

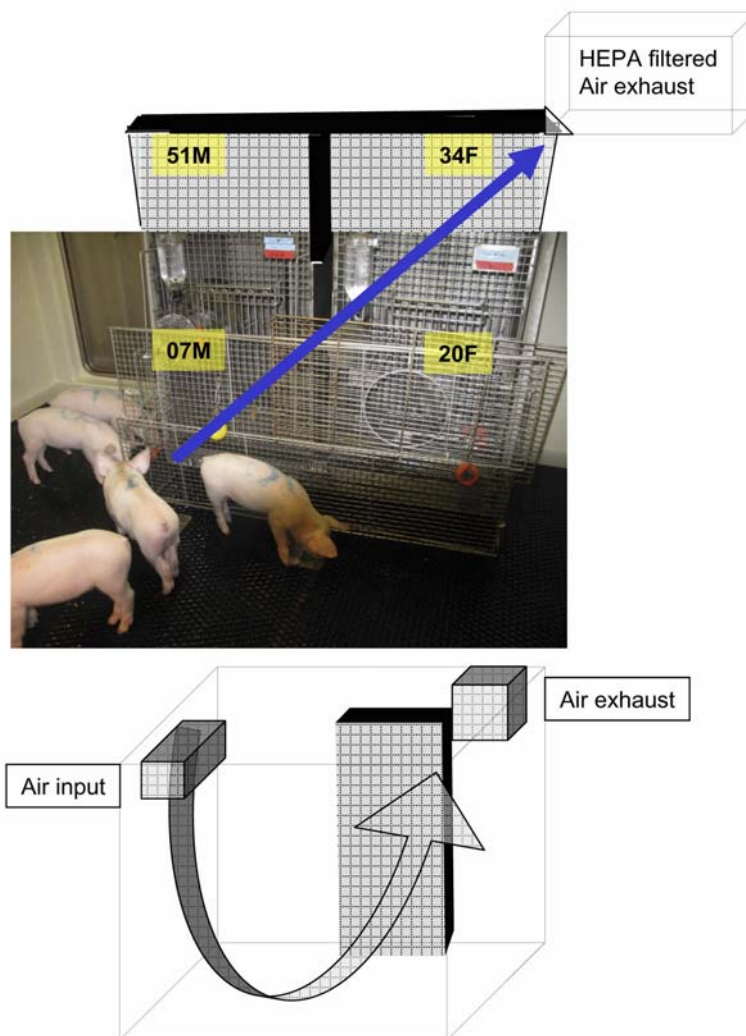
Transmission of Ebola virus from pigs to non-human primates

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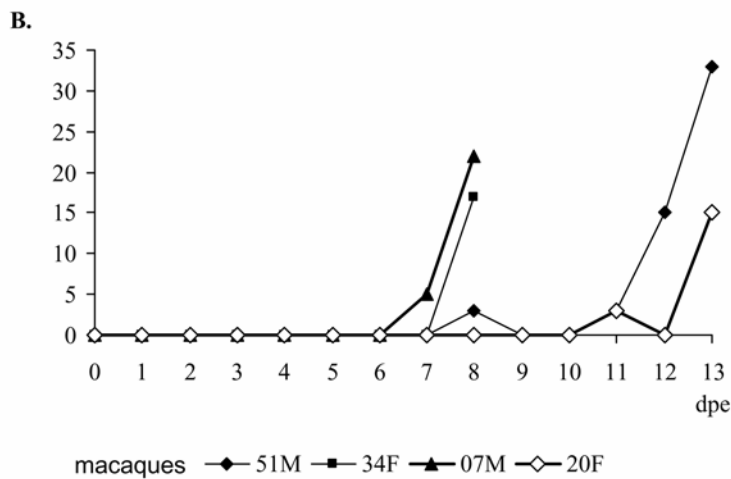
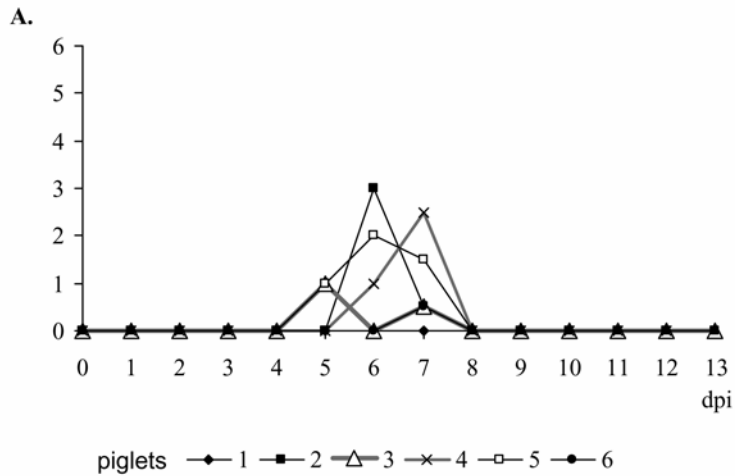
Gary Kobinger^{3,2,*}

Supplementary Information

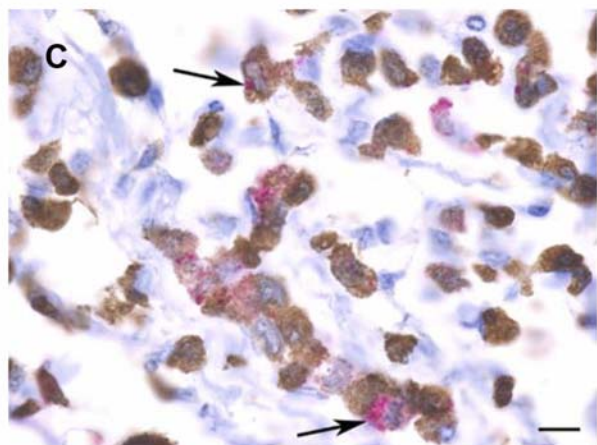
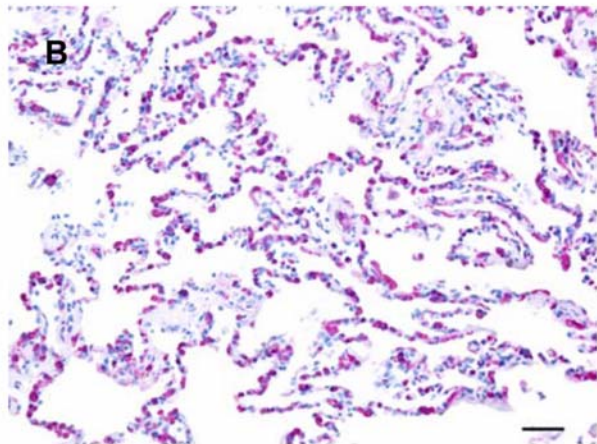
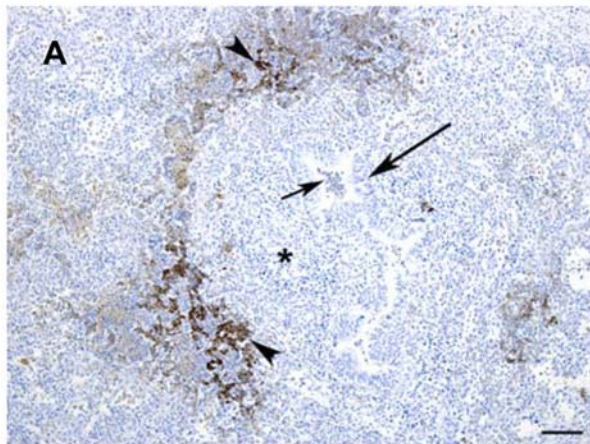
Supplemental Figure S1. Schema of the animal cubicle layout with indicated airflow (blue arrow in front of the cages in the upper image and arrow leading from the air input to the air exhaust in the lower image).



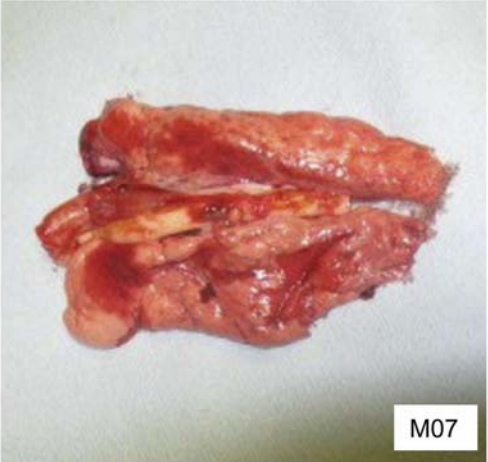
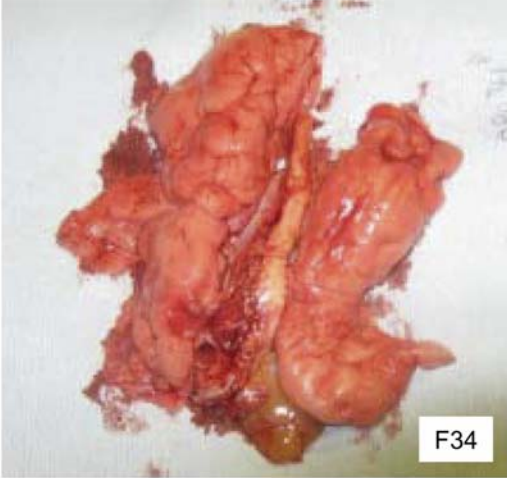
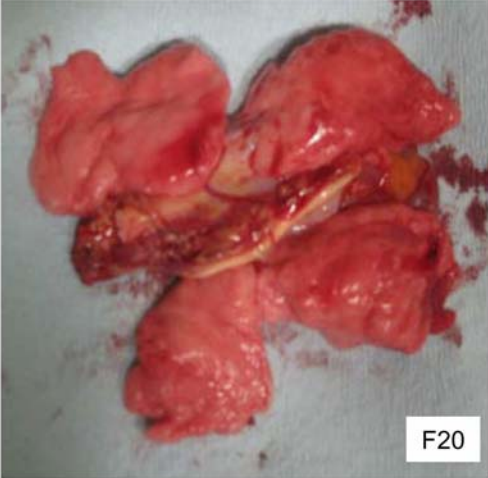
Supplemental Figure S2. Scoring of the clinical signs. Clinical scores in piglets are summarized in **Figure S2.A**. Food intake, urine and feces could be rated only as a group, unless scouring is observed on a specific pig, or the pig appears to be dehydrated or not gaining weight. In this group the above parameters (e/d/u/f) were normal throughout the experiment. Neurological signs were not expected, and not observed in this study. Humane endpoint was set at cumulative scoring of 6, and at 3 if reached in any rated category. Clinical scores for the macaques are summarized in **Figure S2.B**. Humane endpoint was set at cumulative score of 25. In this study, euthanasia was performed prior to reaching the humane endpoint when possible. Guide to the scoring system is provided as Supplementary Table S1.



Supplemental Figure S3. Figure S3.A. Detection of Ebola antigen in lungs of infected piglet No. 4 at 12 dpi by immunohistochemistry using rabbit polyclonal anti ZEBOV VP40 antibody (brown staining). Positive staining in necrotic areas surrounding a bronchiole (arrowhead), positive immunostaining in the luminal exudate (short arrow) and the areas of lymphohistiocytic infiltrate (star*). Long arrow points to the area of hyperplasia of the bronchiolar epithelium. Bar=50 μ m. **Figure S3.B.** Lungs; macaque No. 07M. Infiltrating cells within the alveolar septa were identified as macrophages using immunostaining with antibody Mac387 against calprotectin (L1 antigen) on activated monocytes/macrophages (red staining). Bar=50 μ m. **Figure S3.C.** Lung; monkey No. 07M. Double immunostaining detected simultaneous expression of the monocyte/macrophage marker (brown; antibody Clone Mac387) and Ebola virus antigen (pink, rabbit polyclonal anti-ZEBOV VP40 antibody) within the same cell indicating the presence of viral antigen within macrophages (arrows). Bar=10 μ m.



Supplemental Figure S4. Lungs of NHPs. Multifocal petechial to coalescing areas of red discoloration (hemorrhages) were present over the entire area of lung, most prominently in the caudal lobes.



Supplemental Table 1. Scoring of clinical signs.

Scoring - pigs

1. Depression

- 0 - normal (bright, alert, responsive, curious, attentive, stays with group and plays)
- 1 - inactive and huddles in a group but will readily get up if prompted
- 2 - huddles in a group and very reluctant to get up if prompted
- 3 - extremely depressed, arched back, lowered head, nor responsive to prompt

2. Rectal Temperature (normal temperature for a pig 4- 5 weeks of age is 38.2 - 39.9°C)

- 0 - normal 38.5 – 39.9°C
- 1 - above 40.0 – 40.5°C
- 2 - 40.5 – 41.5°C
- 3 - above 41.5°C and unresponsive to anti-pyretic medication or < 36.0°C

3. Breathing

- 0 - normal (20 - 40 breaths/min)
- 1 - moderately increased (40 - 60 breaths/min); occasional cough
- 2 - increased with some abdominal effort (60 - 80); mild cough
- 3 - increased with strong abdominal effort (>80), cyanotic ears; barking cough

Food intake, urine and feces could be rated only as a group, unless scouring is observed on a specific pig, or the pig appears to be dehydrated or not gaining weight. In this group this parameters (e/d/u/f) were normal throughout the experiment. Neurological signs were not expected, and not observed in this study. Humane endpoint was set at cumulative scoring of 6, and at 3 if reached in any of the above categories.

Scoring - macaques

1. Posture

- 0 - normal
- 3 - decreased activity, decreased normal behavior, pilo-erection
- 5 - huddled, shaking, toes and hands clenched

2. Temperature

- 5 - increase in body temperature above 2°C

3. Weight

- 10 - decrease in body weight of more then 10%

4. Respiration

- 0 - normal
- 2 - increased or decreased
- 10 - laboured, breathing through mouth

5. Feces, urine

- 0 - normal consistency and volume
- 2 - feces absent or dry, decreased urine output, cloudy urine
- 2 - wet pasty or small very dry stool

10 - liquid stool, blood in stool or urine, no urine more than twice (two observations/day)

6. Food, water

0 - normal

1 - slightly decreased (eating fruit and treats, 25% or greater reduction in pellets or water intake)

3 - moderately decreased (reduced intake of fruit and treats, 25 - 50% reduction in pellets/water)

4 - severely decreased (reduced intake of fruit/treats, more than 50% reduction in pellets/water)

10 - seriously decreased (refusing food, dehydration apparent for more than 2 days)

7. Recumbent

0 - no signs

3 - huddled (on camera) but active when people in the room

15 - lies down, will get up when approached or prompted, uses cage for support

25 - lies down, will not get up when approached

8. Attitude

0 - normal

1 - hyperactive or mildly depressed, responds to treats and toys

3 - moderately depressed, response requires prompting; loss of interest in treats and toys

10 - severely depressed, no interest in treats, does not respond to human presence

9. Physical signs

2 - flushed appearance to skin

5 - visible rash

5 - cyanosis

10 - subcutaneous hemorrhages

15 - hemorrhage from orifices

Humane endpoint is set at cumulative score of 25.

Supplemental Table 2. Virus detection in swine tissues by real-time RT-PCR (\log_{10} copy number/ml of 10% w/v tissue homogenate or liquid sample), and virus isolation (\log_{10} TCID₅₀/ml or as positive or negative)

tissue	euthanasia at 12 dpi						euthanasia at 14 dpi					
	pig No. 1		pig No. 2		pig No. 4		pig No. 3		pig No. 5		pig No. 7	
	RNA	vi	RNA	vi	RNA	vi	RNA	vi	RNA	vi	RNA	vi
lung	neg	-	5.45	P	4.9	P	neg	-	neg	-	neg	-
LALN	2.9	N	3.85	N	5.25	P	neg	-	4.4	N	neg	-
SLN	4.5	-	3.25	-	4.5	-	4.05	-	4.45	-	5	-
trachea	neg	-	neg	-	4.4	-	neg	-	neg	-	neg	-
spleen	neg	-	3.35	-	3.15	-	neg	-	neg	-	neg	-
liver	neg	-	3.45	-	neg	-	neg	-	neg	-	neg	-
B 5dpi	3.5	-	3.95	-	3.95	-	neg	-	neg	-	neg	-
B 7dpi	neg	-	4.6	-	4.95	-	neg	-	3.3	-	neg	-
R 1dpi	4	N	4.9	2.65	4.3	N	4.2	N	4.2	N	4.7	2.15
R 5dpi	3.55	N	4	N	3.5	N	3.6	N	neg	N	3.5	N

LALN - lung associated lymph nodes, SLN - submandibular lymph nodes, B – blood, R – rectal swab; (-) indicates virus isolation not attempted, neg - no viral RNA detected, N - negative on virus isolation, **P** - positive on virus isolation.

Supplemental Table 3. Virus detection in porcine swabs. P-positive, N- negative virus isolation

dpi	pig No	nasal swabs			oral swabs		
		sample copy#/ml	virus isolation	2 nd passage copy#/ml	sample copy#/ml	virus isolation	2 nd passage copy#/ml
1	1	neg	N	neg	4.1	N	4.05
	2	neg	N	neg	4.35	N	neg
	3	3.25	N	neg	3.6	P	4.65
	4	4.2	P	9.95	4.35	P	8.6
	5	neg	N	neg	4.9	P	8.05
	6	neg	N	neg	3.3	P	8.9
3	1	3.3	P	7.15	4.05	N	neg
	2	4.15	N	neg	4	N	neg
	3	4.35	P	9.96	3.75	P	5.7
	4	5.1	P	10.2	3.95	P	8.1
	5	3.3	P	10.25	3.95	N	neg
	6	4.05	N	4.65	3.95	N	neg
5	1	4.45	N	4.2	4.15	N	neg
	2	4.75	N	3.8	3.8	N	neg
	3	5.65	P	10.15	3.8	P	8.7
	4	3.6	P	10	3.8	N	neg
	5	4.9	N	5.2	3.2	N	neg
	6	5	P	10.1	3.6	N	neg
7	1	5.05	N	neg	3.8	P	9.3
	2	3.8	N	neg	4.05	N	neg
	3	5	P	8.2	4.05	N	neg
	4	3.9	P	7.8	3.7	N	neg
	5	3.5	N	3.3	3.1	P	9.9
	6	4.2	N	neg	3.3	N	4.2

Supplemental Table 4. Virus detection in the tissues, blood and swabs of macaques by real-time RT-PCR (\log_{10} copy number/g) and virus isolation (\log_{10} TCID₅₀/g) at the time of euthanasia.

tissue	euthanasia at 8 dpi				euthanasia at 13 dpi			
	NHP 07M		NHP 34F		NHP 20F		NHP 51M	
	RNA	vi	RNA	vi	RNA	vi	RNA	vi
blood	8.4	4.6	9.4	4.75	10.8	2	9.4	1.75
rectal swab	7.4	1.85	7.2	1.85	7.45	3.2	8.2	1.85
oral swab	5.95	neg	6.25	4.5	7.2	neg	5.4	neg
nasal swab	3.85	2.85	6.75	2.85	8.7	4.5	6.75	neg
lung 1	10.1	8.1	9.6	8.3	9.75	8.5	9.8	9.2
lung 2	9.4	7.55	10.15	8.25	9.95	8.3	9.6	8.4
BLN	11	8.8	9.8	10	9	8.8	11.05	8
liver	10.2	8.15	11.15	8.85	10.7	9.15	10.8	9.15
spleen	9.5	8.85	9.9	8.95	10.55	9.6	10.9	9.8
intestine	ND	ND	ND	ND	9.95	9.3	9.65	7.85

BLN - bronchiolar lymph nodes,(-) indicates virus isolation not attempted, neg - no viral RNA detected, ND - not done

Two lung sections were tested independently, labeled as lung 1 and lung 2.