Serial Adaptation of A/Michigan Influenza Strain and Confirmatory Pilot LD50 Study in BALB/c Mice

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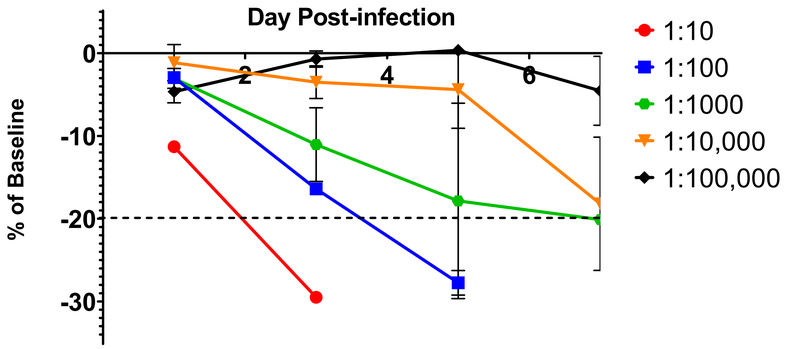
Introduction

Influenza infects millions of people and causes between 291 and 645 thousand mortalities worldwide each year. In the United States alone, there are an estimated 9-36 million illnesses and 12-56 thousand deaths annually; it is estimated that the total economic burden of influenza is approximately $87.1 billion annually. To better support ongoing medical countermeasure efficacy studies against Influenza at MRIGlobal, our team worked to better adapt the A/Michigan flu strain to be used in a mouse model. We wanted to achieve a more productive infection, thus further adaptation of the Influenza strain was required. To this end, we serially passaged infected lung homogenate from infected mice into naïve mice. This allowed the virus to grow to higher titers in mouse lungs and also induce more severe clinical signs to the point of inducing fatal disease. We then further refined the dose of the mouse-adapted, egg-propagated stocks to find a dose that did not cause overtly fatal disease. A more moderate infection facilitates evaluation of medical countermeasures and any improvements they may have on the disease course.

Body Weight Loss



- Animals challenged with 1:10 influenza lost weight very quickly and were euthanized on day 3 post-infection (DPI).
- The 1:100 group, along with one 1:1000 animal were euthanized on 5 DPI due to body weight loss.
- The remaining 1:1000 animal and one 1:10,000 animal reached euthanasia criteria (>20% body weight loss) on 7 DPI.
- The 1:100,000 mice lost an insignificant amount of weight over the 7 day study.

Contact Information

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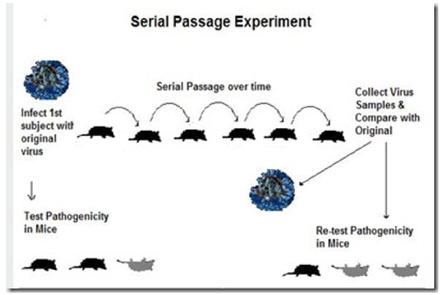
Methods

Serial Mouse adaptation: Groups of 2 female BALB/c mice were intranasally challenged with A/Michigan/45/2015(H1N1) pdm09 stock virus received from International Reagent Resource (IRR). Three days after infection these animals were humanely euthanized and their lungs were collected and homogenized. Tissue debris was pelleted via high speed centrifugation and the virus-rich supernatant was used to infect a subsequent cohort of 2 mice. This was repeated for a total of 4 passages in mice.

The resulting, mouse-adapted influenza was propagated in embryonated chicken eggs to produce a large volume of infectious material for use on future animal challenge studies. In brief, eggs were injected just below the air sac with a syringe containing mouse-adapted influenza. After a 3-4 day incubation period the allantoic fluid containing influenza is collected and aliquoted for future use.



Pilot LD50: 10 female BALB/c mice were used (n=2 per group) were weighed and randomized into 5 separate groups receiving 5 increasing doses of mouse-adapted and egg propagated A/Michigan.

Body weights were collected from all animals on days 1, 3, 5, and 7. Animals that had lost ≥ 20% of their baseline weight were humanely euthanized.

Clinical observations were performed twice daily. Technicians were looking for signs of systemic disease (hunched posture, rough coat, lethargy) and labored breathing.

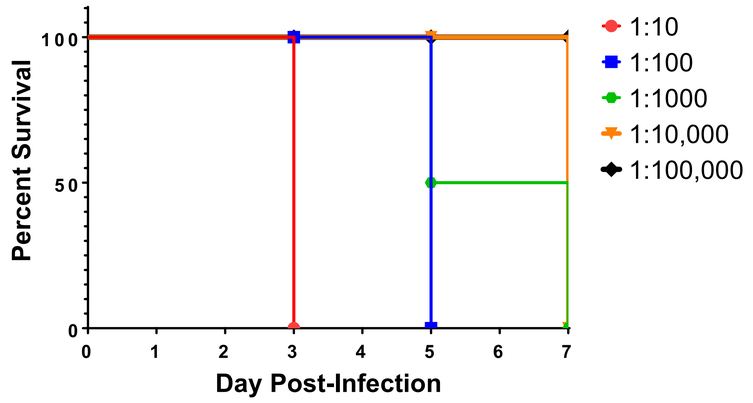
TCID50 assay was performed on Madin-Darby Canine Kidney cells to assess the infectious titer of the generated influenza stocks. Titers increased steadily from $1x10^3$ $TCID_{50}$/mL during mouse passage 3 to $3.16x10^5$ $TCID_{50}$/mL for the final egg-propagated master stock.

Clinical Signs

Group	Clinical Sign	Day First Noted	Day Euthanized	Max Duration
1:10	Labored Breathing	1	3	3
	Systemic	3		1
1:100	Labored Breathing	2	5	3
	Systemic	3		1
1:1,000	Labored Breathing	3	5 and 7	4
	Systemic	6		1
1:10,000	Labored Breathing	3	7	4
	Systemic	6		1
1:100,000	Labored Breathing	3	7	4
	Systemic	6		1

- The 1:10 and 1:100 groups had peracute onset of clinical signs and very rapid deterioration which (along with body weight loss) led to their euthanasia on days 3 and 5, respectively.
- All other groups started displaying signs on day 3, which gradually deteriorated in the 1:1000 and 1:10,000 groups
- The 1:100,000 group never displayed severe clinical signs over 7 days.

Survival



Conclusions

1. **Four serial passages A/Michigan/45/2015(H1N1) pdm09 in female BALB/c Mice transformed the original virus: rather than causing mild/asymptomatic disease it induced a highly fatal pneumonia**
2. **The LD_{50} for the newly generated, mouse-adapted and egg-propagated A/Michigan flu strain is estimated to be approximately $3.16x10^3$ $TCID_{50}$/mL**
3. **Body weight loss as a percentage of a pre-challenge baseline is a great objective measure to track systemic disease and predict outcomes**