# THE EFFECTS OF AGE AT INFECTION AND GENDER ON THE PATHOGENESIS OF THEILER'S VIRUS INDUCED DISEASE – A MODEL OF HUMAN MULTIPLE SCLEROSIS

A Senior Honors Thesis

by

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# ABSTRACT

The Effects of Age at Infection and Gender on the Pathogenesis of Theiler's Virus Induced Disease – a Model of Human Multiple Sclerosis (April 2008)

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Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) affecting 1 in 2000 of the US population. Susceptibility to MS is influenced by environmental, gender, genetic and pathogenic factors. For instance, there is a higher incidence of MS in women than men (2:1) and that there is a sudden onset of the disease between the ages of 15 and 50, suggesting that gender, age and puberty alter the susceptibility to the disease. The etiology of MS is not known, and although viral infection is suspected to be an initiating event.

Theiler's murine encephalomyelitis virus (TMEV) infection in SJL mice causes a biphasic disease in which the chronic phase causes an inflammatory demyelinating disease of the CNS which closely resembles MS. In the current study we utilize TMEV to investigate the influence of both age and gender on disease progression in this viral model of human multiple sclerosis. Here, we tested the hypothesis that age and gender significantly affect the pathogenesis of Theiler's virus-induced disease in which we showed that males displayed worse symptoms than females at later ages of inoculation.

# ACKNOWLEDGEMENTS

First of all, I'd like to thank Andrew Steelman and Colin Young, who spent many hours teaching me how to perform all the techniques needed to complete my research, keeping me on schedule, keeping things interesting and filling me with large quantities of knowledge - whether that knowledge be scientific or even useful for that matter. I'd like to thank Katherine Kuban, another one of the Fellows for this year, who listened to all my complaints, kept me informed on what I was supposed to be doing and called me before Fellows meetings which reminded me that I had one at all. I'd like to thank Dr. Welsh for this opportunity to participate in Research Fellows, especially since I am supposed to be a sophomore and had no prior knowledge of biochemistry, immunology, virology or immunology. She took a huge chance in my ability to learn how to do everything needed and understand the implications of my work. What is even more surprising than her taking that first chance is that she let me run with my experiment as freely as any of her graduate students, allowing me to learn so much more than I would have otherwise, particularly in regard to the planning of an experiment. Thank you for letting me pop into your office whenever I wanted to run through things and whine as well as for being lenient and upbeat even if I had made a huge mistake. Lastly, I'd like to thank the Office of Honors Programs for the opportunity to participate in this great research experience because without their funding and the support it may never have happened nor would it have been so successful.

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# I INTRODUCTION [1]

In order to understand the pathogenesis and treatment of human diseases, it is important to develop animal models. One of the best models for multiple sclerosis (MS) is Theiler's virus infection in mice. Experiments on Theiler's murine encephalomyelitis virus (TMEV) have been performed since its discovery by Max Theiler in 1934 and his continued research in 1937 (Theiler, 1934; Theiler, 1937). The etiology of MS is not known, although viral infection is suspected to be an initiating event. Due to epidemiological studies of MS (Johnson, 1975; McFarlin and McFarland, 1982), "the identification of viral antigens and virus-specific antibodies" (Soldan et al., 1997; Fuller et al., 2007) and the "detection of HHV-6 DNA…as a marker of active viral infection" (Soldan et al., 1997), it is thought that viral agents help create the initial tissue damage that leads to autoimmunity and multiple sclerosis. Comparably, chronic TMEV causes a similar inflammation of central nervous system (CNS) tissues, therefore providing an excellent model for MS (Daniels et al., 1952; Lipton, 1975; Dal Canto et al., 1996; Oleszak et al., 2004; Fuller et al., 2007).

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) affecting 1 in 2000 of the US population. Susceptibility to MS is influenced by environmental, gender, genetic and pathogenic factors. For instance, there is a higher incidence of MS in women than men (2:1) and that there is a sudden onset of the disease between the ages of 15 and 50, suggesting that gender, age and puberty alter the susceptibility to the disease. Sex-dependent effects

[1] This thesis follows the style and format of *Journal of Neuroimmunology.*

on TMEV have been researched, however, the effects of age at infection and gender on the pathogenesis of TVID have not been studied. CD1 mice are highly susceptible to TVID at three weeks of age and their susceptibility declines with age after this (Steiner et al., 1984). More importantly, this study is limited to examining only one and three weeks of age at infection so that the trend before, during and after puberty is not investigated and gender effects are not isolated. Additionally, studies with age related susceptibility to TVID have not been conducted in SJL/J or B10Q mice which is especially important since SJL/J mice develop the highest incidence of MS-like disease following infection with TMEV. If MS is actually caused by a virus, it would be critical to study the disease in the most susceptible model so that it may be appropriate comparison to the most susceptible humans.

In MS, infection with the putative MS infectious agent is thought to occur before the age of puberty (Fischman, 1981). Interestingly, this infectious time period coincides with the onset of puberty in mice as shown by prior studies (Steiner et al., 1984). The physiological changes that occur during puberty may affect susceptibility to TMEV (Fischman, 1981). The experiment being conducted in the current study should clarify whether age at infection and gender are the factors that cause this deviation.

# II METHODS

Over the years, animal models have become the standard for much of scientific research. In regard to a Theiler's virus model or any model for MS in general, some sort of quantitative and qualitative tests must be employed to determine severity of the disease. As shown by review of literature the following tests are largely conducted by most scientists researching TVID, only differing on small details.

**Subjects:** SJL/J mice were used because their high susceptibility to TVID. Breeding SJL/J pairs were purchased from Harlan Laboratories (Indianapolis, IL) and bred in the Comparative Animal Medicine facility at Texas A&M University. Four groups (infected at 1, 2 3 and 4 weeks of age) of approximately 25 mice were randomly assigned using protocols previously described (Campbell et al., 2001; Sieve et al., 2004). Each group consisted of 15 infected (I) and 10 control (C) – with equal numbers of male (M), half female (F) mice. Subgroups taking into account all the previously mentioned variables were labeled as such: 1/F/C, 1/M/C, 1/FM/I (1/F/I + 1/M/I), 2/F/C, 2/M/C, 2/F/I, 2/M/I, 3/F/C, 3/M/C, 3/F/I, 3/M/I and 4/M/I.

**Infection:** The BeAn strain of Theiler's virus was propagated and amplified in BHK-21 cells. The culture supernatant containing infectious virus was aliquoted and stored at -80°C before use (Welsh et al., 1987; Sieve et al., 2004). Mice were anesthetized with isoflurane inhalation and either injected intracerebrally with $5.0 \times 10^5$ plaque forming units of the BeAn strain of TMEV (provided by Dr. H. L. Lipton, Department of Neurology, Northwestern University) in 20 µl of infection media or 20 µl of sterile phosphate buffered saline (PBS).

**Physiological Indices:** Clinical signs of infection and body mass were obtained weekly through the duration of the experiment, beginning at infection and lasting until the time of sacrifice (Day 84 p.i.). The mice were scored on behavioral indications of encephalitic-like symptoms during the disease's acute phase as follows: "0=no behavioral signs of illness, 1=ruffled fur, 2=ruffled fur and slightly hunched posture, 3=ruffled fur, very hunched posture, and lethargic, 4=moribund" (Campbell et al., 2001; Sieve et al., 2004). The mice are observed and scored based on behavior in the disease's chronic phase: "0=no behavioral impairment, 1=weakness in hind limbs, 2=slightly wobbly gait, 3=definitely wobbly gait, 4=very wobbly gait, hunched posture, and loss of righting reflex, 5=all of the previously mentioned symptoms and incontinence, 6=moribund" (Borrow et al., 1998; Sieve et al., 2004).

**Rotarod Analysis:** All groups were tested for decreased performance on rotarod starting at week 6 p.i. using an Ugo Basile Model 7650 accelerating rotarod treadmill (McGavern et. al., 1999; Zoecklein et. al., 2003). Prior to beginning the rota-rod analysis mice were familiarized with three untimed sessions on rota-rod in order to remove errors in times due to the mice being unaccustomed to the procedure. Each mouse completed two sessions on each test date which were averaged and recorded for each session. Times were taken biweekly.

**Tail Bleeds**: The mice were bled via the tail vein once every three weeks. The mice were placed in a recovery cage until all the mice were bled and returned to their home cage. They were not disturbed until the next week's weighing and scoring to reduce the effects of stress on the experiment.

**Sacrifice:** At day 84 p.i., all groups were anesthetized with pentabarbitone. Approximately half of the samples from each group were used for histological analysis whereas the other half were stored for viral recovery to run antibody assays to determine TMEV levels.

**Viral Purification:** Briefly, TMEV was used to infect L2 cells at an MOI of 0.1. Approximately 48 hours the cells were frozen and thawed 3x. Next, the cell debris was cleared by centrifugation at 3,000 x g for 20 minutes. The supernatant was collected and virus pelleted by ultracentrifugation at 80,000 x g for 3 hours. The purified virus was resuspended in 0.1M sodium phosphate buffer. The virus was determined to be at ≥70% purity by SDS-PAGE.

**Antibody Levels to TMEV:** The serum Ig levels of mice at time of sacrifice (Day 84 p.i.) was determined by ELISAs using virus that was purified as described previously and as done in previous studies (Young et al., 1983; Dolimbek et al., 2002; Steelman et. al.). Viral sample was diluted to 1.0 μg/ml in pH 9.6, carbonate buffer (1.589 g $Na_2CO_3$; 2.941 g $NaHCO_3$; 0.191 g $MgCl_2$; fill to 1L with RO-$H_2O$). High protein binding Costar 3590 96-well plates were coated with 0.1 mL of virus/well and incubated at 4ºC for 48 hours. The plates were then washed 3X by filling each well with wash buffer (500 mL 1X PBS; 0.250 mL Tween-20) using a squirt bottle and then rinsed again using a separate squirt bottle containing RO- $H_2O$. Plates were then blocked with SuperBlock buffer (containing no BSA) according to manufacturer's instructions (Pierce). Serum samples were then diluted in pH 7.2, complete assay buffer consisting of 99 mL Assay Buffer A (11.4 g Tris-HCl; 3.32 g Tris-Base; 8.7 g NaCl; fill to 1 L with

RO-H2O) and 1 mL Assay Buffer B (95 mL RO-H2O; 1.0 g Non-Fat Dry Milk; 0.5 mL Tween-20) to achieve a starting dilution of 1/100. 0.2 mL of diluted samples were across row 1 and 0.2 mL of assay buffer was added only to column 12 to serve as background for subtraction. 0.1 mL of assay buffer was added to remaining wells and samples were serially diluted down each column by transferring 0.1 mL volumes between the wells. Plates were then incubated at room temperature for 90 minutes. The plates were then washed 3X by filling each well with wash buffer (500 mL 1X PBS; 0.250 mL Tween-20) using a squirt bottle and then rinsed again using a separate squirt bottle containing RO- $H_2O$. Secondary antibody was diluted 1/500 in complete assay buffer and 0.1 mL was added to each well. Plates were then incubated at room temperature for 60 minutes. The plates were then washed 3X by filling each well with wash buffer (500 mL 1X PBS; 0.250 mL Tween-20) using a squirt bottle and then rinsed again using a separate squirt bottle containing RO- $H_2O$. Reaction was then developed in the dark using 0.1 mL OPD substrate (Sigma) according to manufacturer's instructions for 10 minutes and stopped by adding 0.05 mL of 2.0 M sulfuric acid. OD was determined at 490 nm using a FLUOstar Optima (BMG Inc., Offenburg, Germany) and background was subtracted from each column.

**Histology:** At termination mice were euthanized with pentabarbitone and perfused via the left ventricle with PBS followed by 10% formalin. The brains were removed and dehydrated and embedded in paraffin by the Histology Laboratory, Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University. The brains were sectioned coronally into

four blocks**.** Five micron sections from each block were routinely stained with hematoxylin and eosin (H&E) for microscopic examination.

**Statistical Analyses:** Using repeated measure analyses of variance (ANOVA), all data was tested at 0.05 level of significance.

This study seeks to add to the current knowledge on Theiler's virus as an animal model for Multiple Sclerosis, by clarifying the effect that age of infection has on TVID in each gender. It does not seek to change any previous methods, but to employ them in order to expand the knowledge base in order to better understand the pathogenesis of TVID and to hopefully apply that knowledge to better understand multiple sclerosis.

# III DATA AND RESULTS

**Effects of Infection at 1 Week of Age:** Most mice infected at one week of age were moribund while all were showing severe symptoms. Figure 1 (p.9) reflects survival until time of necessary sacrifice (6-10 days p.i.). Mice were not yet sexed, so no gender results were obtained. Table 1 shows that this observation is in general accordance with several studies results (Steiner; Theiler, 1934; Theiler, 1937). At one week of age the immune system of the mouse is not developed and therefore they are not able to mount a sufficient immune response to the virus. If trying to compare these results to MS, paralysis of the limbs and/or death in young children would be an indicative sign of a possibly undiagnosed MS case.

***Table 1 - Effects of Infection at 1 Week of Age:***

| | |
|---|---|
| Average Score at Sacrifice | 3.23 |

| **Symptoms** | **Number of Mice** |
|---|---|
| Bilateral Front Limb Paralysis | 1 of 15 |
| Bilateral Rear Limb Paralysis | 1 of 15 |
| Unilateral (Left) Rear Limb Paralysis | 1 of 15 |
| Unilateral (Right) Rear Limb Paralysis | 3 of 15 |

***Figure 1 - Effects of Infection at 1 Week of Age:***

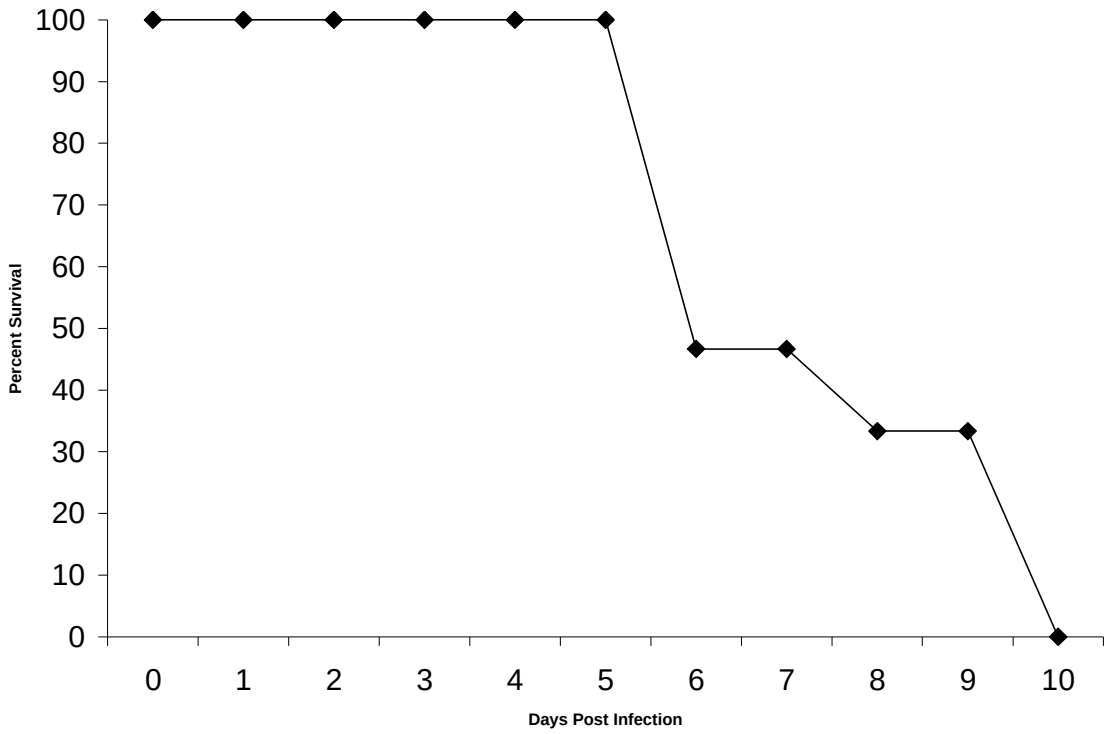
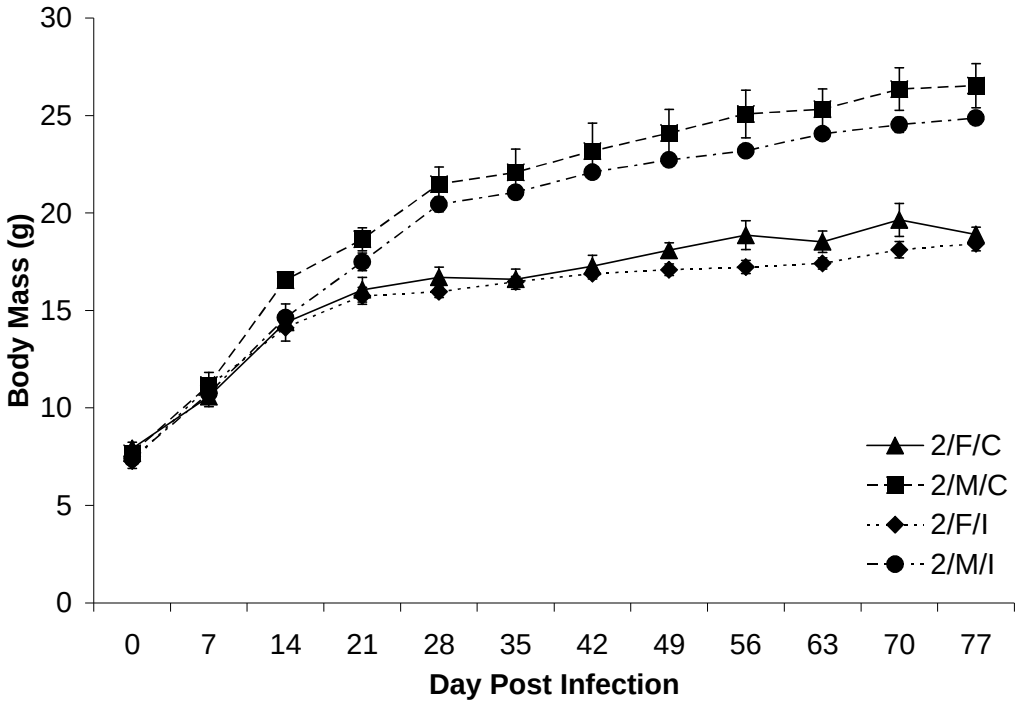


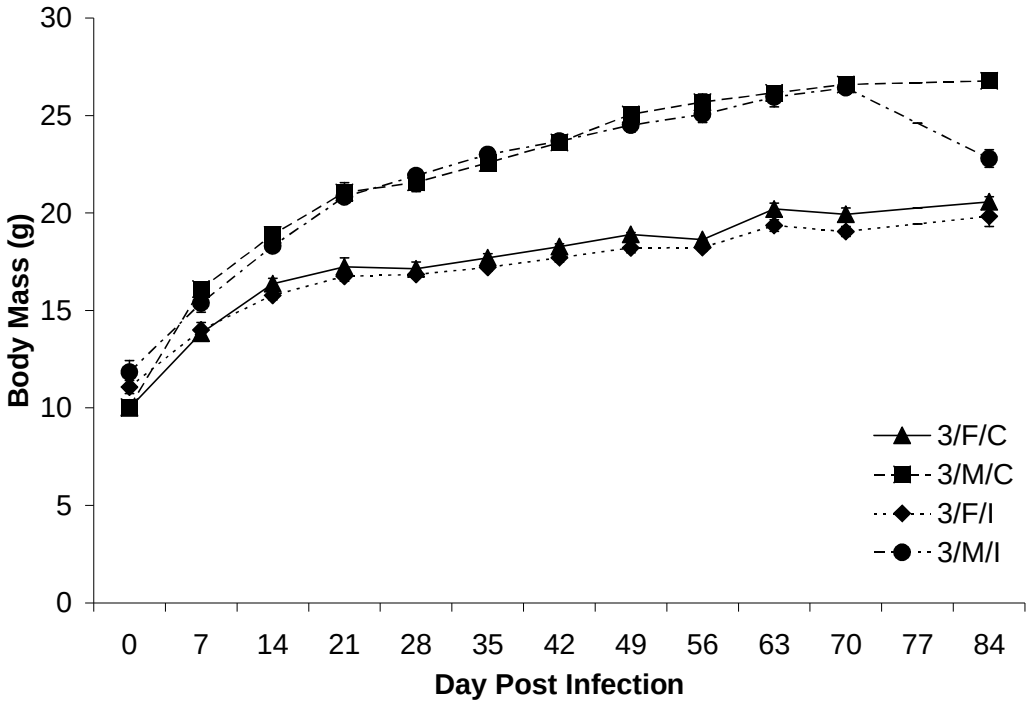
***Figure 2 - Effects of Infection at 2 Weeks of Age on Mass:***




**Legend: 2** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 2 Weeks of Age on Mass:** During the experiment, the body mass of infected mice did not differ statistically from controls during the acute (<49 days p.i) or chronic (≥49 p.i.) phases (all ps>0.05) and males maintained their normally heavier body mass than females (all ps<0.05).

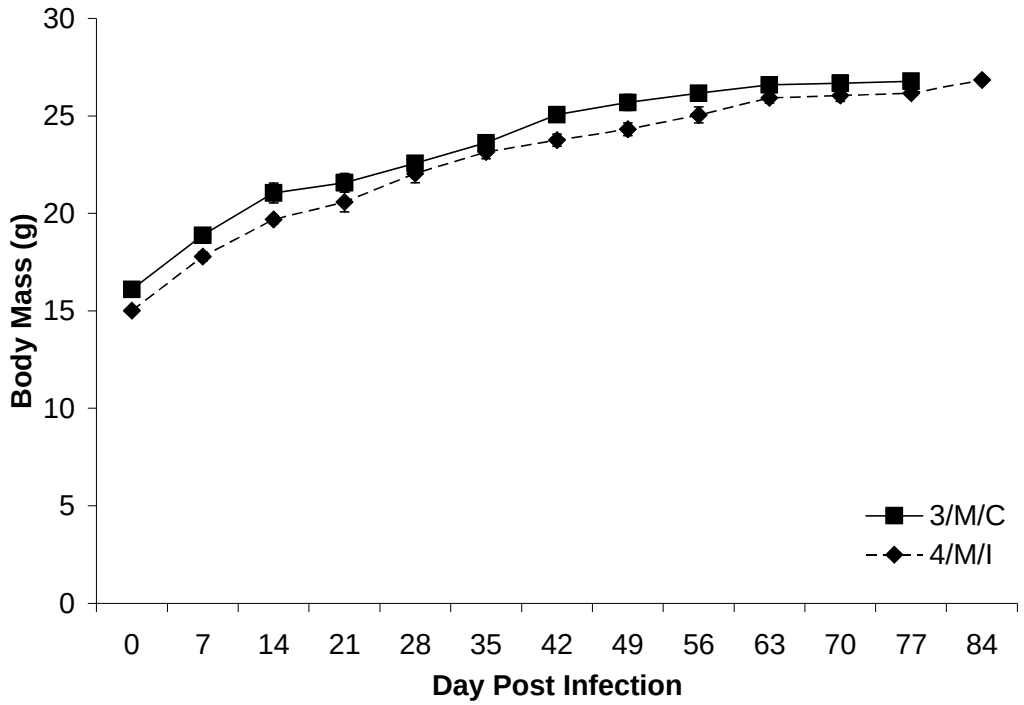
***Figure 3 - Effects of Infection at 3 Weeks of Age on Mass:***




**Legend: 3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 3 Weeks of Age on Mass:** During the experiment, the body mass of infected mice did not differ statistically from controls during the acute (<49 days p.i) or chronic (≥49 p.i.) phases (all ps>0.05) and males maintained their normally heavier body mass than females (all ps<0.05).

***Figure 4 - Effects of Infection at 4 Weeks of Age on Mass:***

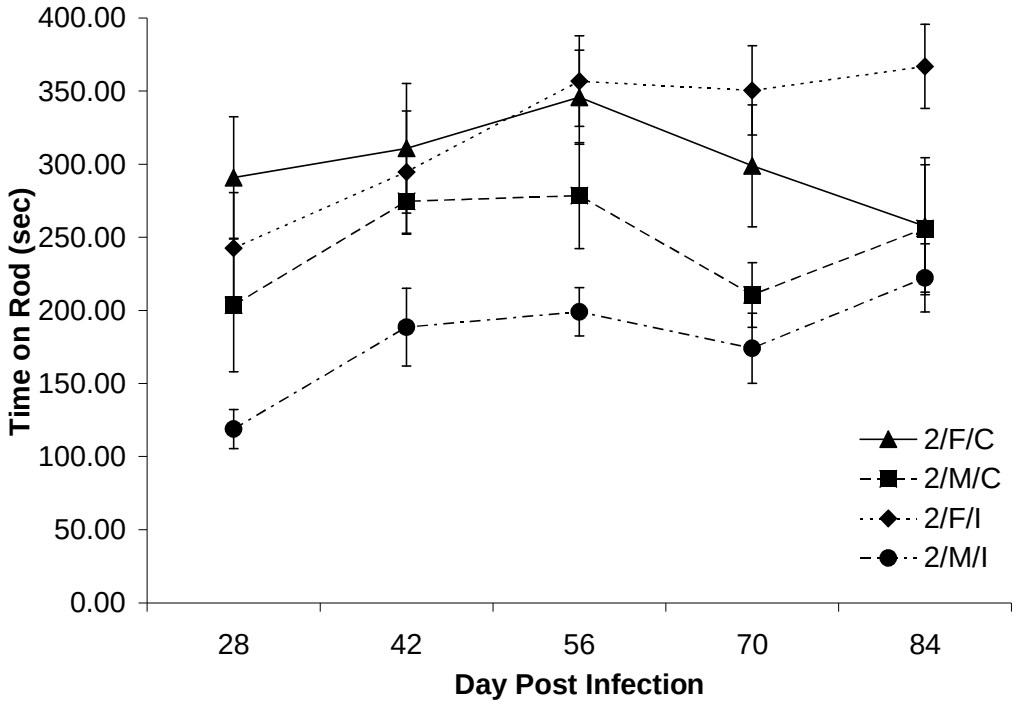



**Legend: 3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 4 Weeks of Age on Mass:** During the experiment, the body mass of infected mice did not differ statistically from controls during the acute (<49 days p.i) phase or chronic (≥49 p.i.) phase (ps>0.05).

**Body Mass Summary:** Infection did not cause mice to differ statistically from their control counterparts during the acute (<49 days p.i) or chronic (≥49 p.i.) phases and males maintained their normally heavier body mass than females. Due to the large difference in the weights of the males compared to females, the males remain much heavier than the females.

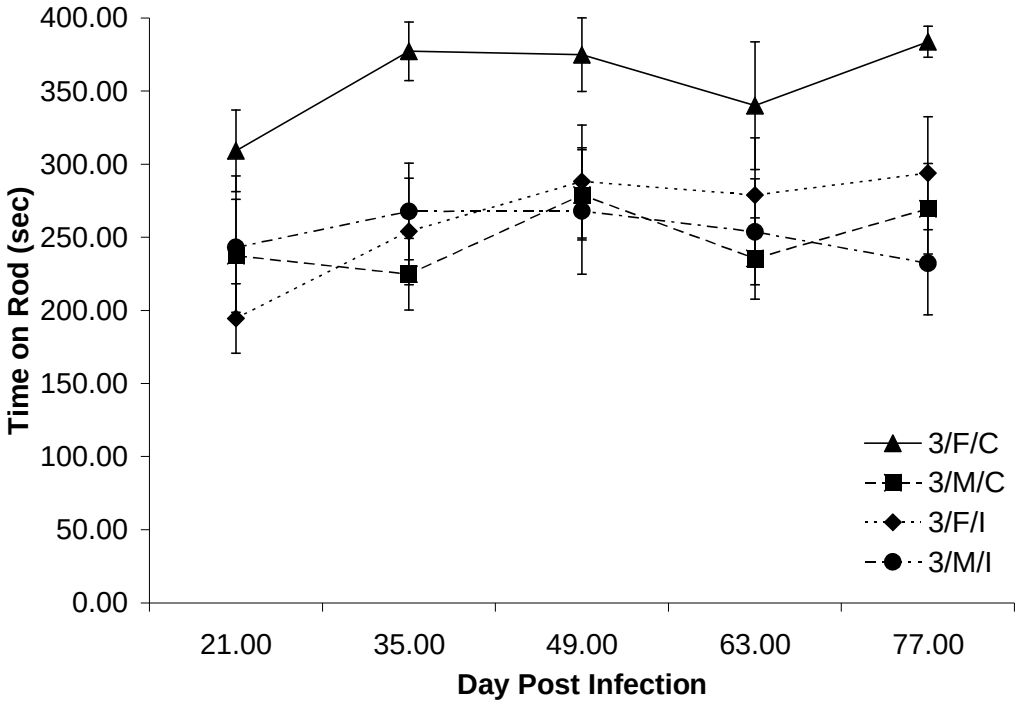
***Figure 5 - Effects of Infection at 2 Weeks of Age on Rotarod Time:***




**Legend: 2** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 2 Weeks of Age on Rotarod Time:** Only the males infected at 2 weeks of age differed from their control counterparts (p=0.0173). Males infected at two weeks of age had lower times than females infected at two weeks of age (p=0.0001).

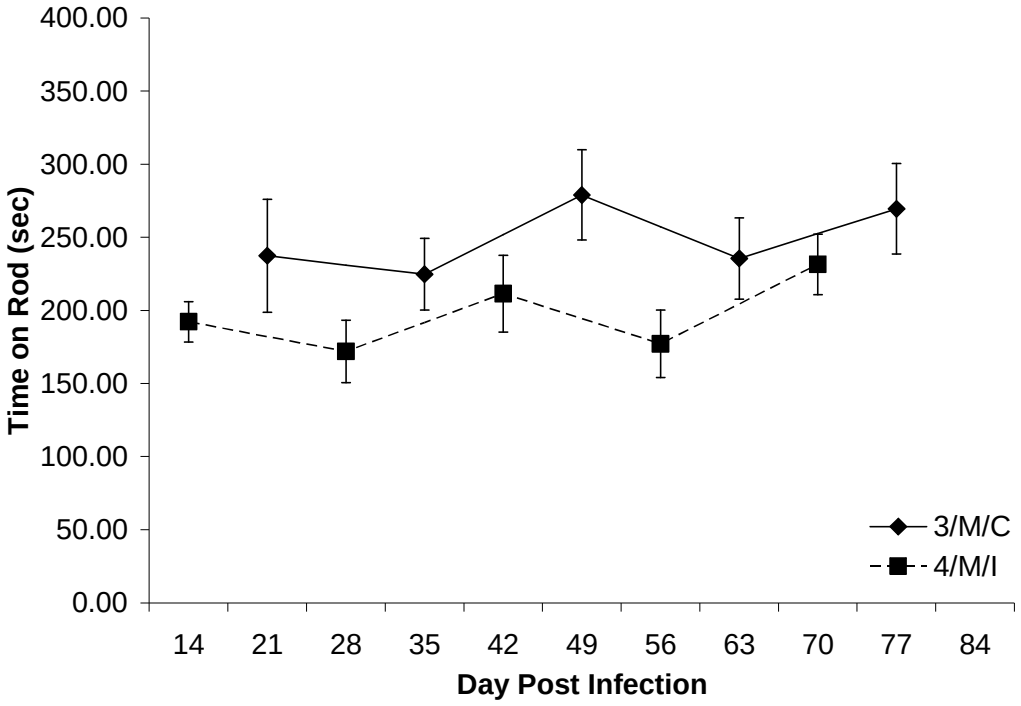
***Figure 6 - Effects of Infection at 3 Weeks of Age on Rotarod Time:***




**Legend: 3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 3 Weeks of Age on Rotarod Time:** Both the male and female groups infected at three weeks of age did not differ from their control counterparts or from one another (ps>0.05)

***Figure 7 - Effects of Infection at 4 Weeks of Age on Rotarod Time:***



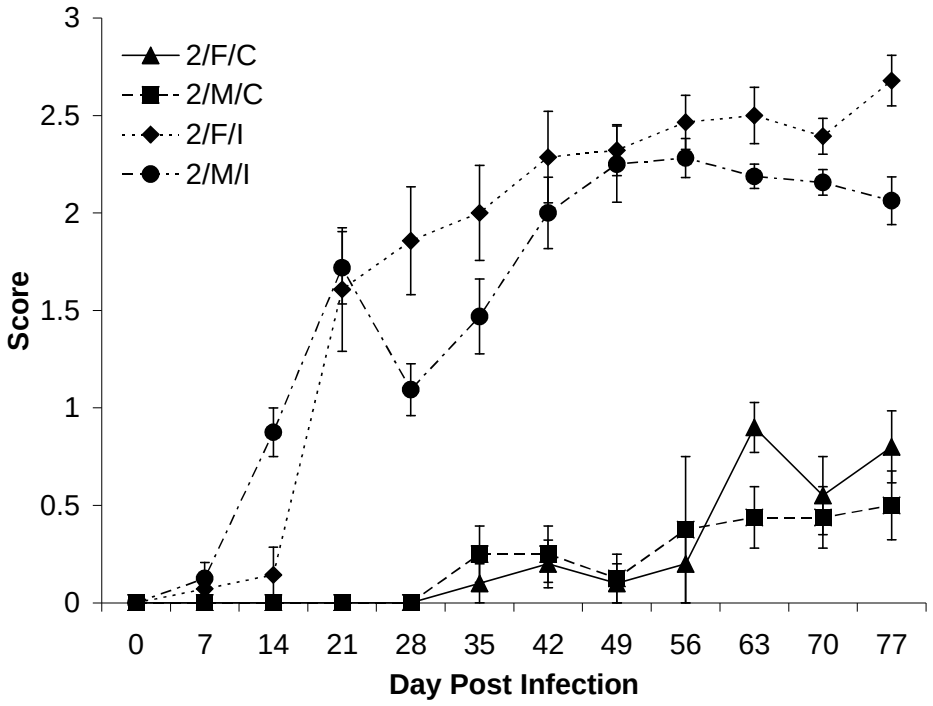

**Legend: 3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 4 Weeks of Age on Rotarod Time:** Males infected at four weeks of age differed from their control counterparts (p=0.0101). As previously explained in the method section, there are no females to discuss at this age of infection.

**Rotarod Summary:** Rotarod analysis has been used with success in many studies as a measure for neurological deficits, however in this experiment it seems that only the males infected at two and four weeks of age actually differed from their control counterparts, meaning that infection generally created no change in rotarod time.

However, when comparing males and females at each age of infection only the males infected at two weeks of age had lower times than the females infected at two weeks of age ($p=0.0001$). Nevertheless, it is still not prudent to use this as a conclusion from the data since the females infected at two weeks of age did not even differ from statistically from their controls. It does not appear that rotarod was indicative of disease in this study.

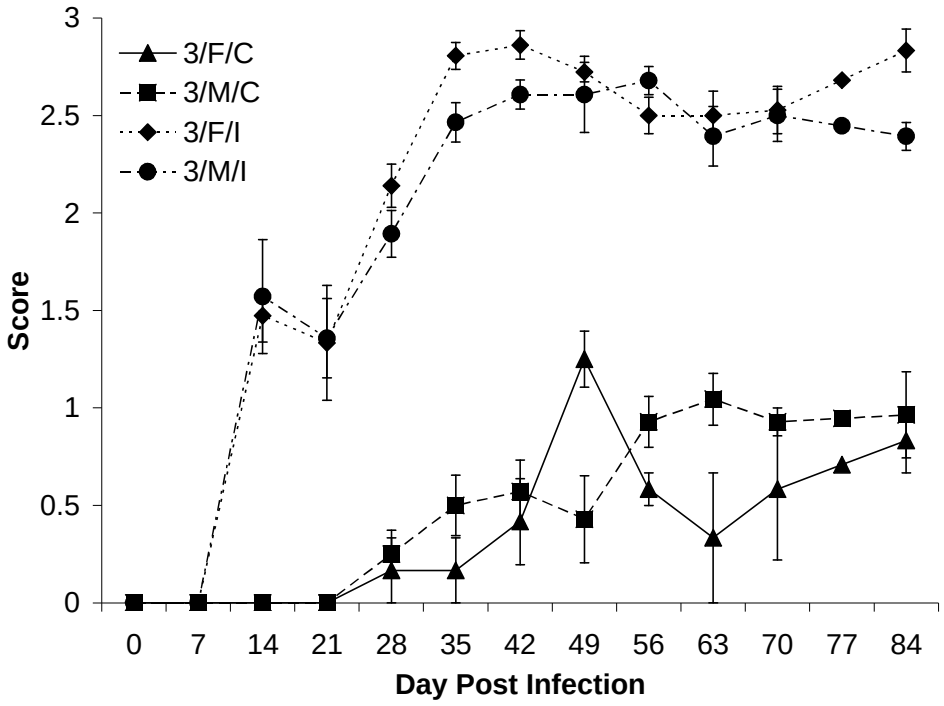
***Figure 8 - Effects of Infection at 2 Weeks of Age on Clinical Score:***




**Legend: 2** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 2 Weeks of Age on Clinical Score:** Both males and females infected at two weeks of age scored higher than their corresponding controls (ps<0.05). Infected males had lower clinical scores than infected females in the chronic phase (p=0.0065).

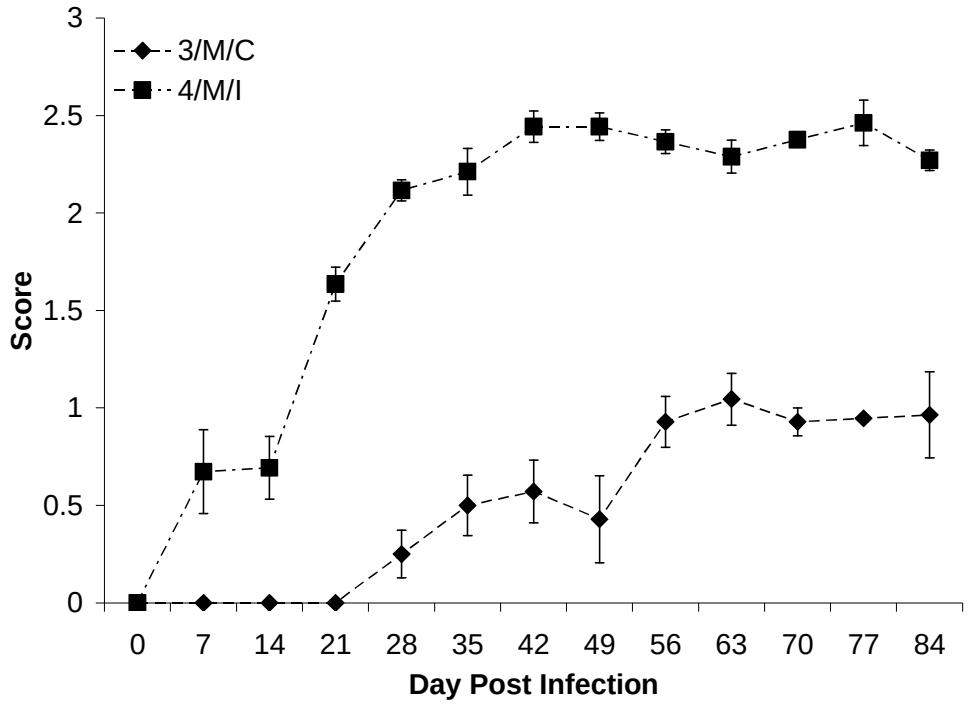
***Figure 9 - Effects of Infection at 3 Weeks of Age on Clinical Score:***




**Legend: 3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 3 Weeks of Age on Clinical Score:** Infected males and females infected at three weeks of age scored higher than their corresponding controls ($ps<0.05$). There was no statistical difference in the scores of infected males and females during the acute and chronic phases ($ps>0.05$).

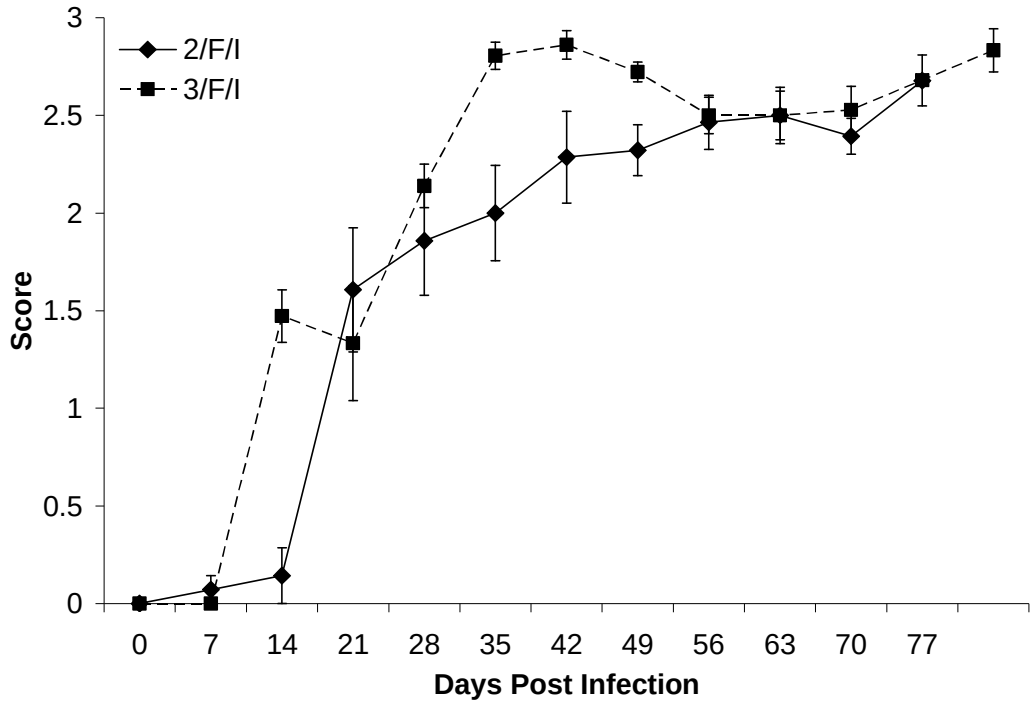
***Figure 10 - Effects of Infection at 4 Weeks of Age on Clinical Score:***




**Legend: 3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 4 Weeks of Age on Clinical Score:** Males infected at four weeks of age scored higher than their corresponding control group in both the acute and chronic phases (p=0.0001).

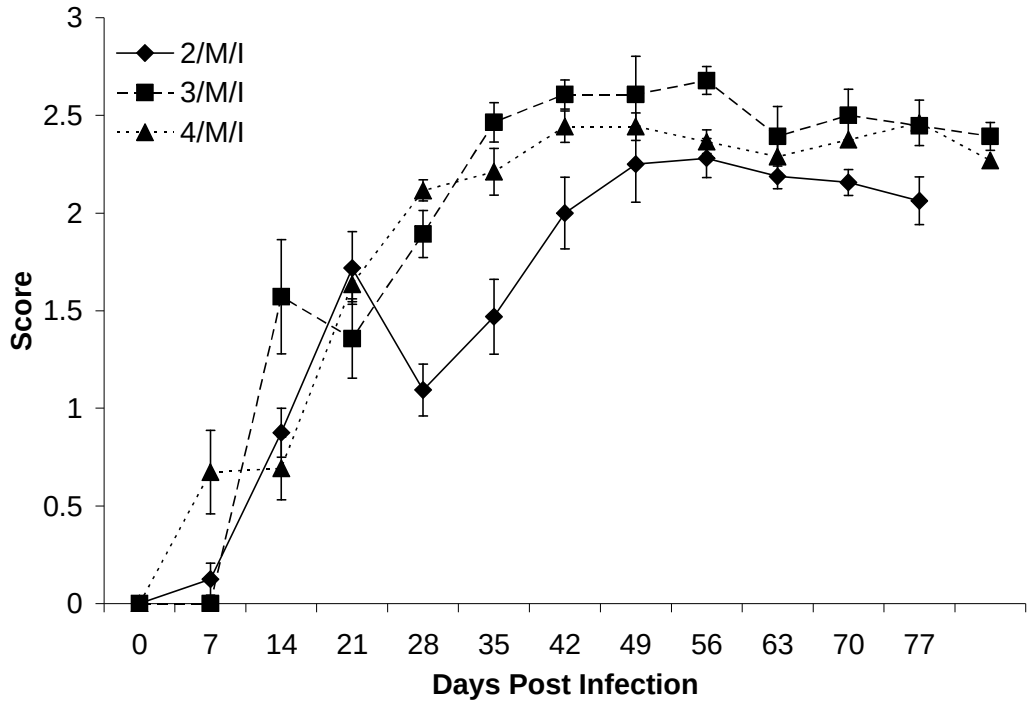
***Figure 11 - Effects of Infection on Clinical Score in Females:***




**Legend: 2/3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection on Clinical Score in Females:** Females infected at two weeks of age had lower clinical scores than females infected at three weeks of age during the acute phases of the disease ($p=0.0102$).

***Figure 12 - Effects of Infection on Clinical Score in Males:***

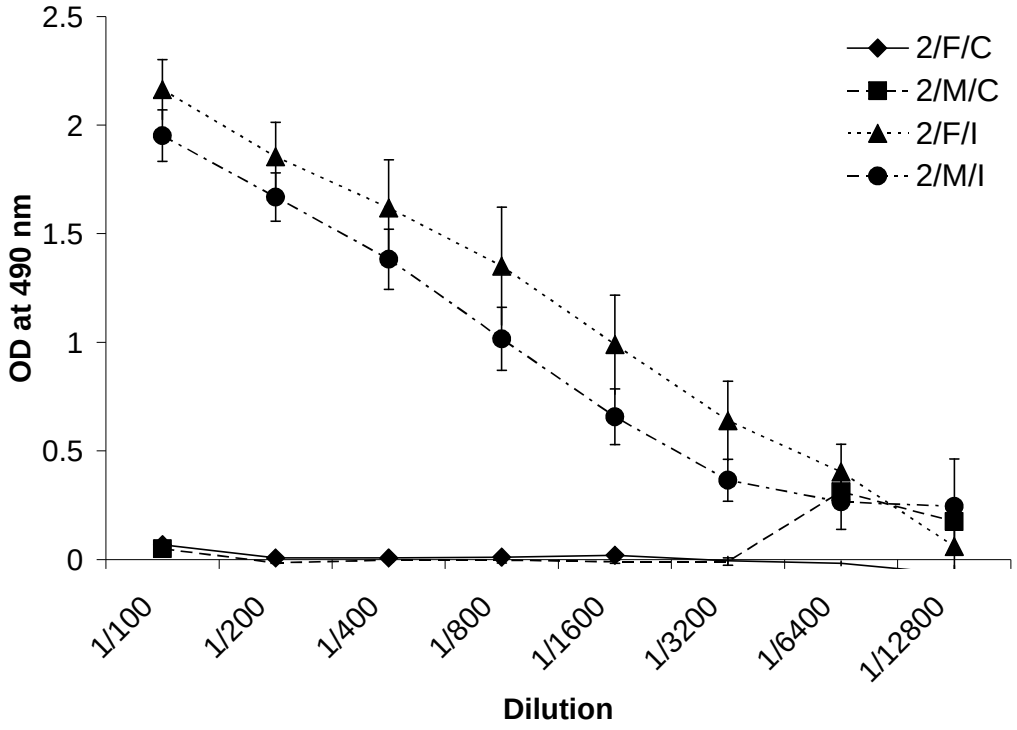



**Legend: 2/3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection on Clinical Score in Males:** Males infected at two weeks of age had lower clinical scores than males infected at three weeks of age in the acute phase ($p=0.0001$). All male groups differed from one another in the chronic phase with those infected at three weeks of age scoring highest, four weeks of age in the middle and two weeks of age scoring lowest ($ps<0.05$).

**Clinical Score Summary:** All infected groups differed statistically from their control counterparts in both the acute and chronic phases of the disease. However, between infected groups there was some variability. Males infected at two weeks of age had lower clinical scores than females infected at two weeks of age in the chronic phase of the disease ($p=0.0065$). Females infected at two weeks of age had lower clinical scores overall than those infected at three weeks of age ($p=0.0102$). Males infected at two weeks of age had lower clinical scores than those infected at three weeks of age in the acute phase ($p=0.0001$). All male groups differed from one another in the chronic phase with those infected at three weeks of age scoring highest, four weeks of age in the middle and two weeks of age scoring lowest ($ps<0.05$). What this means is that infection at two weeks of age for both male and females caused clinical scores to be lower than when infected at three weeks of age. In addition, males clinical scores were lower then females when infected at two weeks of age, but ended up being statistically the same when infected at three weeks of age. It appears that the males are surpassing the females in clinical score as puberty takes place (3-4 weeks of age). Due to unfortunate circumstances no female mice that were to be infected at four weeks of age survived to participate in the experiment. Luckily though, despite the lack of a group of females infected at four weeks of age, another study with C57L/J mice showed a higher severity of disease in males after puberty, reflected in the % of mice infected and the total IgG (Fuller, et. al 2005). This seems to show that there is some physiological change taking place during that either protects females, exacerbates the condition in males or both.

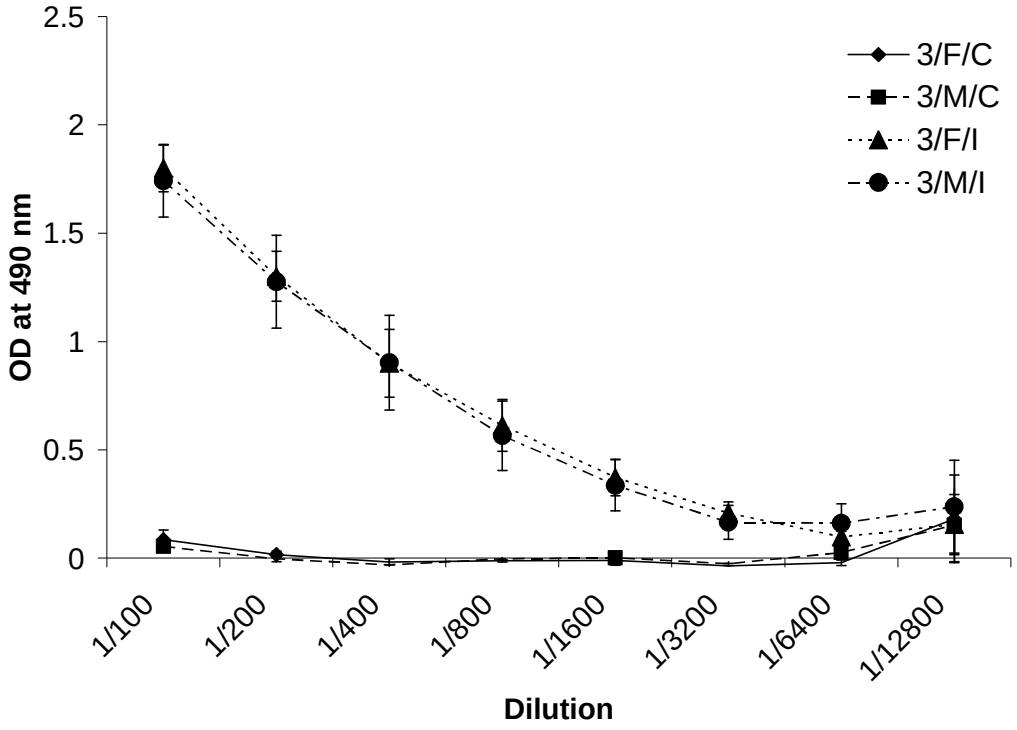
***Figure 13 - Effects of Infection at 2 Weeks of Age on TMEV Antibody Levels:***




**Legend: 2** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 2 Weeks of Age on TMEV Antibody Levels:** Infected mice had higher levels of virus-specific antibody than controls (ps<0.05). Males infected at two weeks of age were the same as females infected at two weeks of age.

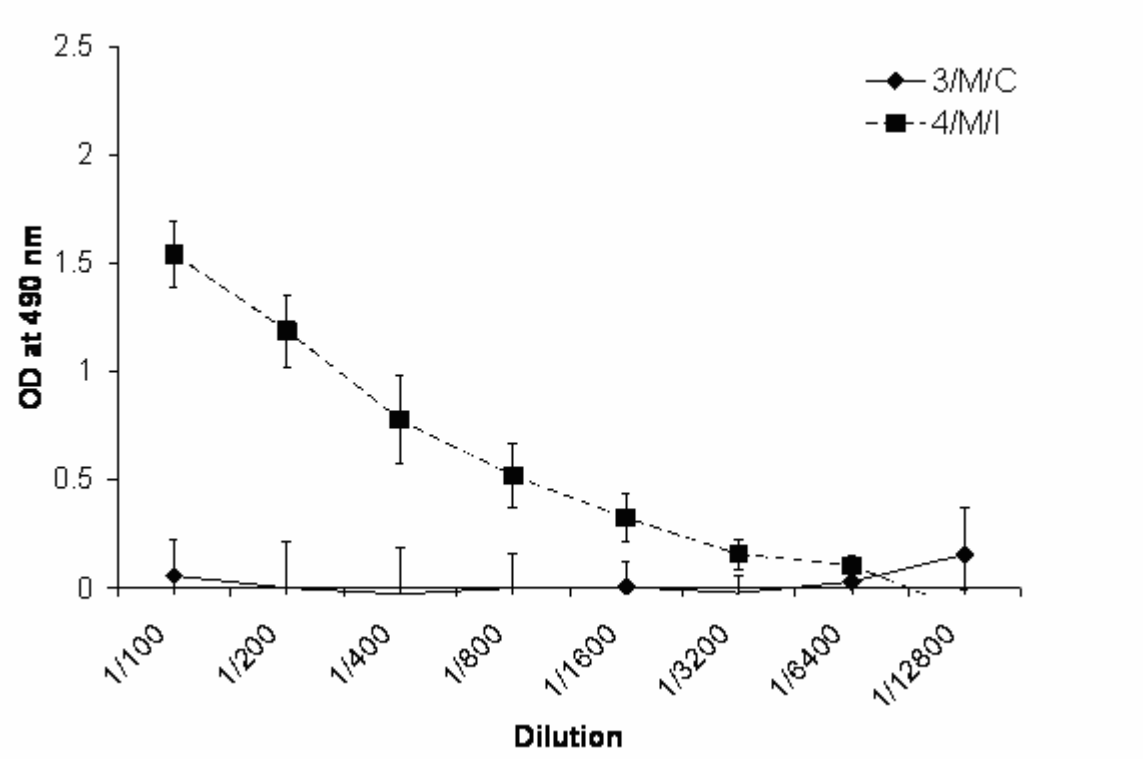
***Figure 14 - Effects of Infection at 3 Weeks of Age on TMEV Antibody Levels:***




**Legend: 3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 3 Weeks of Age on TMEV Antibody Levels:** Infected mice had higher levels of virus-specific antibody than controls (ps<0.05). Males infected at three weeks of age were the same as females infected at three weeks of age.

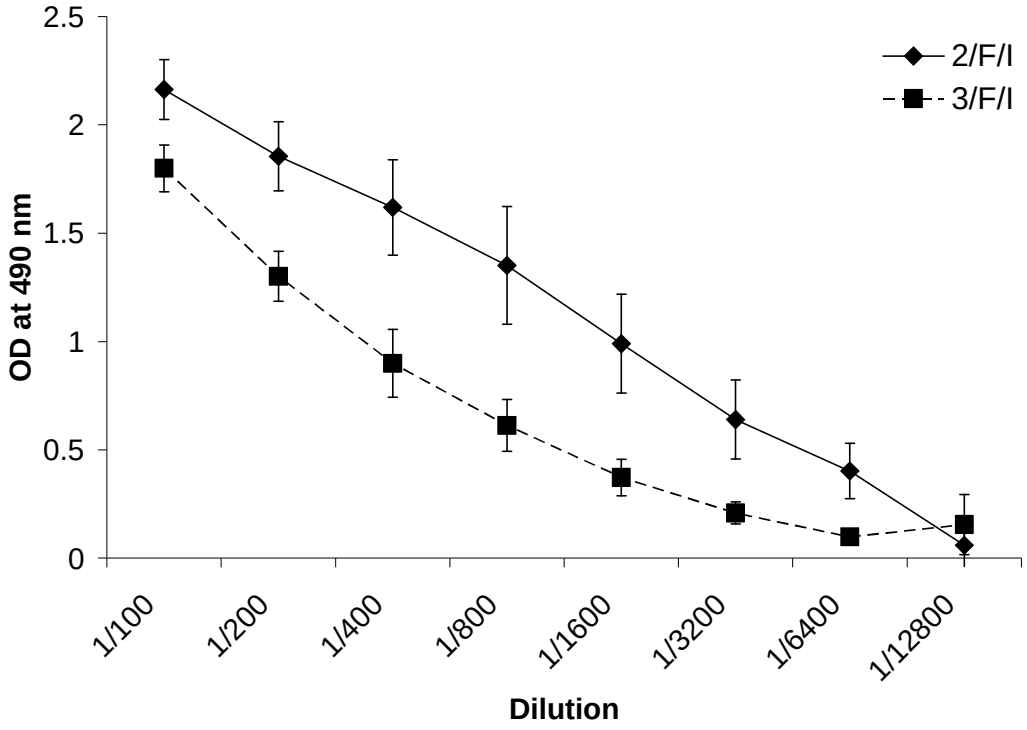
***Figure 15 - Effects of Infection at 4 Weeks of Age on TMEV Antibody Levels:***




**Legend: 3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection at 4 Weeks of Age on TMEV Antibody Levels:** Infected mice had higher levels of virus-specific antibody than controls ($p<0.05$).

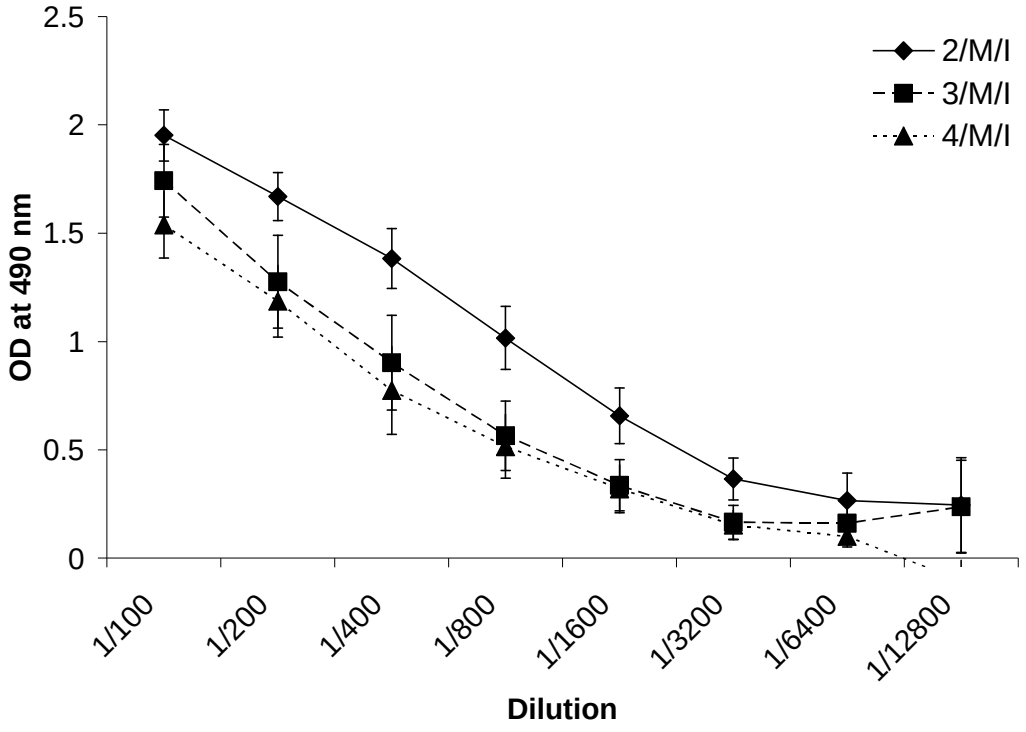
***Figure 16 - Effects of Infection on TMEV Antibody Levels in Females:***




**Legend: 2/3** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection on TMEV Antibody Levels in Females:** Female mice infected at two weeks of age had higher antibody levels than females infected at three weeks of age (p=0.003).

***Figure 17 - Effects of Infection on TMEV Antibody Levels in Males:***




**Legend: 2/3/4** – Weeks Old At Infection, **F** – Female, **M** – Male, **C** – Control, **I** – Infected

**Effects of Infection on TMEV Antibody Levels in Males:** Males infected at two weeks of age had higher antibody levels than males infected at four weeks of age ($p=0.0343$).

**Antibody Assay Summary:** It is interesting to note that the clinical scores somewhat reflect a corresponding change in levels of virus-specific antibody. Firstly, infected mice had higher levels of virus-specific antibody than controls ($ps<0.05$).

Males were the same as females at every age infection, meaning that the difference in clinical score is not directly substantiated by TMEV levels. However, differences in clinical scores were reflected in the fact that female mice infected at two weeks of age had higher antibody levels than those infected at three weeks of age ($p=0.003$). Furthermore, the males infected at two weeks of age also had higher antibody levels than those infected at four weeks of age ($p=0.0343$). This appears to show that a higher antibody response is correlating with a lower overall persistence of the virus in each mouse and a decrease in behavioral symptoms. When a correlation was performed, this hypothesis was correct ($R^2=0.918$).

***Table 2 - Results of Histological Analysis:***

| | # Showing Lesions (Total) | Average Size (%) |
|---|---|---|
| **Controls** | 0 (3) | 0 |
| **2 Weeks of Age** | 4 (7) | 3.06 |
| **3 Weeks of Age** | 5 (7) | 2.41 |
| **4 Weeks of Age** | 5 (6) | 2.41 |

**Results of Histological Analysis:** Control uninfected mice did not develop histological lesions. Brain lesions were seen in some of the infected mice. In order to be able to use these results a higher n value will be needed for each group and also spinal cord tissue should be examined if the experiment is repeated and more mice should be dedicated to histological analysis; 3-4 males and females were used per group, totaling to about 7 as shown above. Due to the small number of mice in each group that actually showed lesions in the brain tissue, nothing can be affirmatively said about the histological results without more data.

# IV DISCUSSION

The results from this study demonstrate that age has a profound effect on the pathogenesis of Theiler's virus infection. Mice that are infected at one week of age develop paralysis and become moribund within 10 days. The immune system is not fully developed at one week which would account for these results. Interestingly, both male and female mice infected at three weeks of age appeared to develop the most severe clinical signs of disease. This finding suggests that other factors contribute to the pathogenesis of Theiler's virus infection.

Overall, since body mass is not affected by infection and rotarod observations are not indicative of disease, the prior observation that a younger age at infection correlates ($R^2$=0.918) to lower clinical scores and a higher antibody response to TMEV at day 84 p.i. is extremely important. Due to the fact that females infected at two weeks of age scored higher than males infected at two weeks of age and the severity of disease for males infected at three weeks of age is equal to females infected at three weeks of age, this difference must be discussed. A possible cause for the discrepancies between males groups compared to the female groups could be (1) estradiol acting as a protective agent against the disease for females, supporting previous studies and conclusions or (2) testosterone acting as a damaging agent to disease progression in males (Fuller et. al 2005). Unfortunately, the after effects of puberty could not be seen in this experiment because of the lack of a group of females infected at four weeks of age, but another study in C57L/J mice shows a higher severity in males after puberty (Fuller et. al 2005). In summary, it appears that clinical scores are lower and antibody response to TMEV is

higher in mice infected at two weeks of age. As time of infection increases, clinical scores increase and antibody response drops, likely due to the physiological addition of estradiol or testosterone, the former potentially causing a protective effect and the latter a negative one. After puberty has passed, the levels of hormones are still high, but not nearly as much as when puberty is taking place, hence the relative drop in clinical scores.

These results fit in very well with previous studies that have shown that males display worse clinical symptoms compared to females (Alley et. al., 2003; Fuller et. al., 2005; Fuller et. al., 2007). This was exactly the case as shown in this study. In addition, two articles discussed puberty in relation to MS – the whole purpose of this research model. One study proposes that multiple sclerosis begins at puberty while the other says that "the data suggest that onset… of MS is dependent on passing… through the puberty period" (Wainerdi, 1961; Fischman 1981). The onset of multiple sclerosis and TVID both occur prior to puberty, however, data from this experiment supports the latter study showing that puberty does have an effect on the pathogenesis, if not the onset of the disease. Also, the data collected shows similarities to the original Theiler studies showing that mice infected at very young ages cannot survive infection as well as similarities to another study by Steiner, in which data was collected on mice infected at one and three weeks of age (Steiner et. al., 1984; Theiler, 1934; Theiler, 1937). Lastly, the overall data in combination with other studies and observations made in the lab point to estradiol as being an important agent in the outcome of the disease (Fuller et. al., 2005).

On that note, the implication of this study with the support of prior research is that further experimentation should take place regarding the effects of puberty. In order to determine what the effects of testosterone or estrogen are on the pathogenesis of TVID, a study involving eight groups should be conducted. First there would be a male and a female control group accounting for two of the groups. Then there would be a male and a female group infected at puberty using methods to determine the onset of puberty as discussed in another study (Takashima-Sasaki et. al., 2006). At the same time, two other male groups and two other female groups would be infected. One male group would be injected with an estrogen supplement and one female group would be injected with a testosterone supplement. The other male group would be injected with a testosterone inhibitor and the last female group would be injected with an estrogen inhibitor. Blood samples should be taken throughout the infection period to determine exact estrogen and testosterone levels which can then be compared with clinical scores and/or antibody levels. These groups with varying degrees of estrogen and testosterone levels would allow researchers to pinpoint whether either of them has an effect on the pathogenesis of TVID, which may have some future value in the treatment of MS patients.

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# Matthew Stephen Propst

## EDUCATION

*College of Veterinary Medicine and Biomedical Sciences*
*Texas A&M University, College Station, TX*
**B.S. in Biomedical Science**
On Foundation and University Honors track
Major GPA: 4.000
Overall GPA: 3.951
Anticipated date of graduation: May 2009

## AWARDS

- Aggie High School Partner Scholarship **2006**
- Charles J Koerth Senior Memorial Scholarship **2007**
- International Education Fee Scholarship **2007**
- John Thomas Robertson Endowed Scholarship **2006**
- Killeen Evening Lions Club Scholarship **2008**
- Loring Cook Foundation Memorial Scholarship **2006**
- McAllen Chamber of Commerce Scholarship **2006**
- McAllen National Bank Scholarship **2006**
- Student Activities Board Scholarship **2006**
- Student Research Week – 3rd in Taxonomy **2008**
- Student Research Week – Session Winner **2008**
- SUURF Scholarship **2007**
- Texas Aggie Scholarship **2007**

## TEACHING EXPERIENCE

Texas A&M University, College Station, TX
**Lab Instructor -** Chemistry 111 **2007**
Collaborated on lab development, met with students upon request, instructed students in lab and graded all their written work, including lab midterm and final papers.

## LANGUAGES

- English – native language
- Spanish – slightly fluent speaker and writer, can read/understand with a moderate proficiency

## MEMBERSHIPS AND OTHER

Aggieland Lions Club – President **2008**
Aggieland Lions Club – Member **2007-2008**
American Red Cross First Aid, CPR and AED Certified **2008**
AMSA Pre-Med – Honored and Distinguished Member **2007**
Alpha Tau Omega – Executive Board Member **2007-2008**
Alpha Tau Omega – Founding Father **2007-2008**
Alpha Tau Omega – Judicial Board Member **2007-2008**
Alpha Tau Omega – Secretary **2007-2008**
American Diabetes Association **1991-2008**
Argentina “Alternative” Spring Break **2007**

| | |
|---|---|
| Camp Discovery – Ropes Activity Staff | **2008** |
| Champions Kids Camp – Counselor | **2008** |
| Fish Camp – Session D, Green, Arenas - Counselor | **2007** |
| Fish Camp – Session D, Aqua, Blum - Freshman | **2006** |
| Friends of IB Organization | **2004-2008** |
| Germany History of Medicine Study Abroad Student | **2007** |
| Habitat for Humanity | **2008** |
| Hillel – Board Member | **2006-2007** |
| Hillel – Freshman Representative | **2006-2007** |
| Juvenile Diabetes Research Foundation - Member | **1991-2008** |
| Level 1 Certified Ropes Course Facilitator | **2008** |
| Mandated Reporter | **2008** |
| National Society of Collegiate Scholars | **2007-2008** |
| Phi Eta Sigma National Honor Society - Member | **2007-2008** |
| TAMU Pre-Medical Society – Honored and Distinguished Member | **2007** |
| Texas A&M for Darfur | **2006** |
| Texas A&M Honors Research Fellow | **2007-2008** |
| Texas Lions Camp – Assistant Team Leader (“Rover”) | **2008** |
| Texas Lions Camp – Century Club Member | **2008** |
| Texas Lions Camp – Life Member | **2008** |
| Texas Lions Camp – Medical Staff | **2006, 2008** |
| The Big Event | **2007-2008** |