



FINAL REPORT

Institute of Soil and Environmental Quality and Avian Biosciences Center Graduate Fellowship Program in the Environmental Compatibility of Poultry Production

Award Recipient Kali Kniel

Department Animal and Food Sciences

Date of Award 2006-2007

Title *Fate of Bacterial Pathogens During the Growth of Leafy Greens*

Summary of Findings

The work sponsored by this fellowship is primarily for work utilizing avian pathogenic *E. coli* strains. The strains chosen are also important in terms of public health and food safety. Both *E. coli* O157 and O8 are zoonotic agents and risks for human foodborne illness. *Escherichia coli* O8 has been isolated 26 times from environmental samples and 18 times from diseased humans (Bettelheim, 2007). This work investigates potential environmental transmission of pathogenic *E. coli* from non-ruminants.

Current work has focused on developing appropriate methodology to detect these APEC bacteria from other soil organisms. Detection in various soil amendments was successful using selective media. Various media types including L-EMB, TSAN, SMACN, XLD and HE were evaluated and we founds that these do not sufficiently inhibit background microflora from soil, manure or biosolids, making it difficult to conduct experiments under real environmental conditions. For successful use of these media, soil and manure samples should be sterilized. We wish to construct "real-world" experiments and for this reason and the ability to use non-autoclaved soil we have started to transform the APEC strains with a plasmid containing green fluorescent protein (gfp). We hope this will aid in the detection of these organisms over a 60 day survival study in different soil amendments.

Interestingly we found successful detection using immunomagnetic separation by the Pathatrix machine. Surprisingly O157 antibody-coated beads are able to capture APEC O8 and O157 strains without interference from contaminating microorganisms; making real life study of pathogenic *E. coli* possible. We have also assessed the APEC strains for the genetic ability to create shiga toxins (*stx I* and *stx II*) and the effacing and adhering pathogenic factor (*eae*) and

found them all to be negative. In a Vero cytotoxicity assay one APEC strain secreted a toxin capable of killing the cells, but the others did not. The APECs do have the *rfb* gene which is associated with the O157 genotype, and this may correlate with the binding to the antibody-coated beads.

Publications Resulting From Research

An abstract was published in the proceedings from the International Association for Food Protection Annual Meeting, held in August 2008, in Columbus Ohio.

Additional Grant Support Received as a Result of ABC Grant

None at this time, although along with a collaborator at the USDA-ARS (Dr. Manan Sharma) we plan on using some of this data in the proposal submission for the BARD in 2008 and for the 2009 USDA-AFRI projects.
