

# Endocrine™ 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 Endocrine™ 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

## Objective

Objective to evaluate the protective effect of nasal influenza vaccines formulated with Endocrine™ 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 Endocrine™
- Parenteral administration: 2x (s.c), 3 wks apart, Fluorix® season 2010/2011 (including A/H1N1/California/7/2009)
- Intratracheal challenge: 10<sup>6</sup> 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 + Endocrine™   | i.n.  | 3     |
| 4     | Split Ag 15µg + Endocrine™  | i.n.  | 3     |
| 5     | Split Ag 30µg + Endocrine™  | i.n.  | 3     |
| 6     | Whole Vir 15µg + Endocrine™ | i.n.  | 3     |

## RESULTS

Table 1

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

|                        |   | Group          |                |                |               |               |               |
|------------------------|---|----------------|----------------|----------------|---------------|---------------|---------------|
|                        |   | 1              | 2              | 3              | 4             | 5             | 6             |
| <b>Clinical score</b>  | 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) |
| <b>Virology</b>        | Lung virus load [log <sub>10</sub> TCID50 /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 [log <sub>10</sub> TCID50 /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 [log <sub>10</sub> TCID50/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 [log <sub>10</sub> TCID50/g] | 10 (6/6)       | 10 (6/6)       | 0.0 (1/6)      | 0.14 (1/6)    | 0.0 (1/6)     | 4.2 (5/6)     |
| <b>Gross pathology</b> | 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

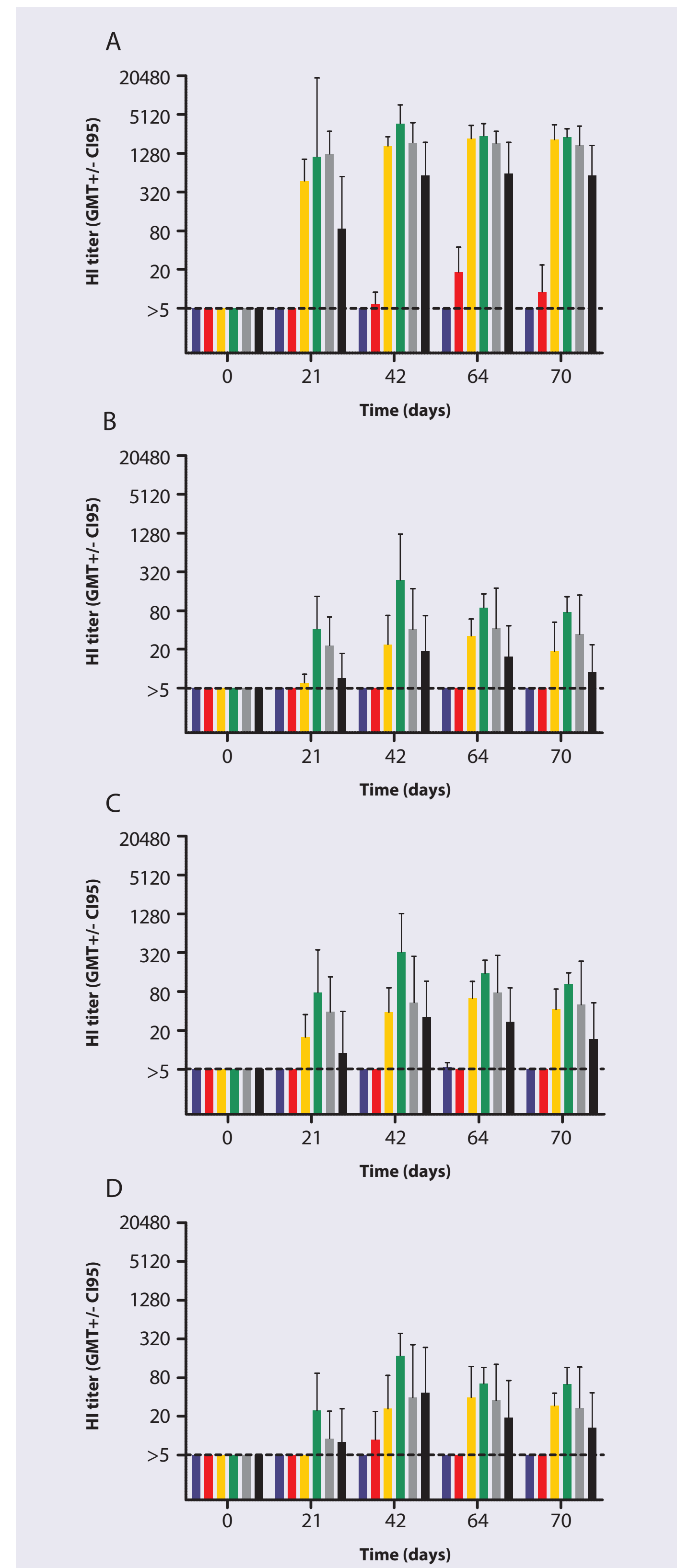


Figure 1. Development of HI antibody titers against H1N1 A/Ned/602/09 (A), A/Swine/Ned/25/80 (B), A/Swine/Italy/14432/76 (C) and A/New Jersey/08/76 (D). Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days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

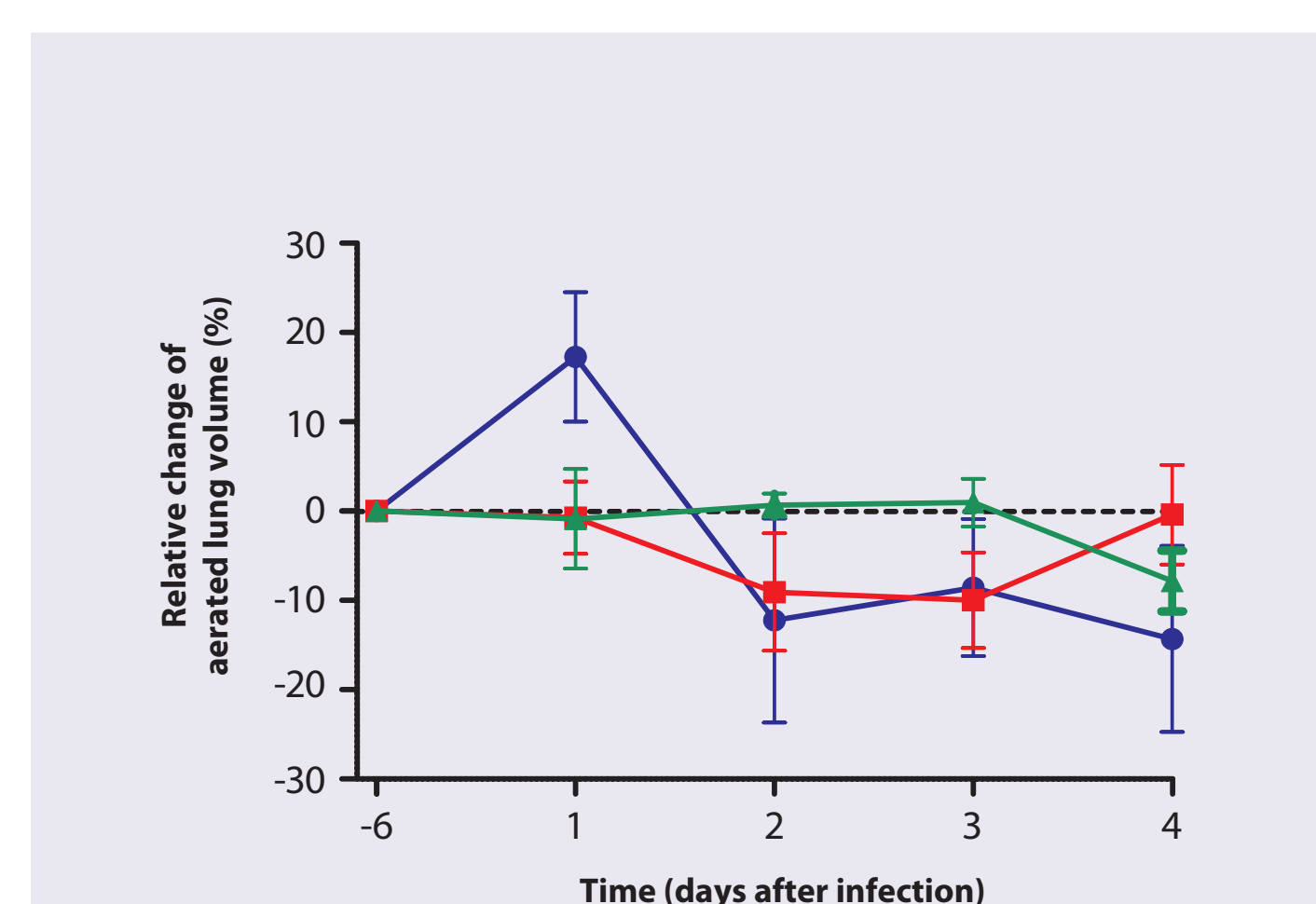


Figure 3. Changes in aerated lung volume after infection with H1N1 A/Netherlands/602/2009. Blue; group 1 (control, i.n. saline), red; group 2 (s.c. TIV), green; group 4 (i.n. Endocrine™ adjuvanted split antigen at 15 µg HA). The aerated lung volume was calculated using lower and upper thresholds in substance 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 10<sup>6</sup> TCID50 H1N1 A/The Netherlands/602/2009 on day 0.

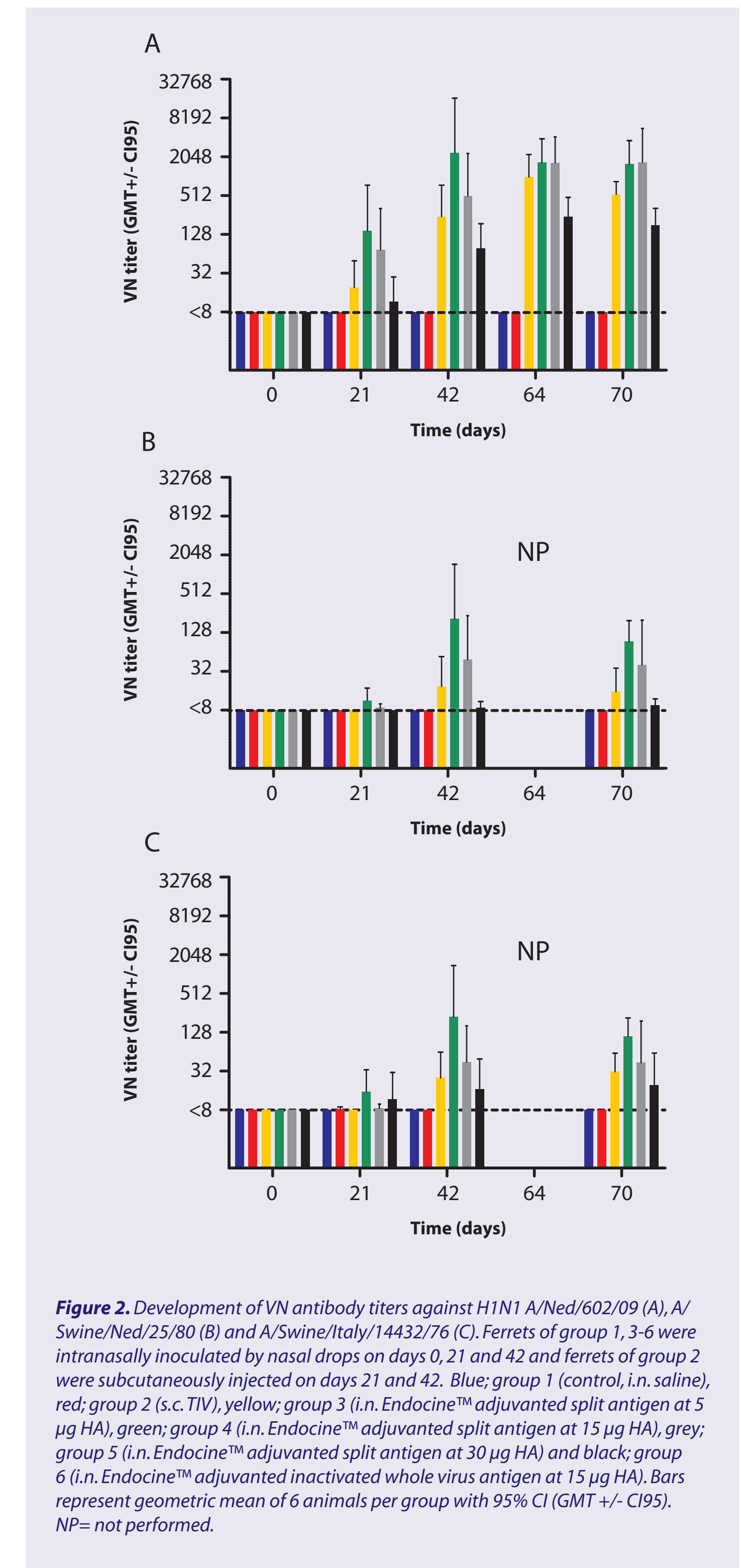


Figure 2. Development of VN antibody titers against H1N1 A/Ned/602/09 (A), A/Swine/Ned/25/80 (B) and A/Swine/Italy/14432/76 (C). Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. Blue; group 1 (control, i.n. saline), red; group 2 (s.c. TIV), yellow; group 3 (i.n. Endocrine™ adjuvanted split antigen at 5 µg HA), green; group 4 (i.n. Endocrine™ adjuvanted split antigen at 15 µg HA), grey; group 5 (i.n. Endocrine™ adjuvanted split antigen at 30 µg HA) and black; group 6 (i.n. Endocrine™ 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.

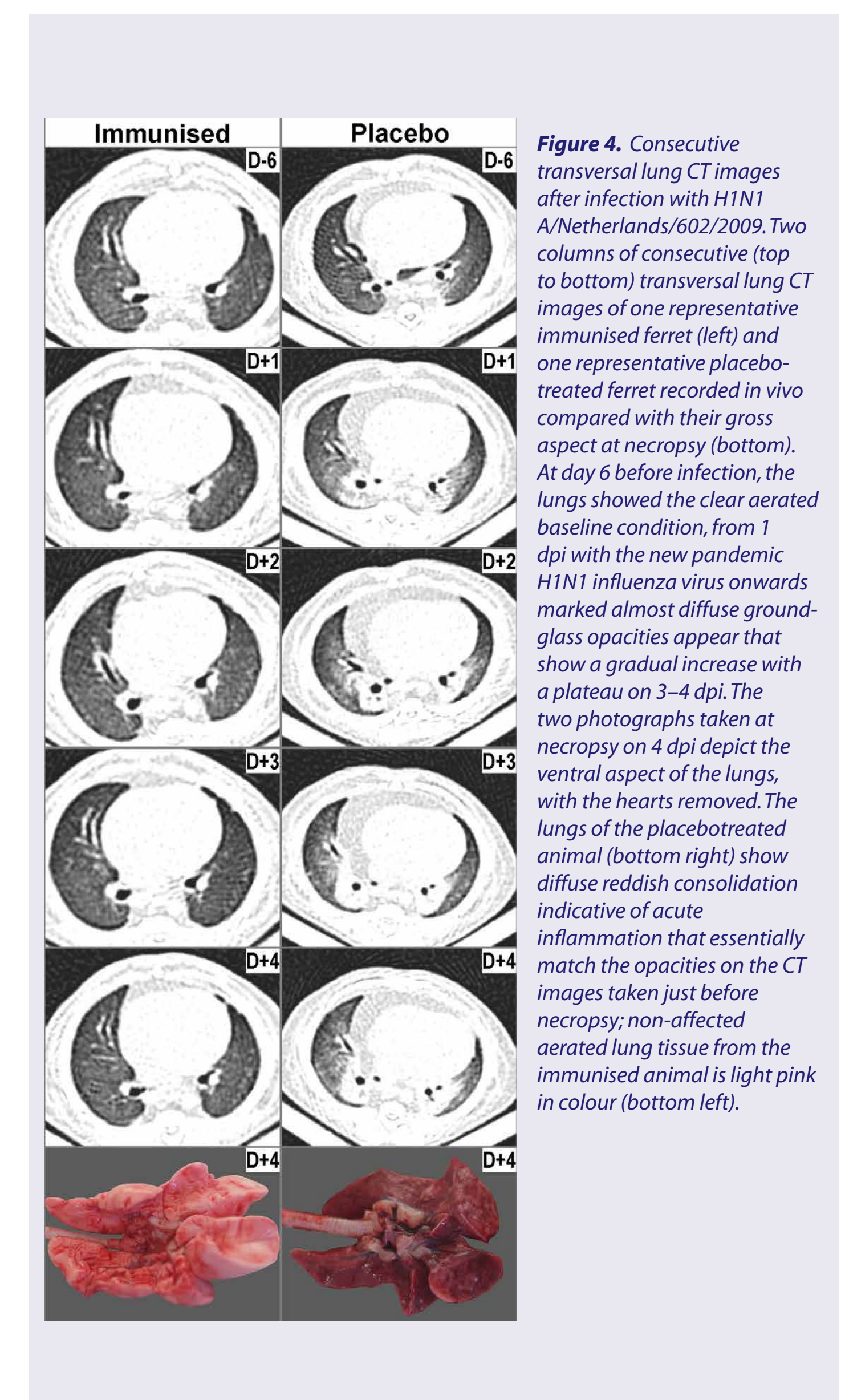


Figure 4. Consecutive transversal lung CT images after infection with H1N1 A/Netherlands/602/2009. Two columns of consecutive (top to bottom) transversal lung CT images of one representative immunised ferret (left) and one representative placebo-treated ferret recorded in vivo compared with their gross 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 placebo-treated 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 Endocrine™ nasal influenza vaccine.
- Split antigen and whole virus based Endocrine™ nasal influenza vaccine were studied.
- Consecutive in vivo CT imaging allows for a day to day read out of vaccine efficacy.
- Endocrine™ nasal influenza vaccine conferred broad and protective immune responses in ferrets

For questions please contact:



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