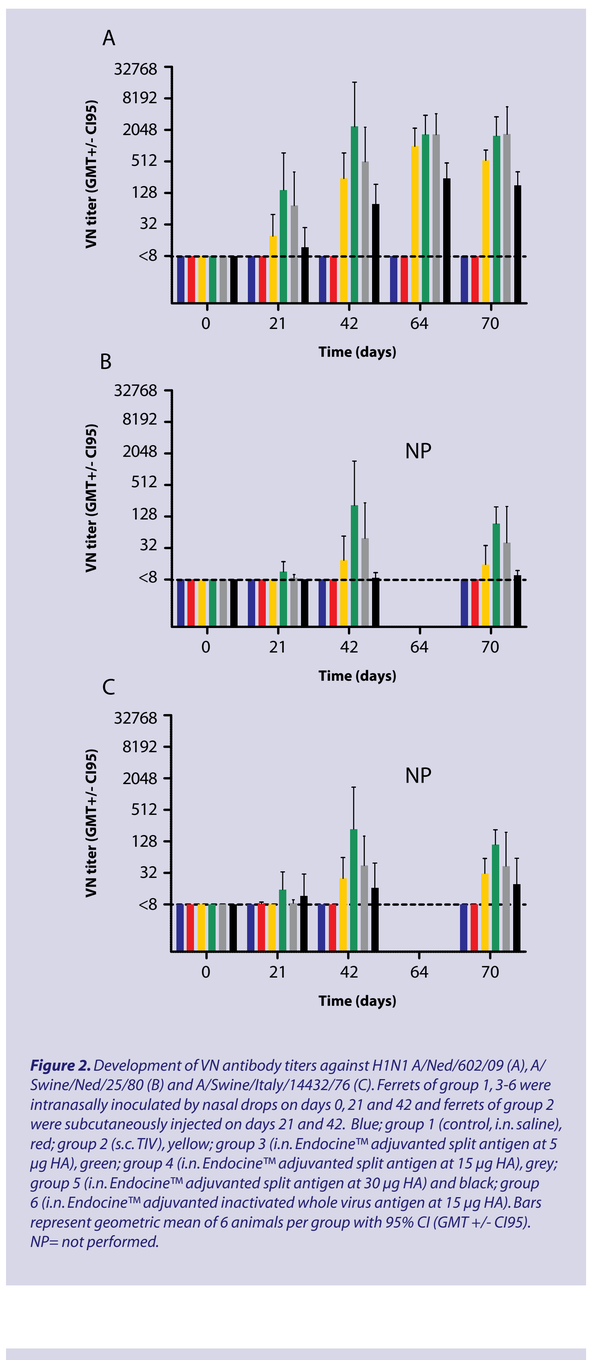

Figure 2. *Development of VN antibody titers against H1N1 A/Ned/602/09 (A), A/Swine/Ned/25/80 (B) and A/Swine/Italy/14432/76 (C). Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. Blue; group 1 (control, i.n. saline), red; group 2 (s.c. TIV), yellow; group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), green; group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), grey; group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and black; group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA). Bars represent geometric mean of 6 animals per group with 95% CI (GMT +/- CI95). NP= not performed.*

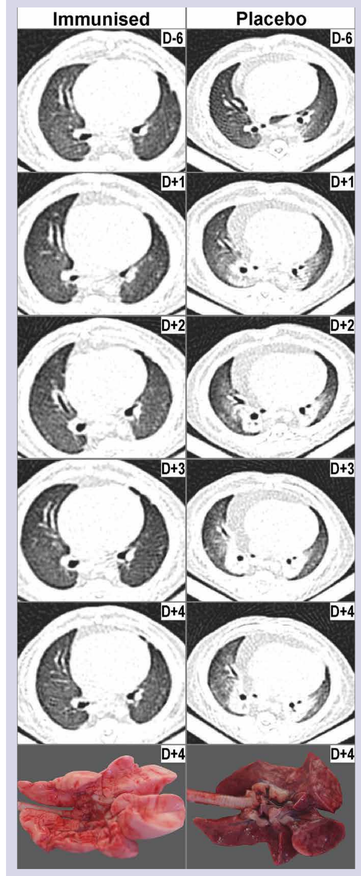

Figure 4. *Consecutive transversal lung CT images after infection with H1N1 A/Netherlands/602/2009. Two columns of consecutive (top to bottom) transversal lung CT images of one representative immunised ferret (left) and one representative placebo-treated ferret recorded in vivo compared with their gross aspect at necropsy (bottom). At day 6 before infection, the lungs showed the clear aerated baseline condition, from 1 dpi with the new pandemic H1N1 influenza virus onwards marked almost diffuse ground-glass opacities appear that show a gradual increase with a plateau on 3–4 dpi. The two photographs taken at necropsy on 4 dpi depict the ventral aspect of the lungs, with the hearts removed. The lungs of the placebotreated animal (bottom right) show diffuse reddish consolidation indicative of acute inflammation that essentially match the opacities on the CT images taken just before necropsy; non-affected aerated lung tissue from the immunised animal is light pink in colour (bottom left).*

CONCLUSION

- **Ferret data support continued development of Endocine™ nasal influenza vaccine.**
- **Split antigen and whole virus based Endocine™ nasal influenza vaccine were studied.**
- **Consecutive in vivo CT imaging allows for a day to day read out of vaccine efficacy.**
- **Endocine™ nasal influenza vaccine conferred broad and protective immune responses in ferrets**

For questions please contact:

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