T regulatory lymphocytes (Tregs) are cells that suppress immune effector cells to prevent an overactive immune response leading to auto-immune disease. In human cancer patients, Tregs are increased in number and function resulting in the tumor cells escaping normal immune surveillance. Since these cells may be a novel cancer treatment target (i.e., decreasing the number of Tregs may allow the normal immune system to fight the cancer better), exploring Tregs in our canine patients would be invaluable. We identify Tregs using flow cytometry with antibodies against their protein markers: CD4+, CD25+, and FoxP3+. However, at the time this research was generated, there was no anti-canine CD25 antibody, so all previous canine cancer Treg studies have been performed using only CD4 and FoxP3 positivity to identify this unique cell subset. Thus, this Master’s research involved cloning the canine CD25 gene and transfecting and expressing the protein into human CD25 negative Hela cells. A commercially available anti-human CD25 antibody was incubated with the transfected cells and definitively recognized the canine CD25 protein. This allowed evaluation of CD4+CD25+FoxP3+ Tregs in normal dogs and dogs with bone cancer. There was no significant difference in the % CD4+CD25+FoxP3+ Tregs between bone tumor bearing dogs and healthy control dogs. However, by definitively validating the use of the anti-human CD25 antibody, there is now a more specific way to identify this unique T cell subset and evaluate them in a variety of different disease settings, specifically different types of cancer.