

In preparation for vaccine development, demonstrate that sheep become resistant to *P. tenuis* by establishing infections followed by challenges to test for resistance using larvae and antigens available from Cornell's colony of *P. tenuis* infected snails.

Ewe lambs born in the March 2013 lambing season, were kept in the barn for the entire 2-year experiment to keep them from being naturally exposed to *P. tenuis*. Snails infected with L1 *P. tenuis* in the Appleton lab 90 days previously were harvested to recover L3 *P. tenuis*. In October 2013, 12 ewe lambs were each orally dosed with 20 L3 (stage 3 larvae) of *P. tenuis* to induce immunity (Infected). Of the 12 ewes, 1 showed signs of *P. tenuis* infection in December 2013. A video of her symptoms is available on-line. She was treated and recovered. 12 Control cohorts of the treated ewes were orally given the suspension, while 7 ewes were kept as Sentinels. Blood samples were collected by jugular venipuncture every two weeks through March 2014 and processed to detect *P. tenuis* antigens. These results were equivocal, with higher levels for Infected ewes compared with Control ewes, but levels in the Sentinel ewes were almost as high as those in Infected ewes. There was a shortage of L3 to challenge all of the Control and Infected ewes in October 2014. Instead, 4 of 12 Control ewes and 5 of 12 Infected ewes were challenged orally with 100 L3; the unchallenged ewes were given the suspension media. Thus, in this Second year of the experiment the Control ewes not challenged with *P. tenuis* were similar to the sentinel ewes that were never treated. Blood was collected the day before challenge with L3 and at days 7, 21, 35, 49, 63, 77, 91, 105, 119, 133, and 146 post-challenge and an ELISA (enzyme-linked immunosorbent assay) was used to determine relative concentrations of IgG antibodies to *P. tenuis* as assessed by optical density (OD). The OD values were 0.51 for unchallenged Control and 0.51 for Sentinel groups (± 0.009). These were compared by analysis of variance that used a statistical model including Challenge, Ewe within Challenge as a random effect, Days (post challenge including day -1) and the Challenge by Days interaction. Only the effect of ewe was different ($P < 0.001$) and we chose to exclude Sentinel ewes from further analysis. No ewes demonstrated symptoms of *P. tenuis* infection in the second year. The ELISA OD values for *P. tenuis* antibodies in serum of the experimental ewes (Unchallenged Control, Challenged Control, Unchallenged Infected, Challenged Infected) were compared by analysis of variance that used a statistical model including Initial infection, Challenge, Ewe within Initial infection and Challenge as a random effect, Days (post challenge including day -1) and the Initial x Challenge, Initial x Days, Challenge x Days, and Initial x Challenge by Days interactions.

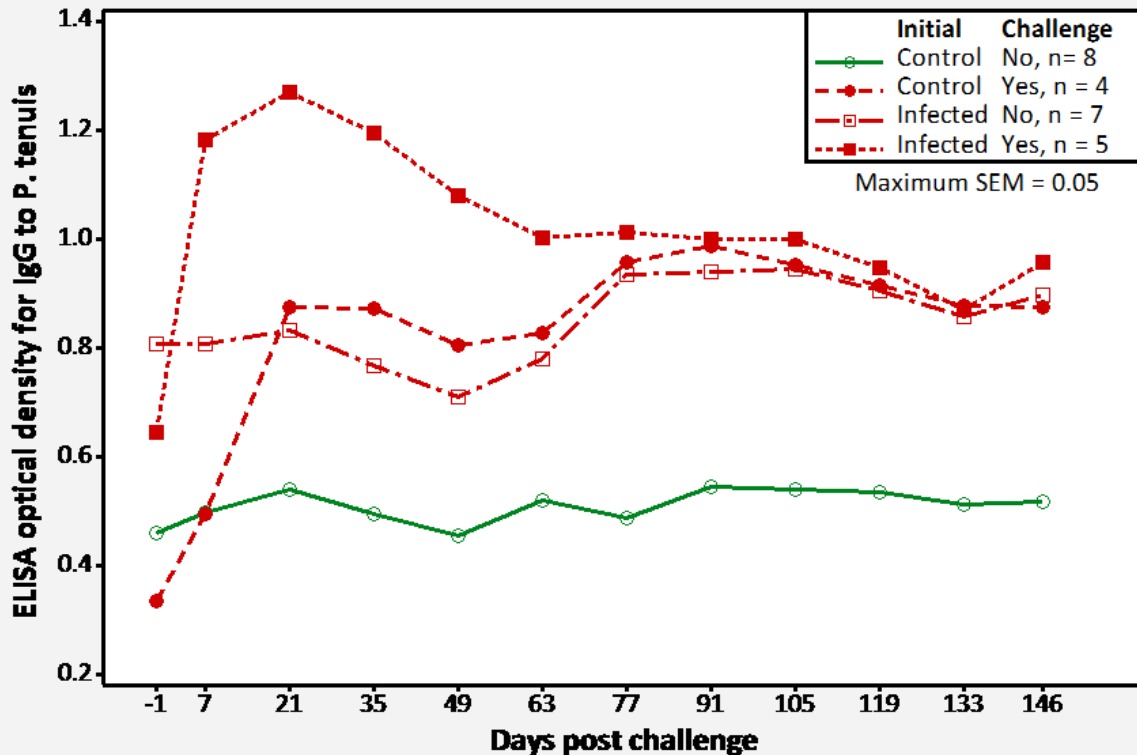


Figure 1. Effect of days post challenge on serum antibody concentrations to *P. tenuis* in ewes initially in control or a group treated with 20 L3 at 7 months of age and then with about half of each group challenged or not challenged with 100 L3 at 19 months of age ($P < 0.001$ for the Initial x Challenge x Day interaction using a repeated measures model with ewe as a random effect).

The Initial x Challenge by Days interaction ($P < 0.001$) indicated that immune responses with increasing days post-challenge were different among the 4 Initial x Challenge groups (Figure 1). Mean OD values (± 0.051) for antibody levels for unchallenged Control ewes did not change much with days post-challenge, varying between 0.46 on day -1 to a high of 0.54 on day 91 and settling on 0.52 by day 146. Challenged Control ewes demonstrated the effect of initial exposure to *P. tenuis*, starting at 0.34 on day -1, increasing rapidly to 0.88 on day 21, then to a high of 0.99 on day 91 and only declining to 0.88 by day 146. Mean OD values for antibody levels for unchallenged Infected ewes didn't change much. Demonstrating an immune response to infection a year earlier; they started high at 0.81 on day -1, increased to 0.95 on day 105 and leveled off to 0.90 by day 146. Challenged Infected ewes demonstrated immunological memory to infection a year earlier; they started high at 0.64 on day -1, and dramatically increased to 1.18 on day 7 reaching a high of 1.27 on day 21, then declining to 1.00 on day 91 and to 0.87 on day 133, then settling to 0.96 on day 146. There was not much difference among the Infected and Challenged groups in OD values from day 77 to through 146, with averages of 0.93, 0.91, and 0.97 for Challenged Control, Unchallenged Infected, and Challenged Infected, respectively. In contrast the average OD value for Unchallenged Control ewes from day 77 through day 146 was 0.52. These results show that sheep develop immunity to *P. tenuis* that could protect them from the paralysis associated with consumption of forage with high concentrations of L3. Although Challenged Control ewes unexpectedly did not exhibit symptoms of infection with *P. tenuis*, the antibody response to challenge was typical for first exposure. Because dendritic cells in the skin are highly effective at presenting antigens to the immune system, it is likely that a vaccine prepared with killed *P. tenuis* L3 would be effective. This would

require, feces from infected white tail deer, infection of a snail colony, and recovery of L3 *P. tenuis*. Alternatively, recombinantly-produced surface proteins on *P. tenuis* L3 could be tested for antigenicity and might make effective vaccines.