

SI Guide

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Supplementary Table 5. RT-qPCR Ct values from selected tissues and swabs from ferret experiment 1.

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Supplementary Figure 1. Cytokine and chemokine responses following *in vitro* infection of primary human airway cells. Titers of IFN- β , CXCL-10, and IFN- λ as determined by ELISA following HPAI infection of three primary human airway cell cultures.

Supplementary Figure 2. Survival and weight loss of BALB/c mice infected with each of the four Canadian HPAI A(H5N1) isolates at different doses.

Supplementary Figure 3. Survival and weight loss of deer mice (*Peromyscus maniculatus*) infected with each of the four Canadian HPAI A(H5N1) isolates at 10⁵ PFU.

Supplementary Figure 4. Schematic of the pilot ferret experiment (experiment 1) cage set-up. Infected ferrets are indicated with a virus above them. One day following infection, cagemates were added. Blue lines represent cages with male ferrets, pink lines represent cages with female ferrets.

Supplementary Figure 5. Weight loss seen in infected and contact ferrets from experiment 1. In the top panel, weight loss from all animals is shown (mean + SEM of both infected and contact animals). In the bottom four panels, weight loss from each individual is shown.

Supplementary Figure 6. Scanned and rectal temperatures of infected and contact ferrets from experiment 1.

Supplementary Figure 7. Schematic of the second ferret transmission experiment (experiment 2) cage set-up. Infected ferrets are indicated with a virus above them. One day following infection, cagemates and aerosol animals were added. Blue lines represent cages with male ferrets, pink lines represent cages with female ferrets.

Supplementary Table 1. Origin of gene segments in each H5N1 isolate

Gene	BWTE/MB/22	RT.Hawk/ON/22	Redfox/PEI/22	RedFox/ON/22	Turkey/ON/22
PB1	North American	North American	Eurasian	North American	North American
PB2	North American	North American	Eurasian	North American	North American
PA	North American	Eurasian	Eurasian	North American	North American
HA	Eurasian	Eurasian	Eurasian	Eurasian	Eurasian
NP	North American	North American	Eurasian	North American	North American
NA	Eurasian	Eurasian	Eurasian	Eurasian	Eurasian
NS	Eurasian	Eurasian	Eurasian	Eurasian	Eurasian
M	Eurasian	Eurasian	Eurasian	Eurasian	Eurasian

Supplementary Table 2. Calculated LD₅₀ values for each of the four Canadian HPAI A(H5N1) isolates in BALB/c mice.

Virus	BALB/c LD₅₀
BWTE/MB/2022 (H5N1)	19.35 PFU
RT.Hawk/ON/2022 (H5N1)	< 1 PFU
Redfox/PEI/FAV-0544/2022 (H5N1)	6.61 PFU
Redfox/ON/2022 (H5N1)	< 1 PFU

Supplementary Table 3. Log₁₀ TCID₅₀/g infectious viral titers in the tissues of euthanized ferrets from experiment 1.

Animal	Virus	DPI/ DPC	Nasal Turbinate	Trachea	Right UL	Right LL	Left UL	Left LL	Heart	Spleen	Kidney	Liver	Brain
Infected M2	BWTE/MB/22	11	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Infected F1	BWTE/MB/22	7	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	3.20	3.80
Infected F2	RT.Hawk/ON/22	7	6.16	4.65	4.11	4.19	4.07	5.44	BLOD	5.30	4.16	5.21	BLOD
Infected F3	RT.Hawk/ON/22	7	5.27	4.45	3.17	4.62	BLOD	5.20	BLOD	BLOD	BLOD	BLOD	3.63
Contact F2	RT.Hawk/ON/22	7	3.60	BLOD	3.41	3.77	BLOD	3.86	BLOD	4.51	BLOD	6.53	BLOD
Infected M3	RT.Hawk/ON/22	4	4.65	NC	5.27	4.74	NC	NC	NC	NC	NC	NC	NC
Contact M2	RT.Hawk/ON/22	11	BLOD	3.63	3.69	4.09	4.01	4.20	BLOD	BLOD	BLOD	BLOD	4.81
Infected M4	Redfox/PEI/22	11	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Infected F4	Redfox/PEI/22	13	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	3.13	BLOD
Infected M5	Redfox/ON/22	7	3.50	3.26	BLOD	3.42	3.51	BLOD	BLOD	BLOD	BLOD	3.62	3.17
Contact M4	Redfox/ON/22	18	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC	NC	NC	NC	NC
Infected F5	Redfox/ON/22	7	3.16	4.37	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	3.30	4.92

Ferret Terminal Tissue TCID₅₀ titers in Log₁₀ TCID₅₀/g. M, male; F, female; UL, Upper Lung; LL, Lower Lung; BLOD, Below limit of detection; NC, not collected; DPI, Day post-infection; DPC, day post-contact.

Supplementary Table 4. Log₁₀ TCID₅₀/g infectious viral titers in the tissues of euthanized ferrets from experiment 2.

Animal	DPI/DPC	Nasal Turbinate	Trachea	Right UL	Right LL	Left UL	Left LL	Heart	Spleen	Kidney	Liver	Brain
Infected M1	5	4.56	6.05	4.36	3.62	4.96	4.13	BLOD	3.35	3.24	5.30	BLOD
Infected M2	5	5.93	6.14	5.64	5.97	5.57	4.83	3.65	4.16	BLOD	6.45	5.35
Infected F1	4	5.28	5.44	4.75	5.80	4.63	5.20	3.07	5.25	BLOD	5.49	BLOD
Infected F2	4	5.62	5.24	5.48	5.52	6.10	6.10	4.10	4.20	BLOD	4.29	5.09
Contact M1	8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC
Contact M2	6	5.00	3.49	4.95	5.57	5.02	5.57	4.29	6.11	3.90	6.44	5.85
Contact F1	8	BLOD	BLOD	3.61	4.63	4.13	3.40	BLOD	4.35	BLOD	BLOD	NC
Contact F2	8	3.40	3.50	5.25	4.86	5.35	4.99	BLOD	5.25	3.31	4.36	NC
Aerosol M1	8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC
Aerosol M2	8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC
Aerosol F1	8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC
Aerosol F2	8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	NC

Ferret Terminal Tissue TCID₅₀ titers in Log₁₀ TCID₅₀/g. UL, Upper Lung; LL, Lower Lung; BLOD, Below limit of detection; NC, not collected; DPI, Day post-infection; DPC, day post-contact/aerosol animal introduction.

Supplementary Table 5. RT-qPCR Ct values from selected tissues and swabs from ferret experiment 1.

Animal	Virus	DPI/DPC	Nasal Turbinate	Lung	Brain	Oral Swab	Nasal Swab
Infected M2	BWTE/MB/22	11 tissues/9 swabs	36.54	33.61	30.75	BLOD	BLOD
Infected F1	BWTE/MB/22	7	29.23	27.50	19.87	36.55	36.01
Infected F2	RT.Hawk/ON/22	7	25.10	24.54	19.66	26.04	36.54
Infected F3	RT.Hawk/ON/22	7	15.81	21.76	24.19	27.74	24.01
Contact F2	RT.Hawk/ON/22	7	29.12	23.79	25.61	34.02	33.04
Infected M3	RT.Hawk/ON/22	4 tissues/ 3 swabs	32.08	18.44	NC	BLOD	23.79
Contact M2	RT.Hawk/ON/22	11 tissues/ 9 swabs	25.53	19.14	31.23	31.18	36.73
Infected M4	Redfox/PEI/22	11 tissues/ 9 swabs	37.30	24.63	32.07	34.61	33.33
Infected F4	Redfox/PEI/22	13 tissues/ 12 swabs	32.04	32.15	33.63	BLOD	31.63
Infected M5	Redfox/ON/22	7	25.01	20.26	21.61	30.28	25.70
Contact M4	Redfox/ON/22	18 tissues/ 12 swabs	36.61	32.89	NC	BLOD	BLOD
Infected F5	Redfox/ON/22	7	25.61	26.36	15.01	33.62	27.08

Ferret Terminal Tissue and swab Rt-qPCR Ct values. M, male; F, female; BLOD, Below limit of detection; NC, not collected; DPI, Day post-infection; DPC, day post-contact.

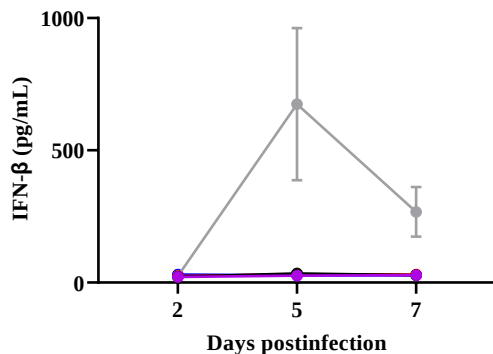
Supplementary Table 6. RT-qPCR Ct values from selected tissues and swabs from ferret experiment 2.

Animal	DPI/DPC	Nasal Turbinate	Lung	Brain	Oral Swab	Nasal Swab
Infected M1	5	20.48	19.22	21.28	31.56	31.19
Infected M2	5	20.89	20.50	15.19	25.11	26.90
Infected F1	4 tissues/ 3 swabs	20.87	15.91	19.10	23.49	23.12
Infected F2	4 tissues/ 3 swabs	17.63	21.94	19.03	29.99	25.99
Contact M1	8 tissues/ 7 swabs	30.58	31.68	NC	BLOD	BLOD
Contact M2	6 tissues/ 5 swabs	20.27	17.04	16.29	29.86	29.72
Contact F1	8 tissues/ 7 swabs	35.73	26.94	NC	36.02	BLOD
Contact F2	8 tissues/ 7 swabs	31.15	20.03	NC	32.61	32.07
Aerosol M1	8 tissues/ 7 swabs	31.07	31.81	NC	BLOD	BLOD
Aerosol M2	8 tissues/ 7 swabs	33.66	34.00	NC	39.50	BLOD
Aerosol F1	8 tissues/ 7 swabs	31.80	27.75	NC	BLOD	38.78
Aerosol F2	8 tissues/ 7 swabs	31.04	32.69	NC	BLOD	BLOD

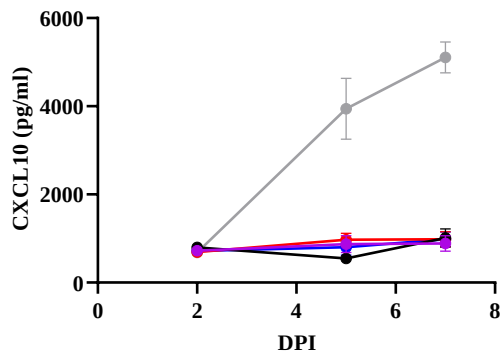
Ferret Terminal Tissue and swab Rt-qPCR Ct values. M, male; F, female; BLOD, Below limit of detection; NC, not collected; DPI, Day post-infection; DPC, day post-contact.

● CTRL
 ● Turkey/ON/22
 ● RT.Hawk/ON/22
 ● Redfox/PEI/22
 ● Redfox/ON/22

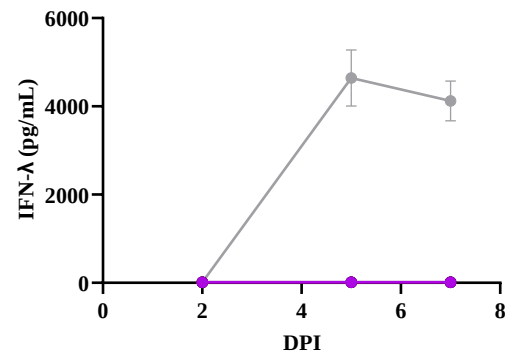
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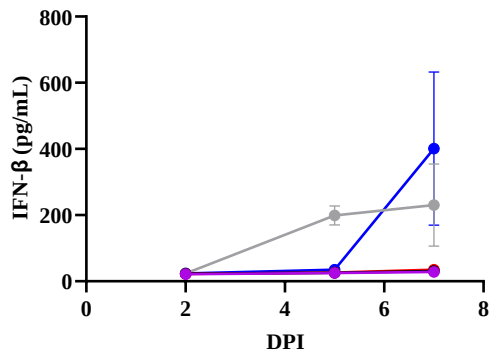
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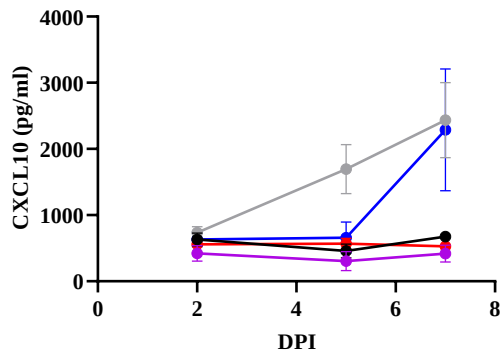
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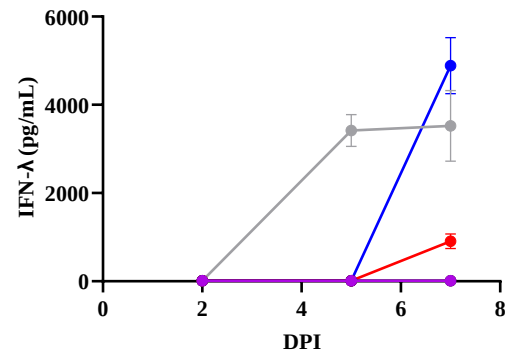
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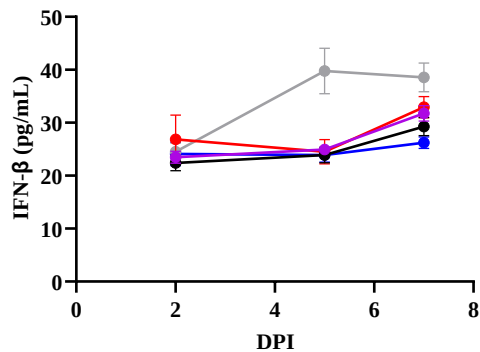
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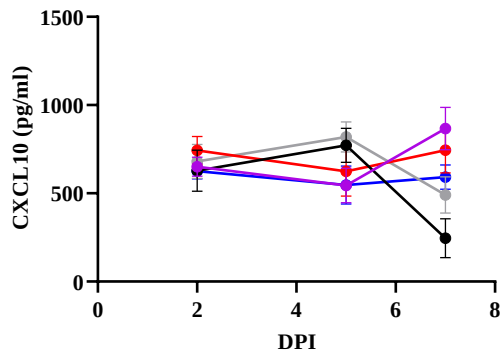
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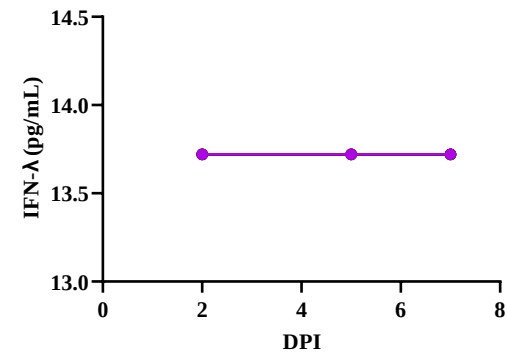
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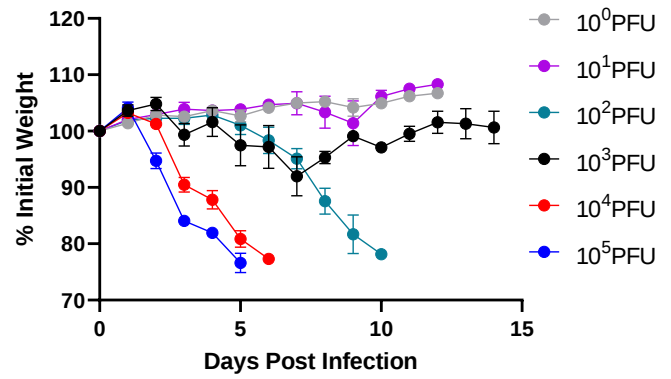
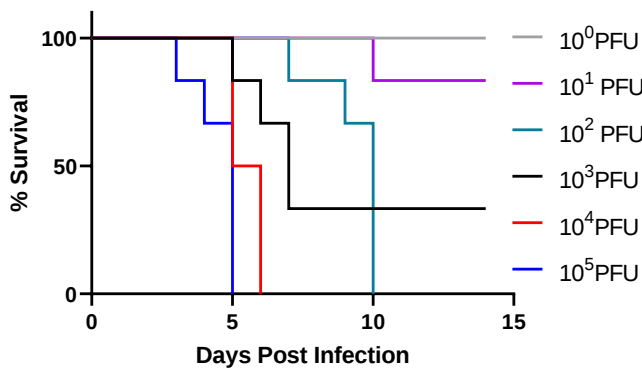
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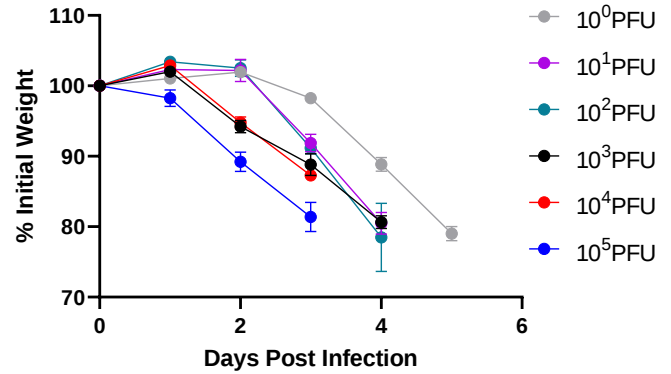
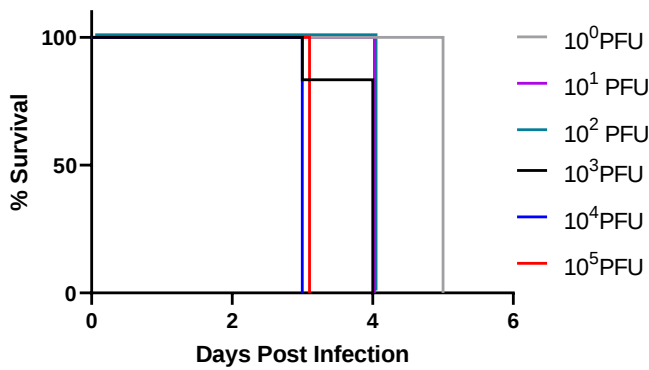
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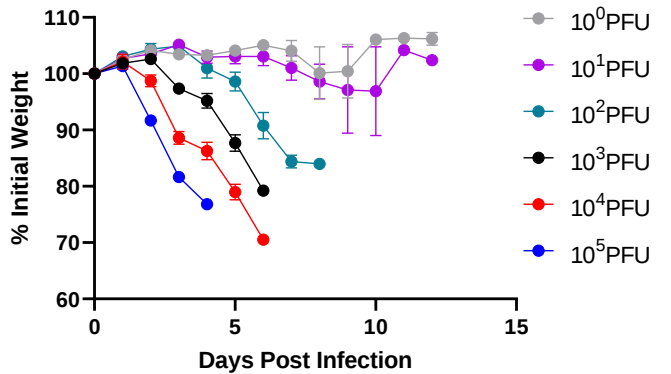
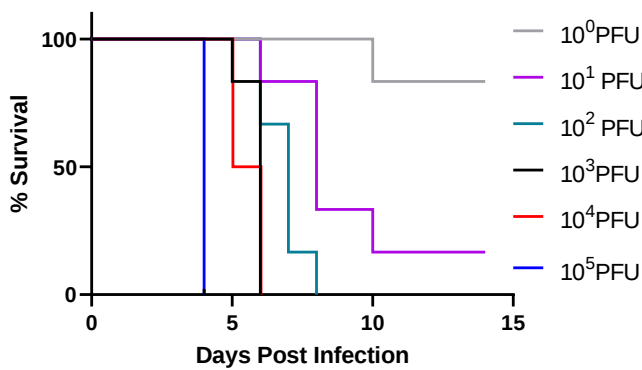
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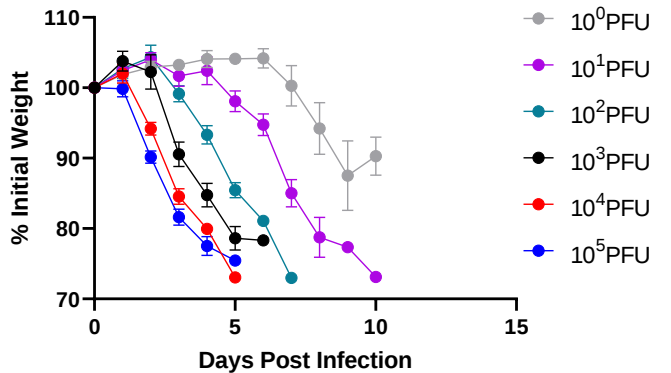
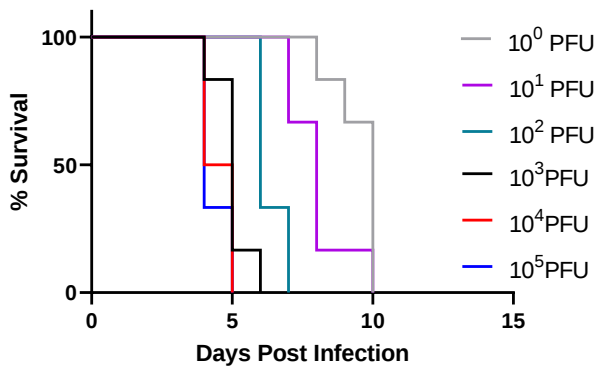
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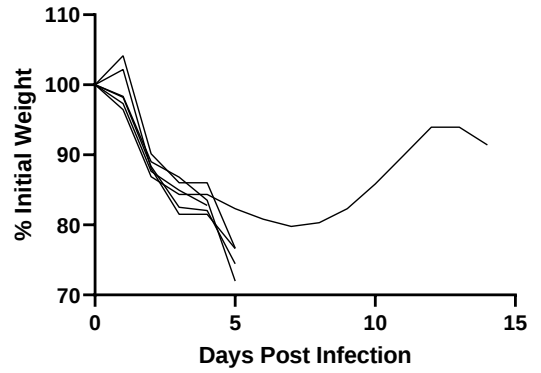
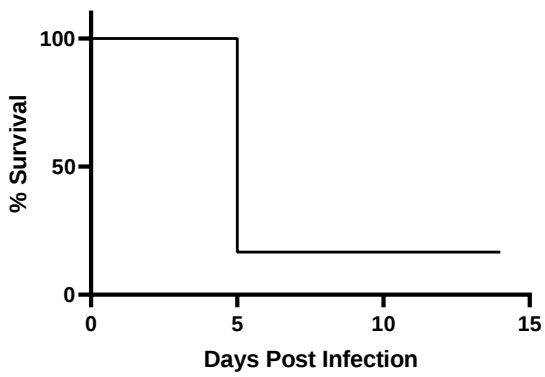
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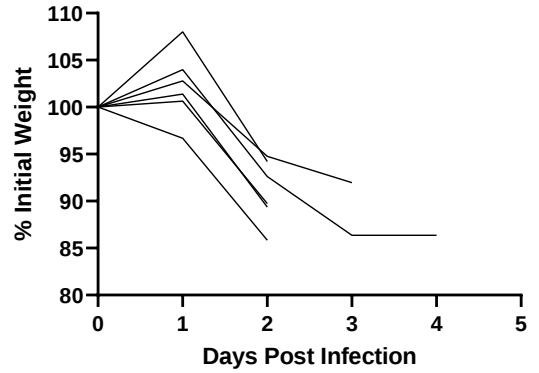
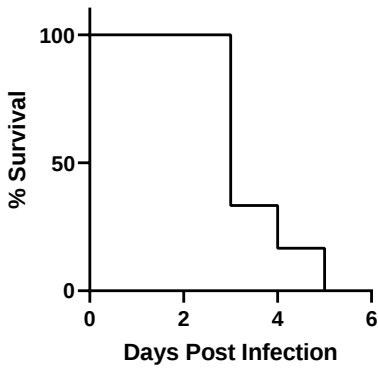
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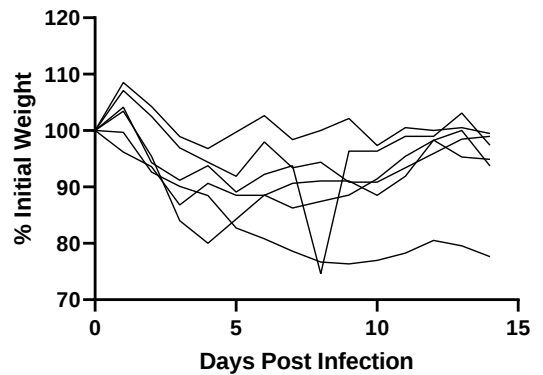
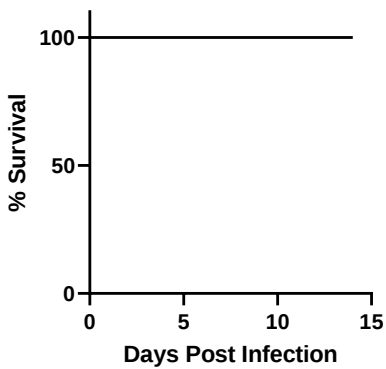
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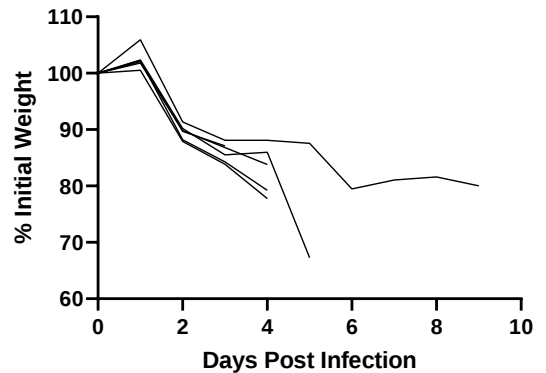
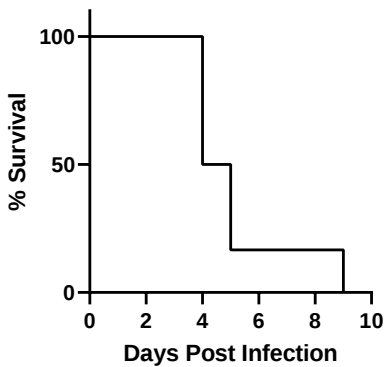
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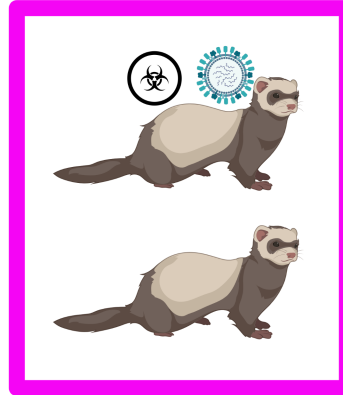
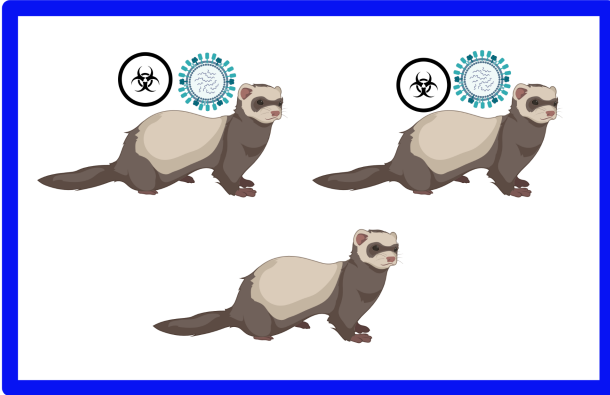


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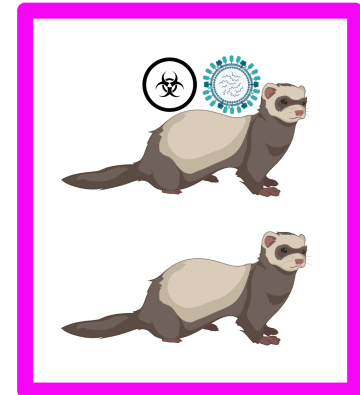
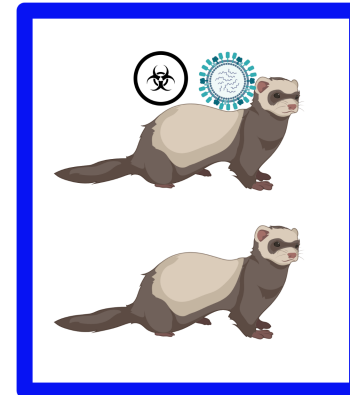


Ferret Transmission Experiment 1

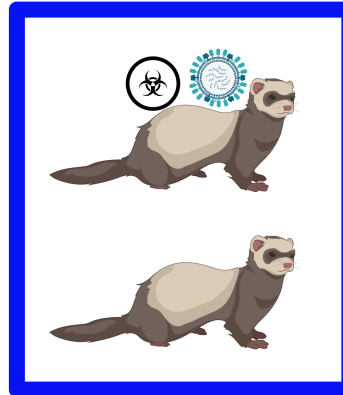
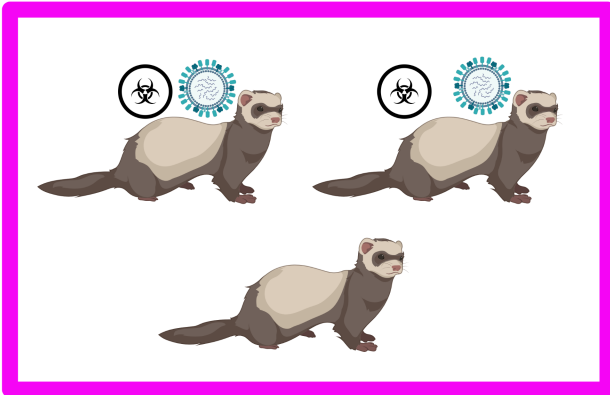
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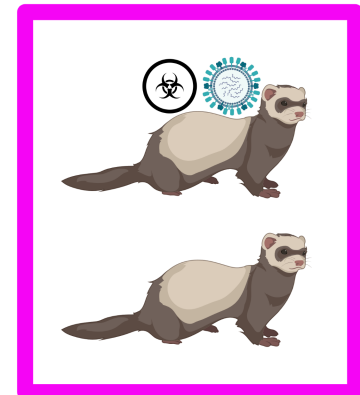
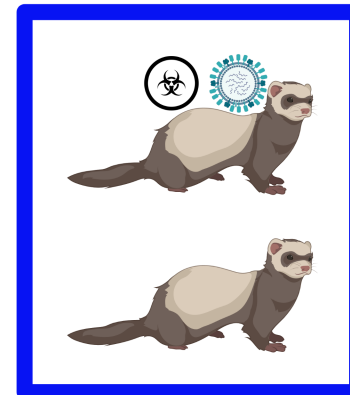
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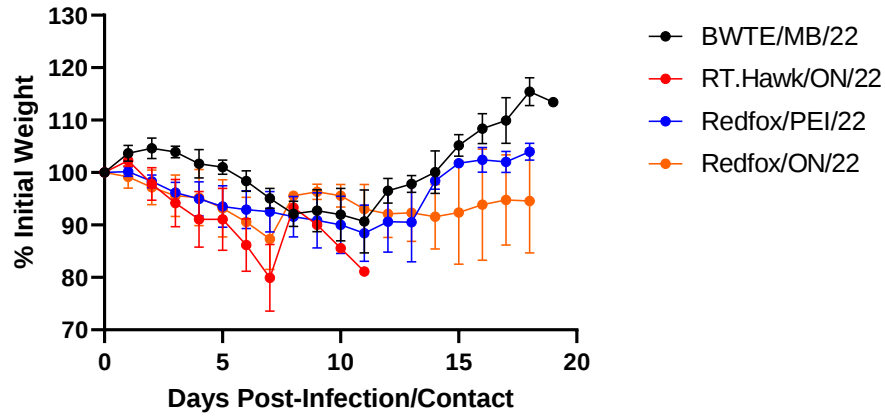
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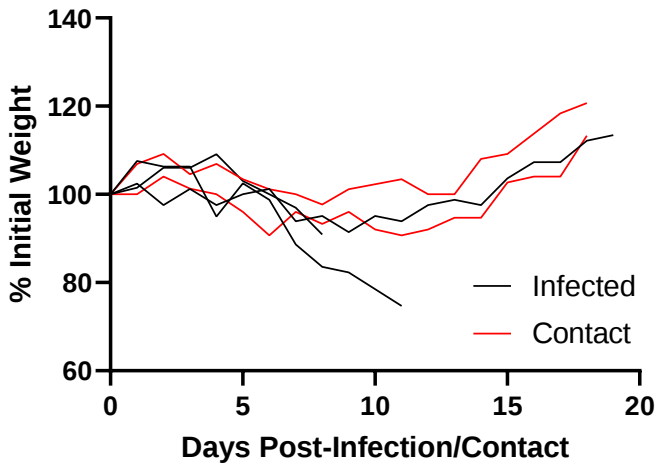
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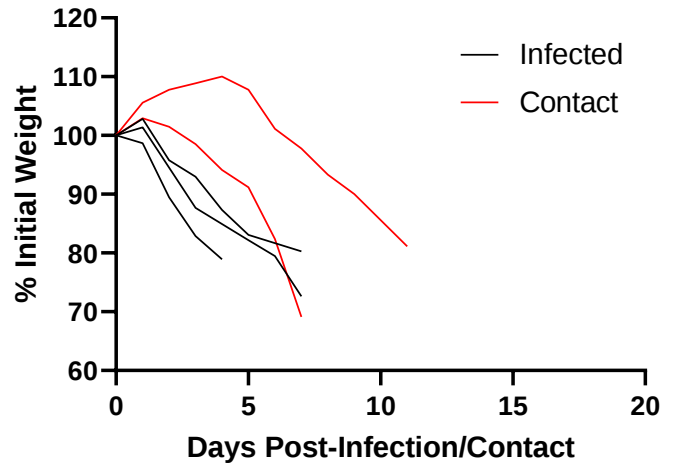
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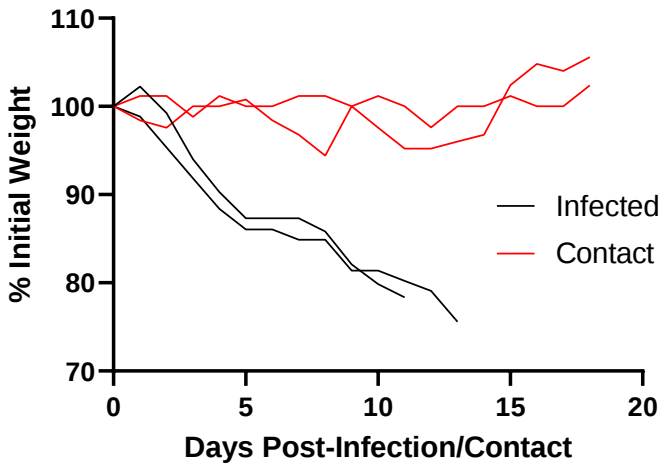
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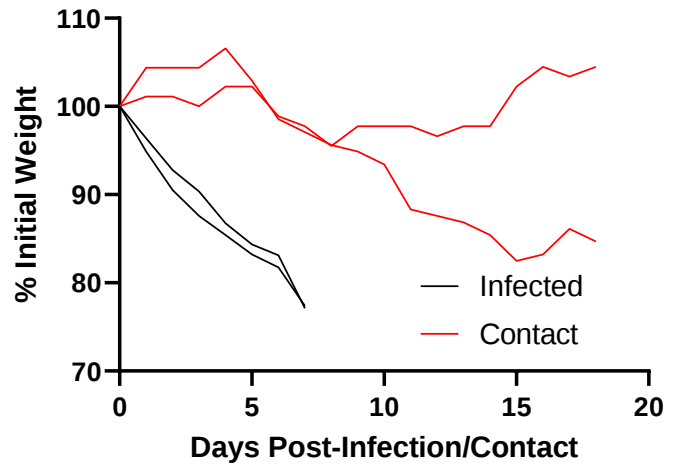
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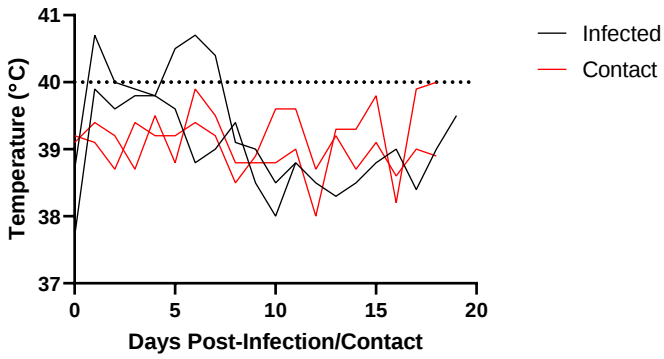


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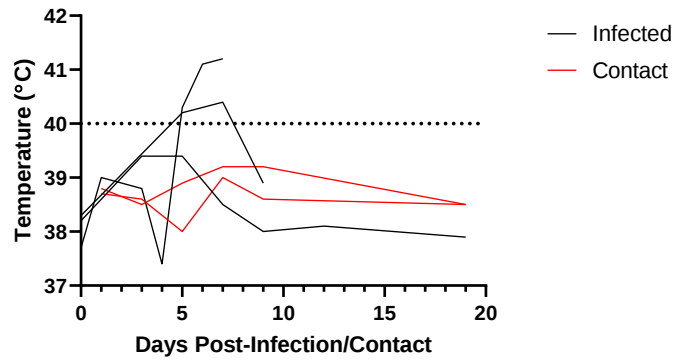


BWTE/MB/22

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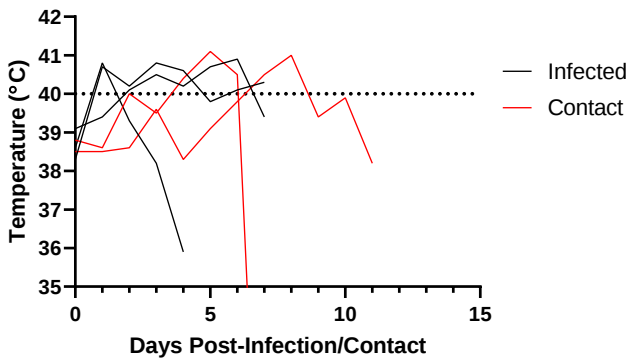


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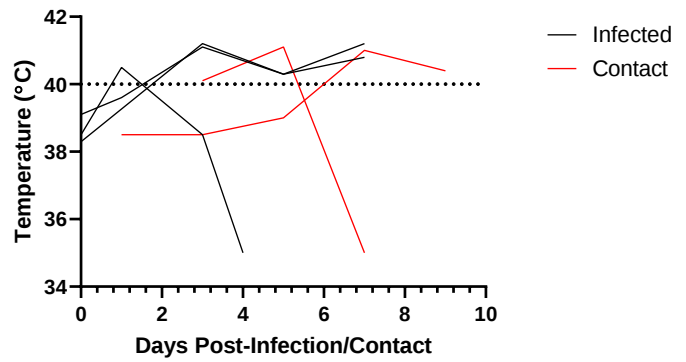


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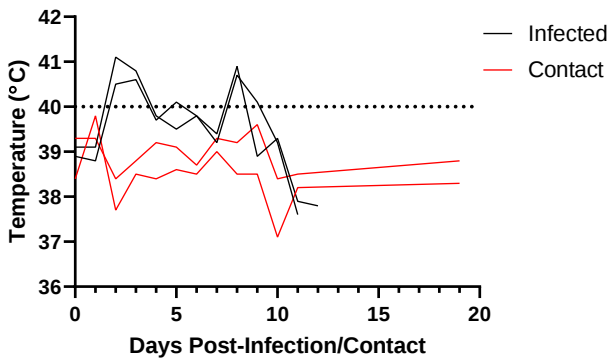


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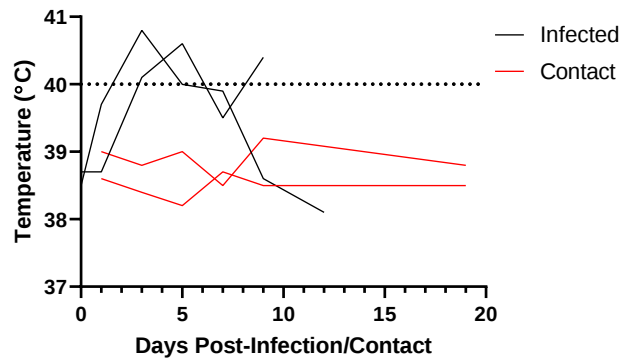


Redfox/PEI/22

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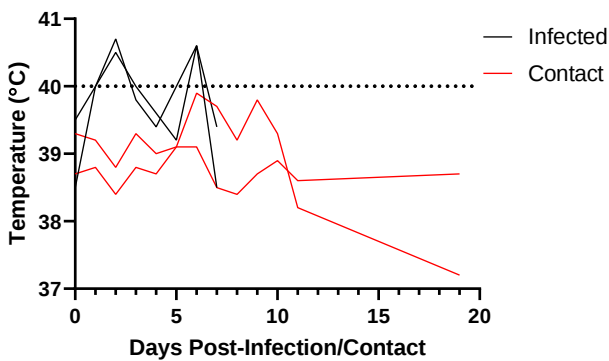


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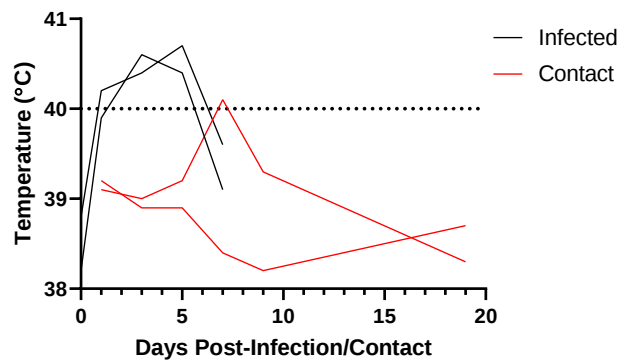


Redfox/ON/22

Scanned Temperature



Rectal Temperature



Ferret Transmission Experiment 2 - RT.Hawk/ON/22

