

Cerba Research

www.cerbaresearch.com

Email: leon.dewaal@cerbaresearch.com



- Influenza virus is member of the *Orthomyxoviridae* family
- Seasonal influenza virus causes yearly epidemics and highly pathogenic avian influenza virus (HPAI) poses a potential pandemic risk
- HPAI is present since 1997 with a global spread in multiple species (CFR of >50%)
- H5N1 A/Grey seal/Netherlands/302603/2023 was isolated from the brain of a seal²
- No licensed vaccine available for currently circulating H5N1 clade 2.3.4.4b

The diagram illustrates the experimental timeline for a SARS-CoV-2 infection study in ferrets. It features a horizontal timeline with key events marked at specific days post-infection (dpi).

- Day -14:** Subcutaneous data logger implantation.
- Day 0:** (IT) Infection with $10^{5.5}$ TCID₅₀/3.0mL. A blue virus icon is shown above this point.
- Day 0 to 3 dpi:** Recovery period. Monitoring includes survival, body weight, temperature, and respiration rate (using the implanted logger). Nose and throat swabs are collected.
- Day of death:** Gross pathology, relative lung weight, tissue samples (virology, histopathology, and immunohistochemistry) are collected.
- Day 3 dpi:** Scheduled euthanasia.

A ferret is shown on the left side of the timeline.

- Virus strains used:
 - H5N1 A/Indonesia/05/2005 (n=3, A/Indo/2005)
 - H5N1 A/Grey seal/Netherlands/302603/2023 (n=6, A/grey seal/NL/2023)

A

Survival (%)

Day post-infection

—●— A/grey seal/NL/2023
—●— A/Indo/2005

B

Body weight loss (%)

C

Body temperature ($^{\circ}\text{C}$)

Infection

04:00 -1 d.p.i. 16:00 -1 d.p.i. 04:00 0 d.p.i. 16:00 0 d.p.i. 04:00 1 d.p.i. 16:00 1 d.p.i. 04:00 2 d.p.i. 16:00 2 d.p.i. 04:00 3 d.p.i.

D

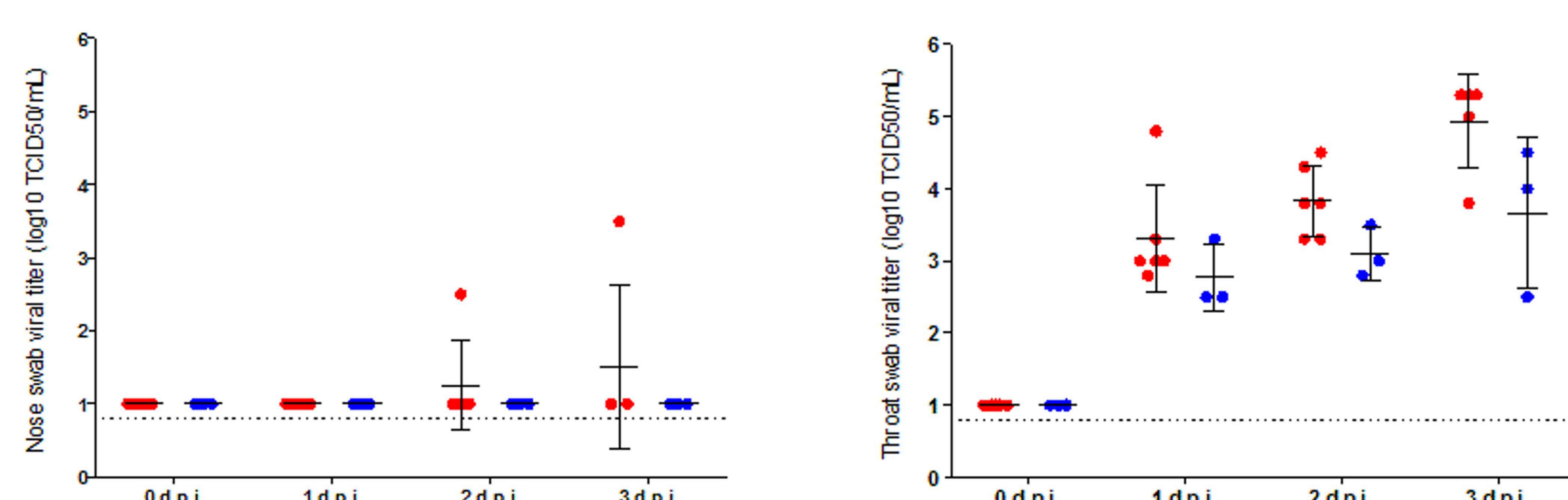
Respiration rate per minute

Infection

04:00 -1 d.p.i. 16:00 -1 d.p.i. 04:00 0 d.p.i. 16:00 0 d.p.i. 04:00 1 d.p.i. 16:00 1 d.p.i. 04:00 2 d.p.i. 16:00 2 d.p.i. 04:00 3 d.p.i.

Figure 2 is a dot plot showing viral titers (log₁₀ TCID₅₀/g) for two influenza A virus strains, A/grey seal/NL/2023 (red dots) and A/Indo/2005 (blue dots), across six tissues: Lung, Nasal turbinate, Spleen, Olfactory bulb, Cerebrum, and Cerebellum. The y-axis represents the viral titer on a log₁₀ scale from 0 to 8. Horizontal lines with error bars indicate the mean and standard deviation for each group. Asterisks (*) above the Lung and Nasal turbinate plots indicate significant differences (p < 0.05) between the two strains. Dashed horizontal lines are drawn at 1 and 2 on the y-axis.

Tissue	A/grey seal/NL/2023 (Mean ± SD)	A/Indo/2005 (Mean ± SD)
Lung	5.8 ± 0.2	5.2 ± 0.3
Nasal turbinate	3.8 ± 1.2	1.3 ± 0.1
Spleen	3.7 ± 1.8	2.0 ± 0.8
Olfactory bulb	2.2 ± 0.8	2.5 ± 0.9
Cerebrum	2.2 ± 0.8	1.2 ± 0.2
Cerebellum	1.5 ± 0.8	1.3 ± 0.1



A

Affected lung tissue (%)

● A/grey seal/NL/2023
● A/Indo/2005

Group	Individual Values (%)	Mean (%)	SD (%)
A/grey seal/NL/2023	80, 82, 85, 88, 90, 92, 95, 98, 100	~88	~10
A/Indo/2005	60, 75, 78, 80, 82, 85, 88	~75	~10

B

Relative lung weight ($\times 10^{-2}$)

Group	Individual Values ($\times 10^{-2}$)	Mean ($\times 10^{-2}$)	SD ($\times 10^{-2}$)
A/grey seal/NL/2023	1.5, 1.9, 1.9, 2.3, 2.5, 3.2	~2.2	~0.5
A/Indo/2005	1.8, 1.9, 1.9, 2.0	~1.9	~0.1

Inflammatory changes within lung, trachea and liver

(A) Lung necrosis (N) and multifocal, lymphohistiocytic, partly neutrophilic, perivascularly accentuated infiltrates (*)

(B) peribronchial cuffing in ferret (*)

(C) Mild lymphohistiocytic inflammation within the trachea

(D) No infiltrates within the trachea

(E) Oligofocal to multifocal areas of necrosis (N) in the liver

(F) Mild lymphohistiocytic pericholangitis (*) within the liver

scale bar: 100µm

Detection of AIV NP antigen in lung, trachea and liver

(A) AIV NP antigen in single bronchial epithelial cells in lung tissue (>)

(B) AIV NP antigen in single or small clusters of cells within the trachea epithelium (>)

(C) Moderate to high AIV NP antigen (>) expression in hepatocytes

(D) Focal AIV NP expression in hepatocytes.

scale bar: 100 μ m
scale bar insert: 20 μ m

- H5N1 A/grey seal/Netherlands/602203/2023 (A/GS) can infect and replicate in (lungs of) ferrets
- H5N1 A/GS is more pathogenic than historical H5N1 A/Indo
- Replication of H5N1 A/GS more obvious in extra-respiratory organs and upper respiratory tract than historical H5N1 A/Indo
- Ferrets infected with H5N1 A/GS provide a window of opportunity to investigate new intervention strategies to prevent infection and/or disease with a relevant H5 pandemic strain

¹ Guilfoyle et al. “Highly Pathogenic Avian Influenza Virus A/H5N1 subclade 2.3.4.4 b isolated from a European grey seal (*Halichoerus grypus*) is highly virulent in ferrets.” J Infect Dis 2025

² Mirolo et al. "Highly pathogenic avian influenza A virus (HPAIV) H5N1 infection in two European grey seals (*Halichoerus grypus*) with encephalitis." *Emerging Microbes & Infections* 2023

