Figure S1. Determining correlation between infectious virus titre and real time RT-PCR copy number. Correlation of infectious virus titres as measured by $TCID_{50}$ assay and copy number as measured by real time RT-PCR, detecting influenza A matrix (Flu A M), influenza B non-structural (Flu B NS), H1 HA or H3 HA genes, for the same samples. Red boxes indicate the last sampling day when the nasal wash sample tested by $TCID_{50}$ assay was still positive (above background).

Figure S2. Visual summary of virus shedding after primary infection and challenge. Virus shedding for each animal is shown, with the primary infection represented by an arrow and the challenge infection represented by grey bars. The length of the arrow indicates the duration of primary infection, the length of grey bar indicates the duration of challenge infection. No grey bar indicates the challenge infection was prevented. The dashed lines indicate the day of primary inoculation, the dotted line indicates the day of challenge and the solid lines indicate the day on which peak challenge virus shedding occurs.

Figure S3. **Growth curves of A(H1N1)pdm09→B.** Real time RT-PCR detected influenza A matrix gene for A(H1N1)pdm09 virus and influenza B NS gene for influenza B virus.

Figure S4. Growth curves of B→A(H1N1)pdm09. Real time RT-PCR detected influenza A matrix gene for A(H1N1)pdm09 virus and influenza B NS gene for influenza B virus.

Figure S5. **Growth curves of B→A(H3N2).** Real time RT-PCR detected influenza A H3 HA gene for A(H3N2) virus and influenza B NS gene for influenza B virus.

Figure S6. **Growth curves of A(H3N2)→B.** Real time RT-PCR detected influenza A H3 HA gene for A(H3N2) virus and influenza B NS gene for influenza B virus.

Figure S7. Growth curves of A(H1N1)pdm09→A(H3N2). Real time RT-PCR detected influenza A H1 HA gene for A(H1N1)pdm09 virus and influenza A H3 HA gene for A(H3N2) virus.

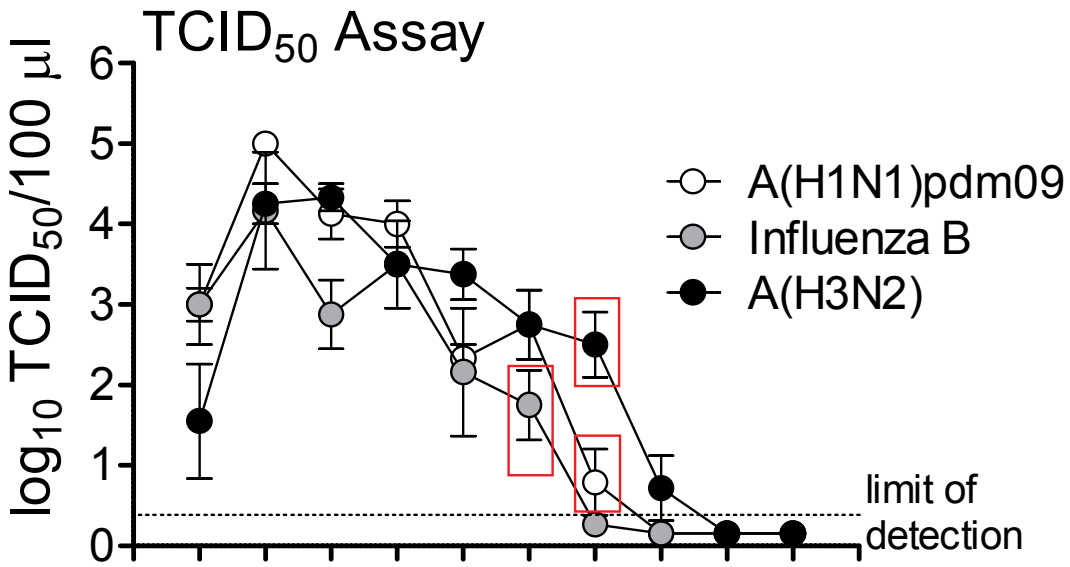
Figure S8. Growth curves of A(H3N2)→A(H1N1)pdm09. Real time RT-PCR detected influenza A H3 HA gene for A(H3N2) virus and influenza A H1 HA gene for A(H1N1)pdm09 virus.

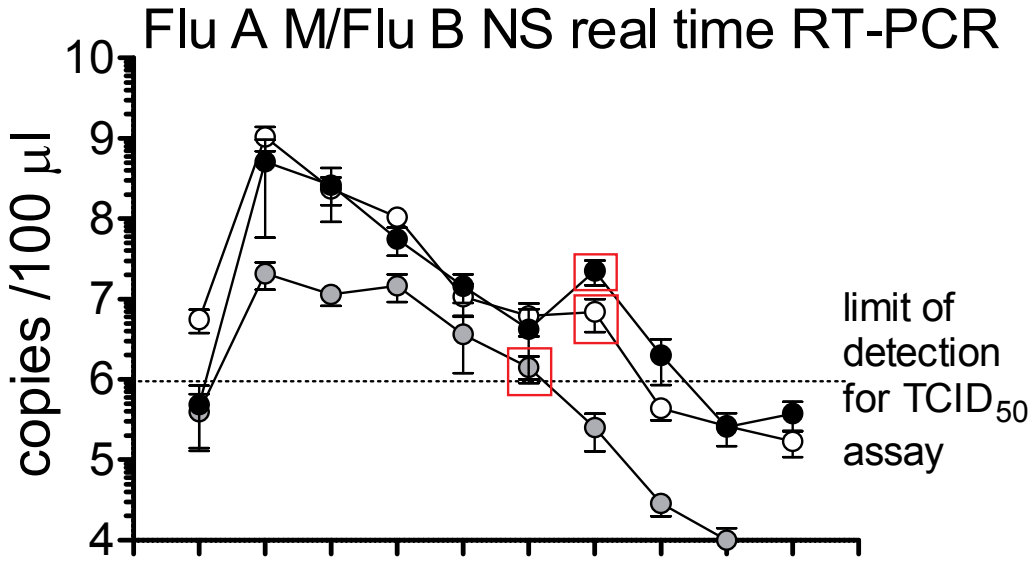
Figure S9. Shedding of the challenge virus is prevented or co-infections may occur in short intervals. Ferrets were infected with virus then challenged with a different influenza A subtype (primary virus → challenge virus). Graphs indicate the outcome of the challenge infection, where ferrets were infected with challenge virus (black), protected from infection with challenge virus (white), or co-infected with challenge and primary virus (striped) (n=3-6).

Figure S10. The presence of primary virus shedding immediately after challenge can delay shedding of the challenge virus between influenza A virus subtypes. Each ferret was categorised as shedding primary virus the day after challenge (present), or not (cleared), and the number of days to the start (A, B), or the peak (C, D), of challenge virus shedding was determined. Line indicates median.

Figure S11. The delay of virus shedding after challenge is variable between influenza A virus subtypes. For each ferret that was shedding primary virus the day after challenge, the number of days after challenge for which the primary virus shedding was still detected (x-axis) was plotted against the number of days to the start (A, B), or the peak (C, D), of challenge infection (y-axis). A line of best fit by linear regression was plotted for every virus pair to determine the delay for an infection to begin, or to peak.

Figure S1


TCID50 Assay
log10 TCID50/100 µl
6
5
4
3
2
1
0
A(H1N1)pdm09
Influenza B
A(H3N2)
limit of
detection


Flu A M/Flu B NS real time RT-PCR
copies /100 µl
10
9
8
7
6
5
4
limit of
detection
for TCID50
assay

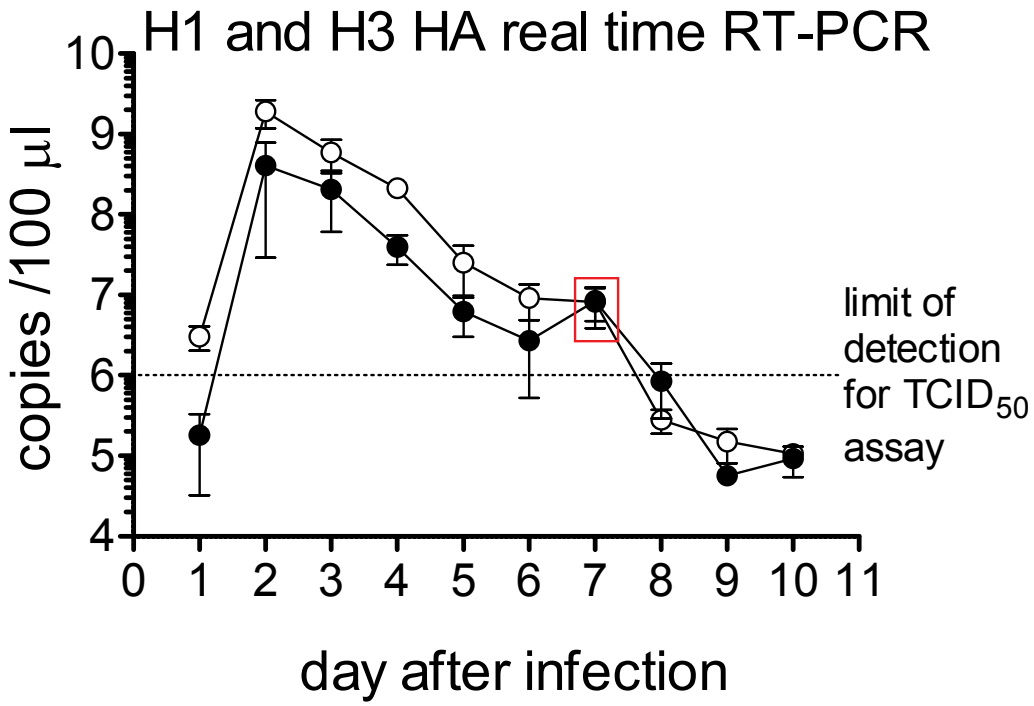

H1 and H3 HA real time RT-PCR
copies /100 µl
10
9
8
7
6
5
4
limit of
detection
for TCID50
assay
0 1 2 3 4 5 6 7 8 9 10 11
day after infection

Figure S2

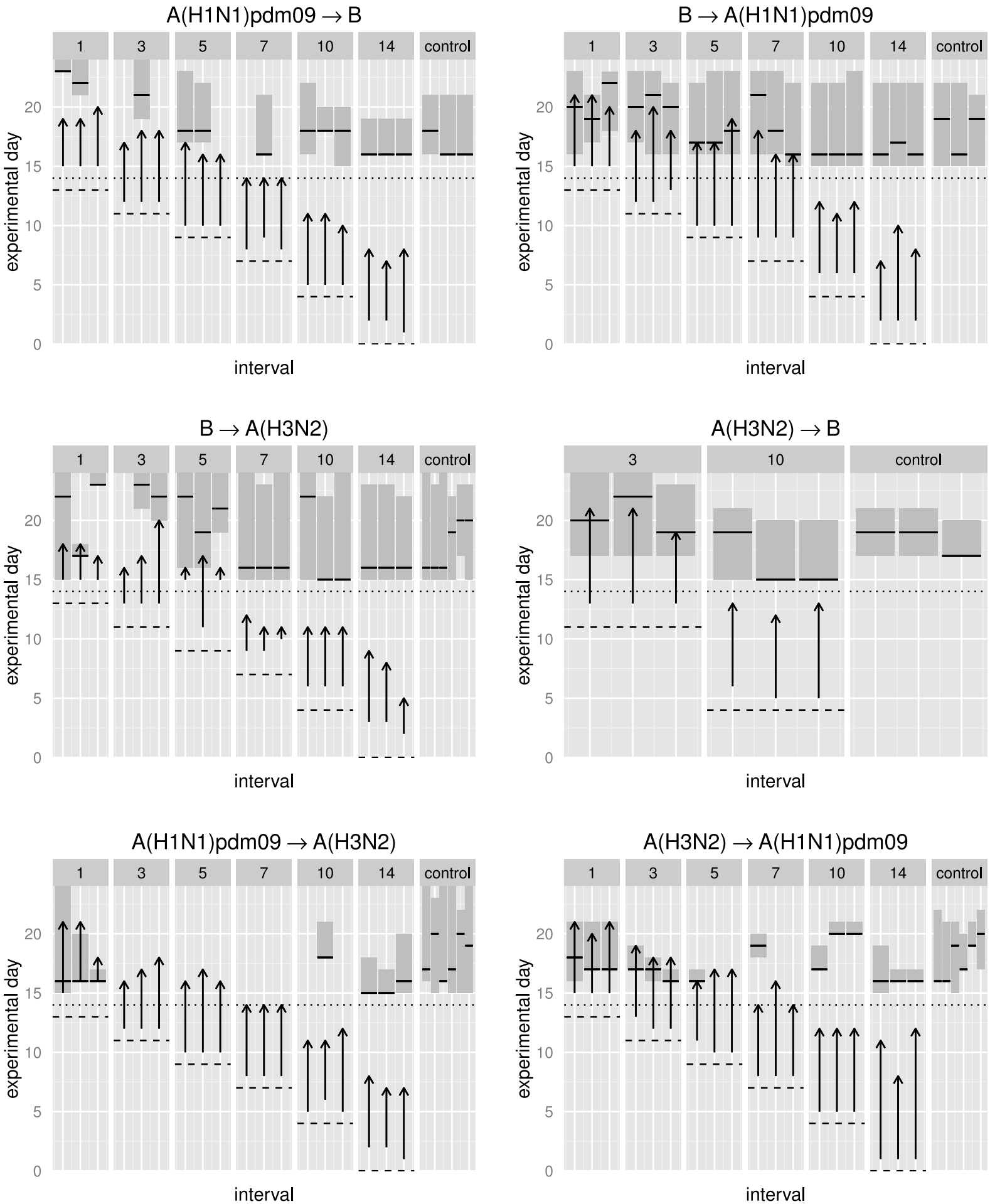

A(H1N1)pdm09 → B
B → A(H1N1)pdm09
B → A(H3N2)
A(H3N2) → B
A(H1N1)pdm09 → A(H3N2)
A(H3N2) → A(H1N1)pdm09
1
3
5
7
10
14
control
experimental day
interval
0
5
10
15
20

Figure S3

A(H1N1)pdm09 -> B (A/Tasmania/2004/2009 ->B/Brisbane/1/2007)

FER-13-02 - real time RT-PCR detected with CDC Flu A and B NS primer/probes

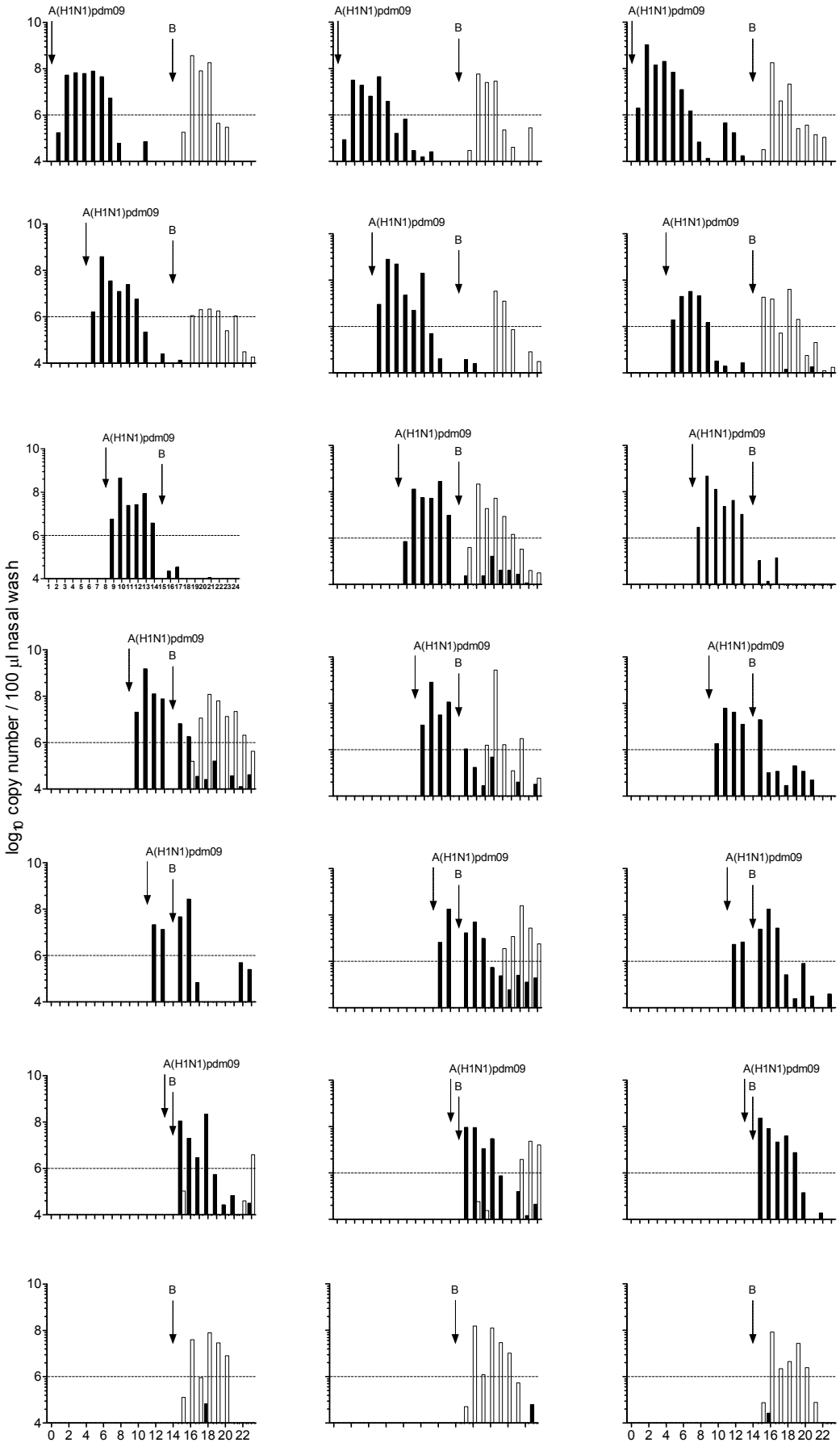


Figure S4

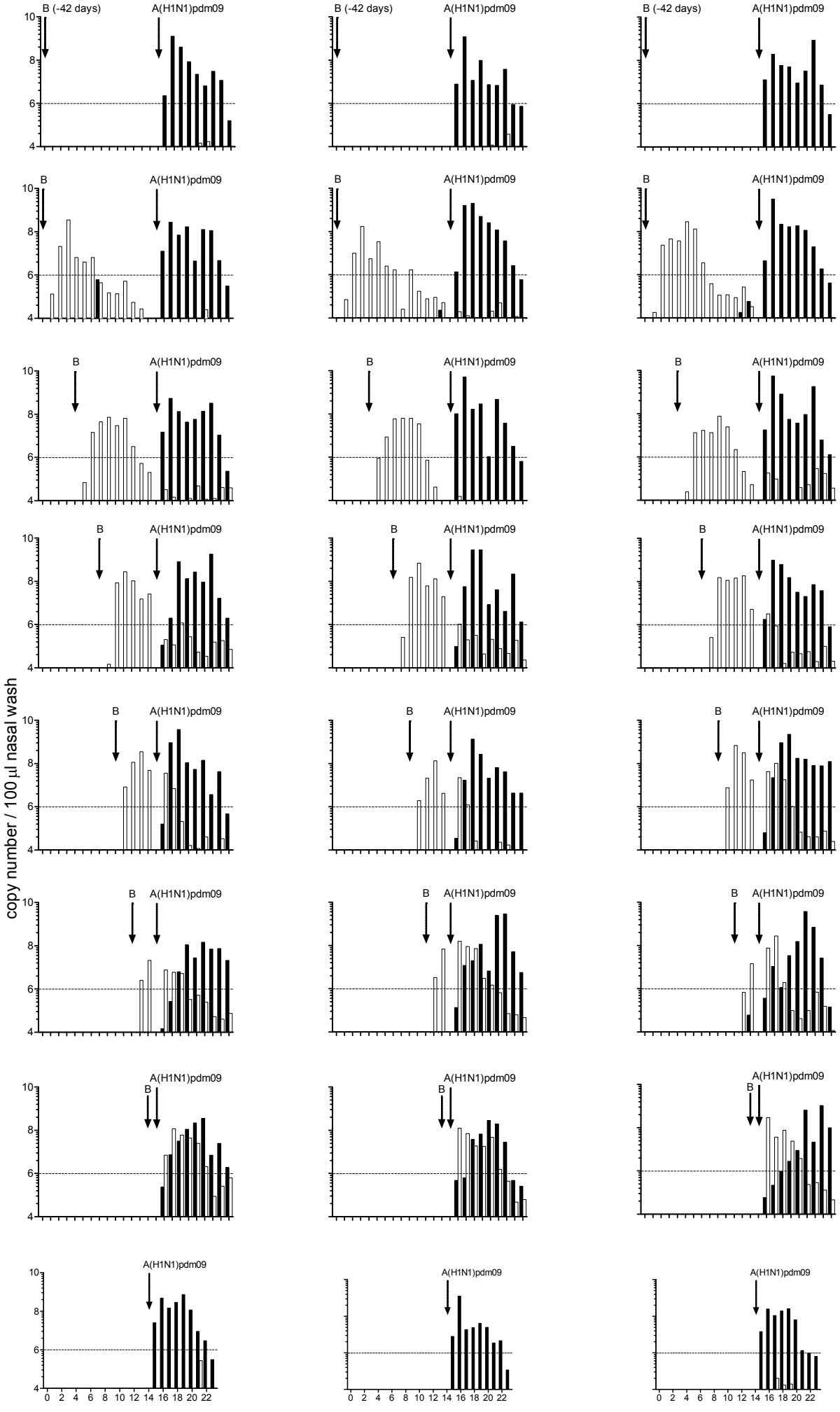

day 56 interval
day 14 interval
day 10 interval
day 7 interval
day 5 interval
day 3 interval
day1 interval
control
B (-42 days)
B
A(H1N1)pdm09
copy number / 100 µl nasal wash
experimental day

Figure S5

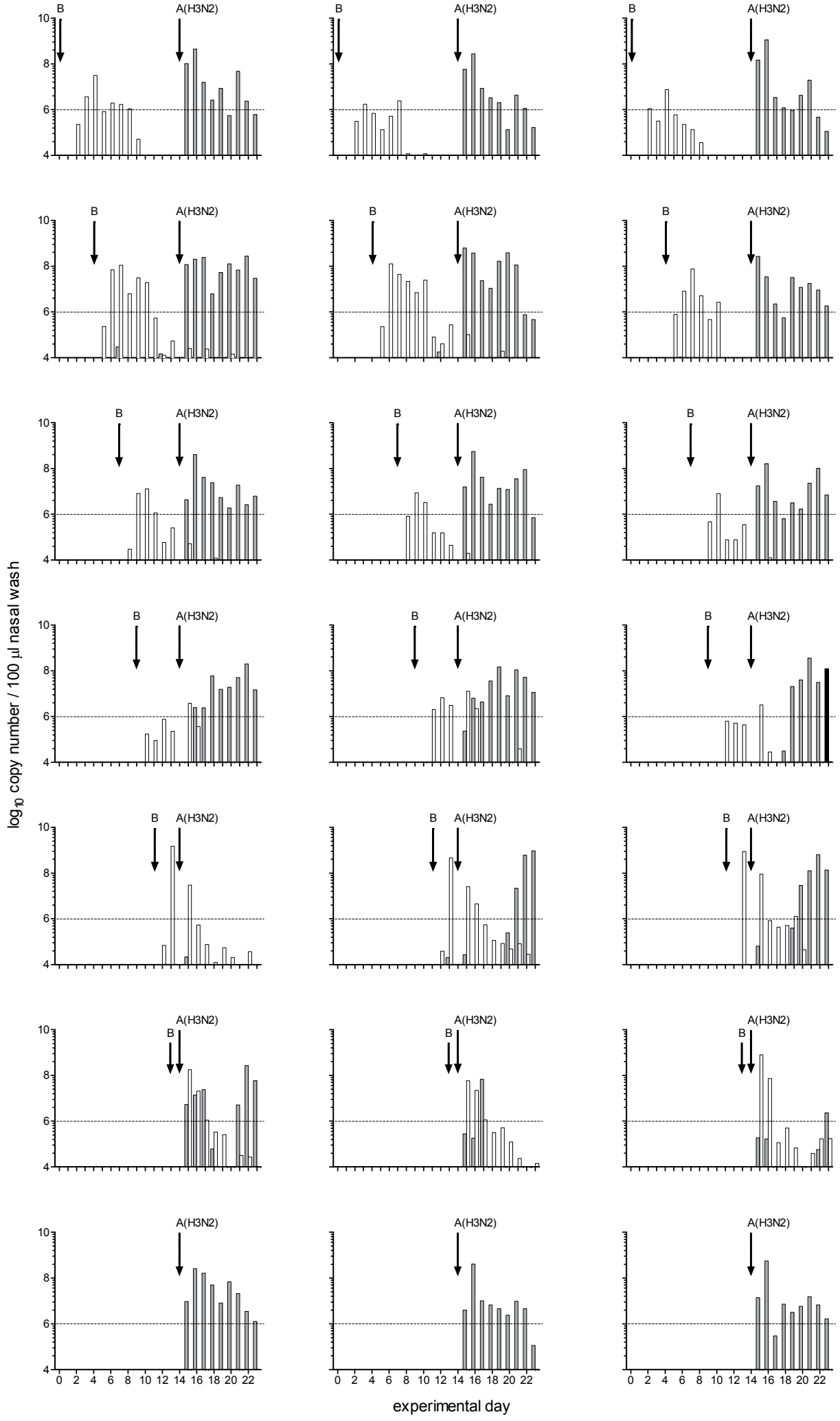

day 14 interval
day 10 interval
day 7 interval
day 5 interval
day 3 interval
day1 interval
control
B
A(H3N2)
log10 copy number / 100 µl nasal wash
experimental day

Figure S6

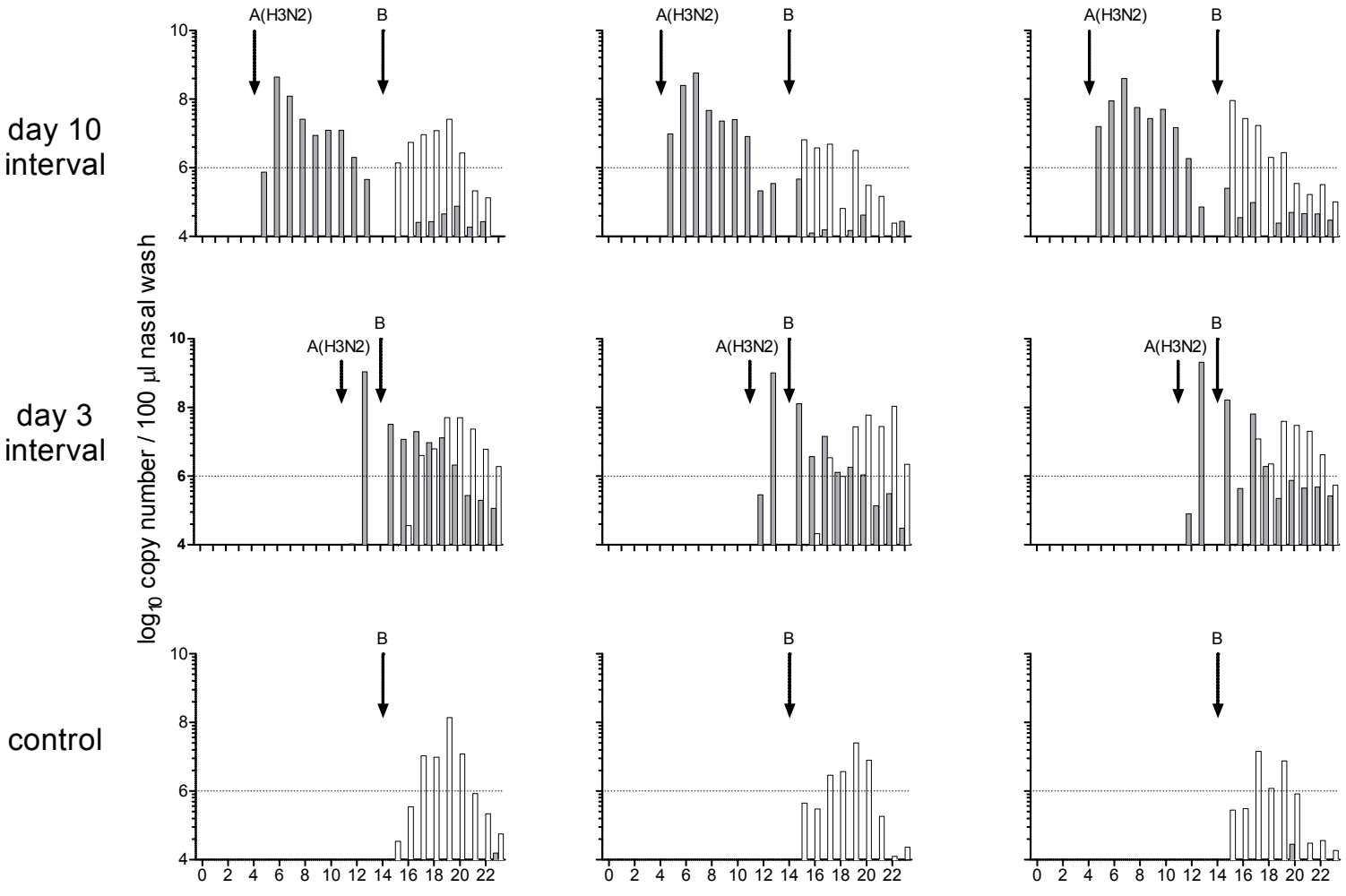

day 10 interval
day 3 interval
control
A(H3N2)
B
log10 copy number / 100 µl nasal wash
experimental day

Figure S7

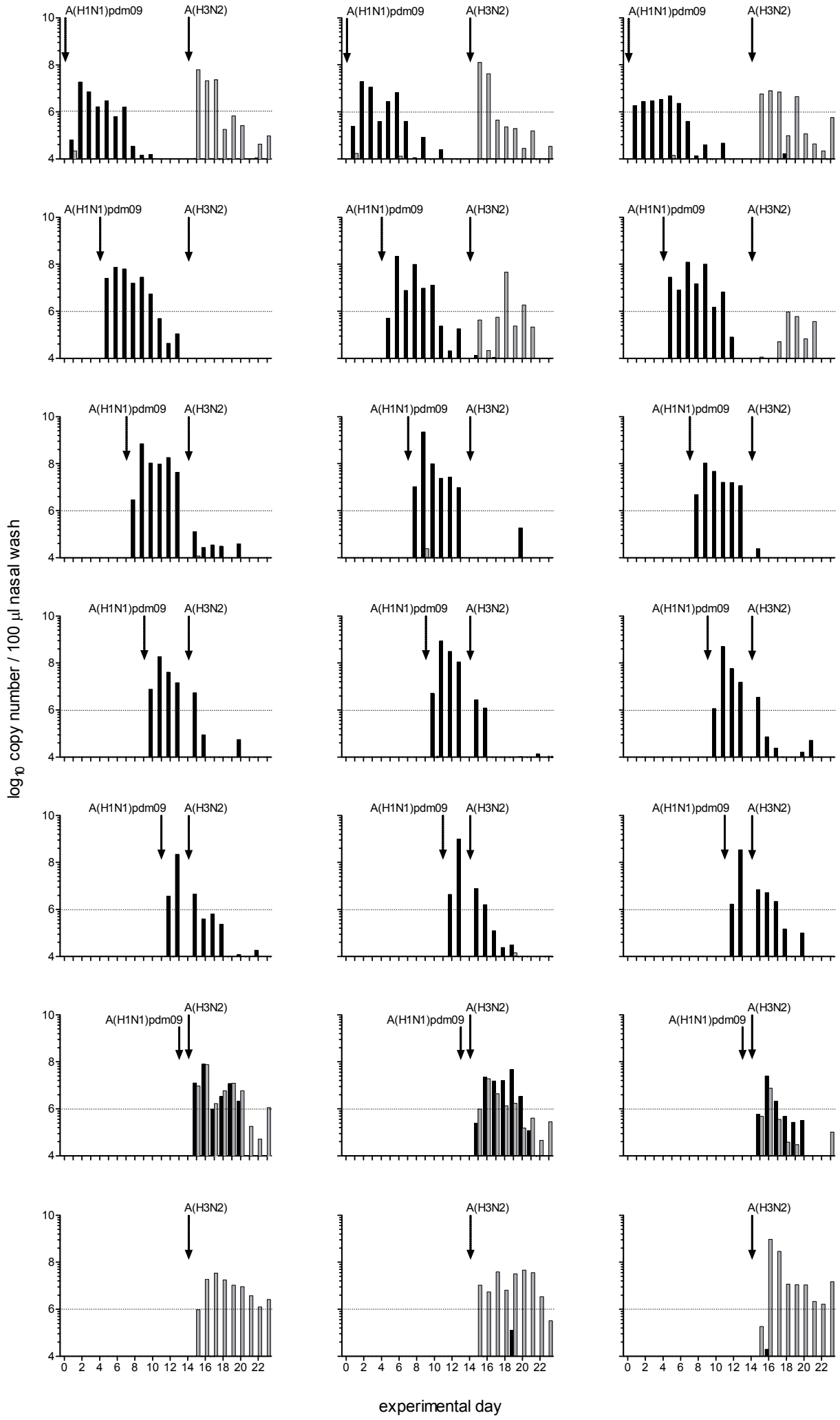

day 14 interval
day 10 interval
day 7 interval
day 5 interval
day 3 interval
day1 interval
control
A(H1N1)pdm09
A(H3N2)
log10 copy number / 100 µl nasal wash
experimental day

Figure S8

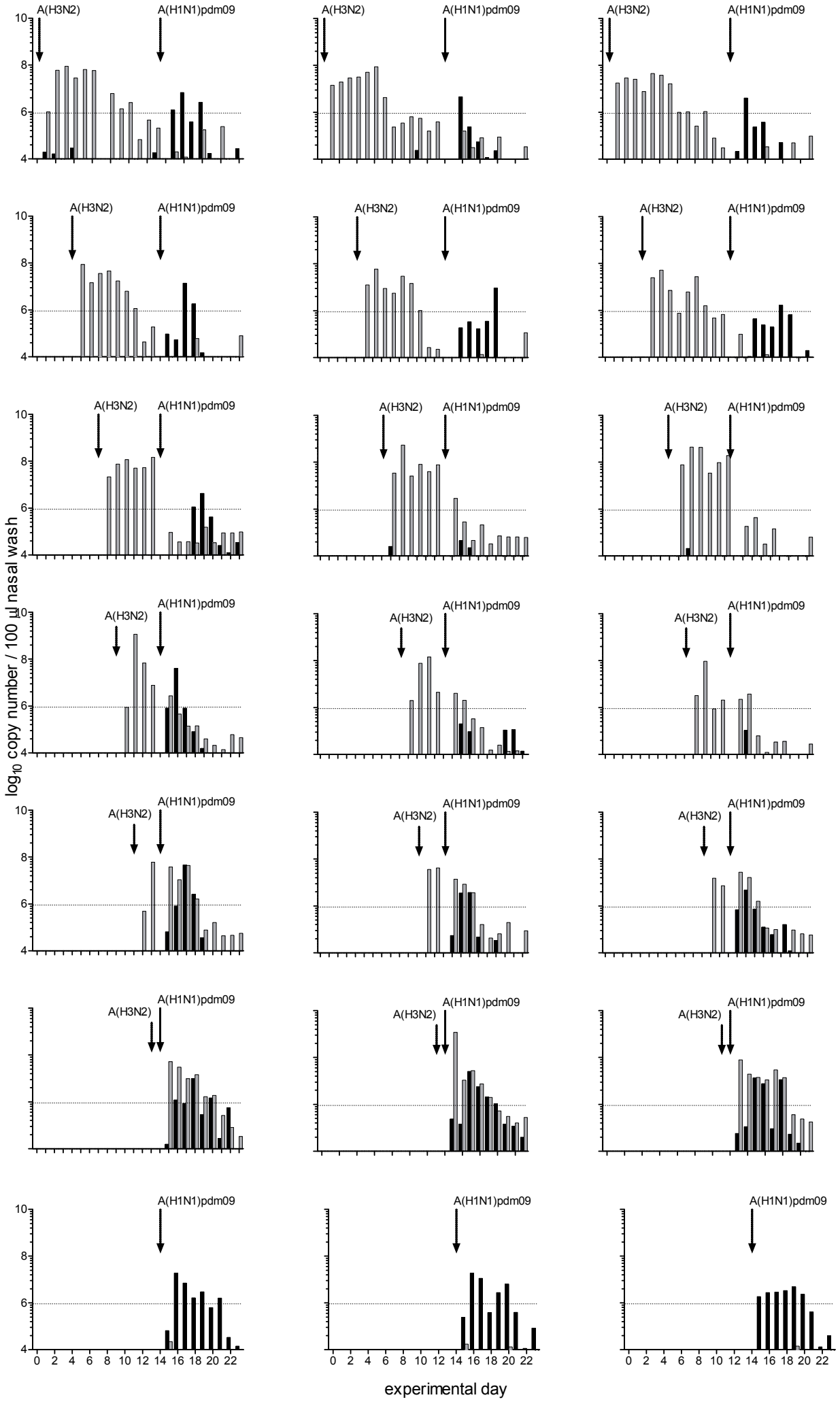

day 14 interval
day 10 interval
day 7 interval
day 5 interval
day 3 interval
day1 interval
control
A(H3N2)
A(H1N1)pdm09
log10 copy number / 100 µl nasal wash
experimental day

Figure S9

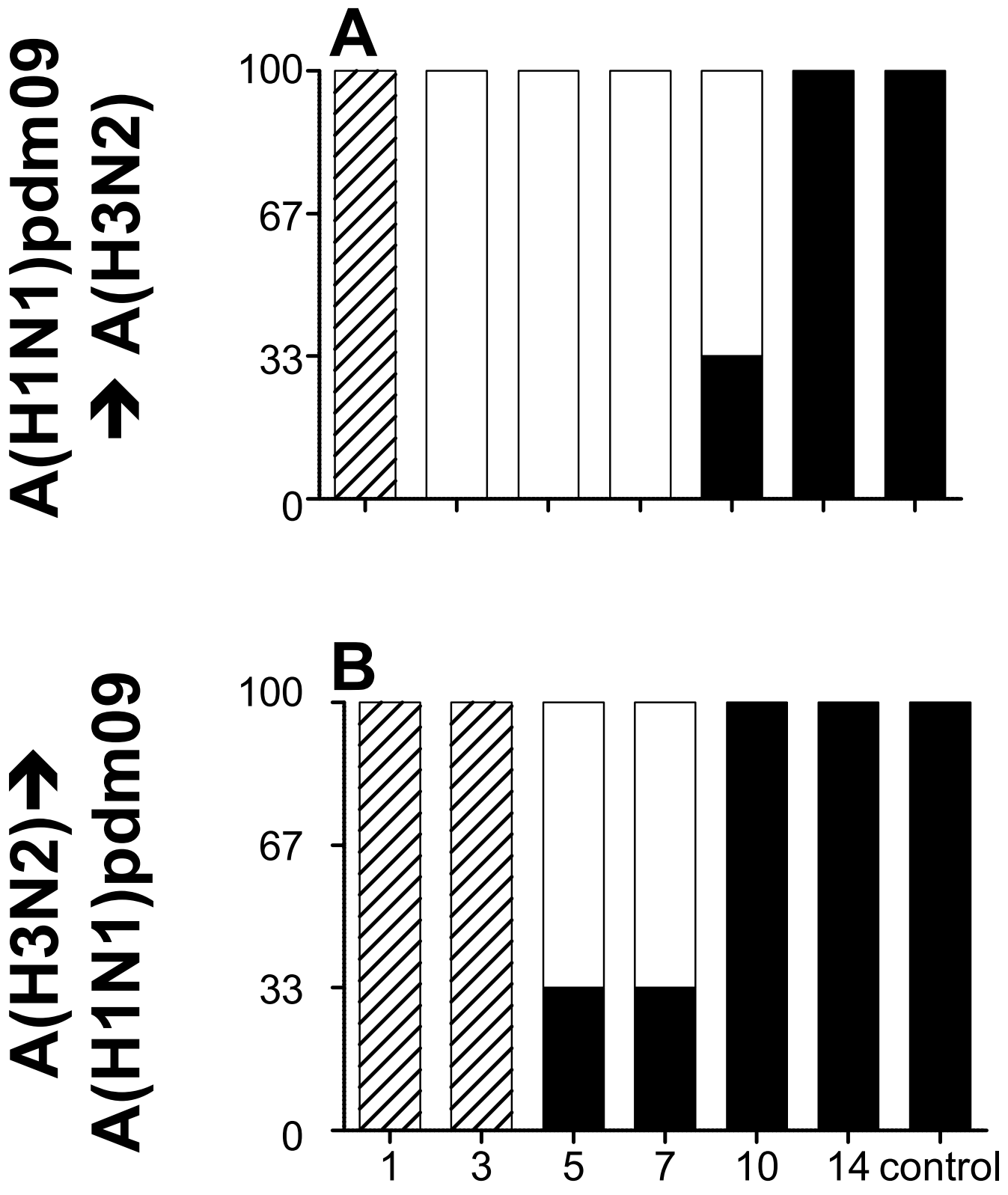

A
A(H1N1)pdm09
➔ A(H3N2)
100
67
33
0
B
A(H3N2)➔
A(H1N1)pdm09
100
67
33
0
1
3
5
7
10
14
control
interval between primary infection
and challenge (days)

Figure S10

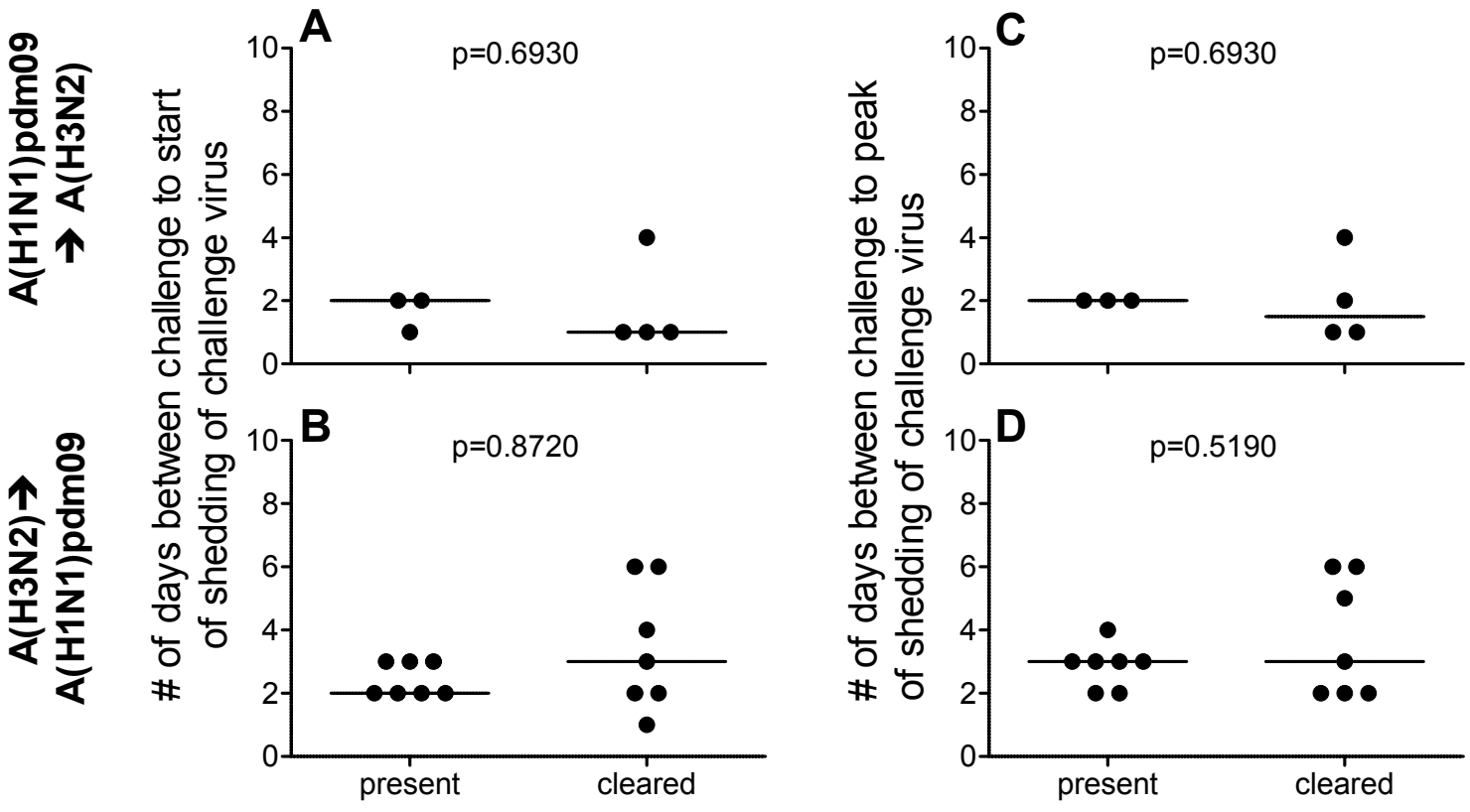
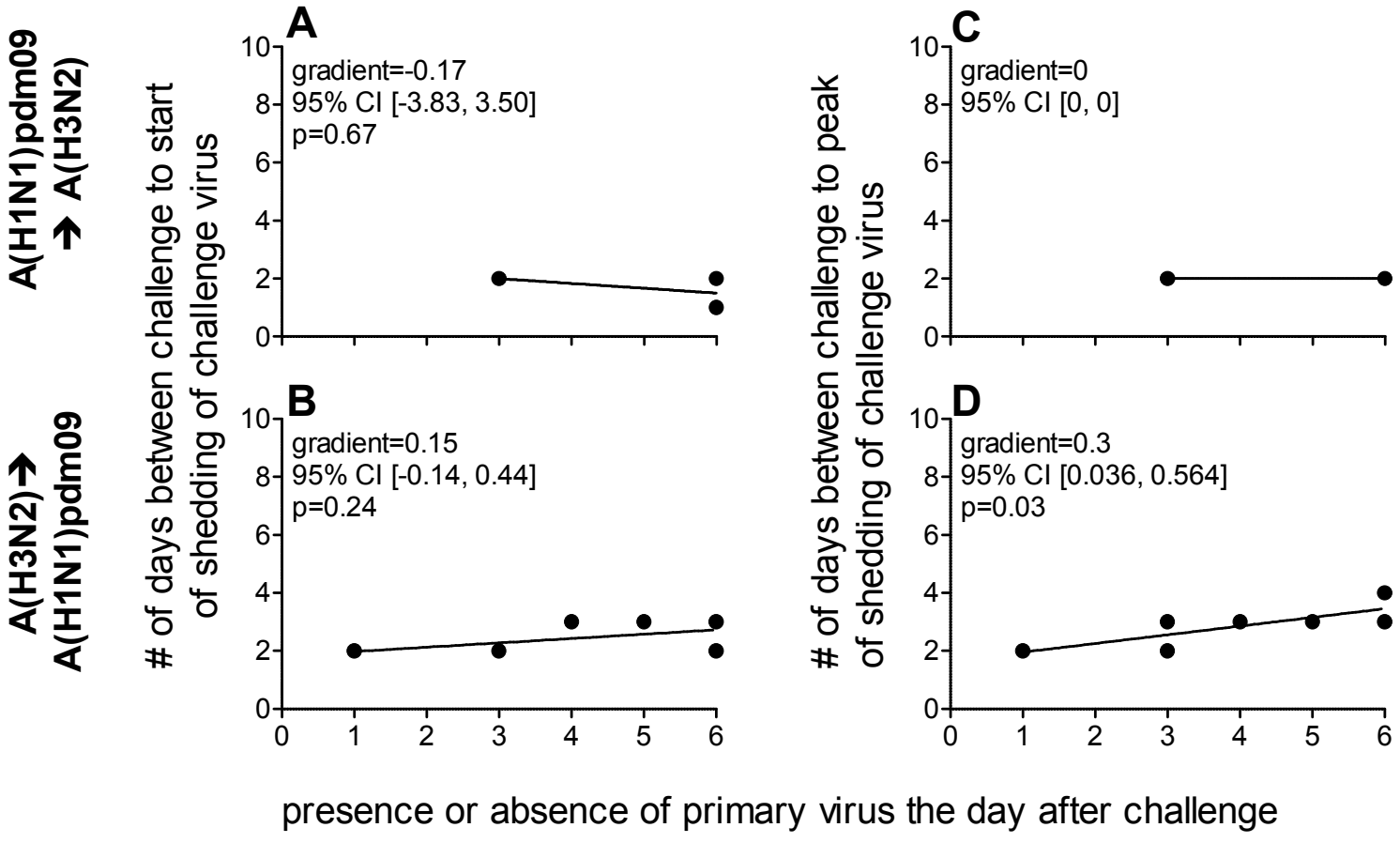

A(H1N1)pdm09 ➔ A(H3N2)
A(H3N2)➔ A(H1N1)pdm09
A
B
C
D
p=0.6930
p=0.8720
p=0.6930
p=0.5190
# of days between challenge to start of shedding of challenge virus
# of days between challenge to peak of shedding of challenge virus
0
2
4
6
8
10
present
cleared
presence or absence of primary virus the day after challenge

Figure S11


A(H1N1)pdm09 ➔ A(H3N2)
A(H3N2)➔ A(H1N1)pdm09
A
gradient=-0.17
95% CI [-3.83, 3.50]
p=0.67
B
gradient=0.15
95% CI [-0.14, 0.44]
p=0.24
C
gradient=0
95% CI [0, 0]
D
gradient=0.3
95% CI [0.036, 0.564]
p=0.03
# of days between challenge to start of shedding of challenge virus
# of days between challenge to peak of shedding of challenge virus
presence or absence of primary virus the day after challenge