**United States Department of Agriculture**
**Project Initiation**

# Project Data for Accession Number 1031382

## Project Title

Probiotic Lactobacillus-Based Recombinant Oral Vaccine Against Clostridial Dermatitis In Turkeys

## Project Details

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|---|---|
| **Sponsoring Institution**<br>National Institute of Food and Agriculture | **Project Status**<br>ACTIVE |
| **Funding Source**<br>NIFA Non Formula | **Grant Year**<br>2023 |
| **Grant No.**<br>2023-77045-41024 | **Cumulative Award Amt.**<br>$465,000.00 |
| **Proposal No.**<br>2023-06330 | **Multistate No.**<br>(N/A) |
| **Project Start Date**<br>Sep 15, 2023 | **Project End Date**<br>Sep 14, 2026 |
| **Program Code**<br>[LHTR] Laying Hen and Turkey Research Program | |

## Project Director

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## Recipient Organization

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## Performing Department

(N/A)

## Non Technical Summary

A challenging question in the 21st century is "How to feed the world, which is rapidly growing and projected to reach a population of 9.1 billion by 2050?" To address this problem, animal agriculture, specifically the poultry sector that provides a stable source of protein, has intensified its production to meet the global demand for food. Alongside chicken (meat and egg) production, turkey production also contributes significantly to the overall poultry economy. However, in recent times, the use of antibiotics in raising poultry has come under great scrutiny, as overuse of antibiotics increases the risk of the spread of antimicrobial resistance (AMR), which in turn has led to the re-emergence of certain devastating infectious (bacterial) diseases. One such disease is Clostridial dermatitis (CD), also called Gangrenous dermatitis, in turkeys; this disease is characterized by gangrene and peeling of the skin and sudden deaths of birds; thus, it has become a major economically important health problem faced by turkey growers. Certain non-antibiotic control strategies such as improved farm management practices and using killed bacterial

vaccines, as well as prebiotics and probiotic feed additives, have been tried and tested with some degree of success in controlling CD. Unfortunately, there are no effective non-antibiotic means, such as vaccines, currently available to prevent this disease in turkeys. Therefore, the goal of this research is to develop an effective oral vaccine that can replace antibiotics and provide long-term immunity against CD in turkeys.Probiotics are live microorganisms that provide health benefits when administered in adequate quantities. Specifically, the Lactobacillus species of probiotic bacteria have been used extensively in research-based commercial applications to show that such probiotics given orally to poultry can promote gut health and reduce inflammation in the intestine, and, thus, help in reducing gut infections, including those caused by Clostridial bacteria. On the vaccine research aspects, our laboratory has identified a vaccine candidate called 'ntATX-D2', which has shown promising prospects for preventing CD disease in turkeys. The unique and novel concept of this project is to combine the 'gut health benefits of Lactobacillus probiotic bacteria' and 'vaccine-induced disease-specific immunity' into a single platform to develop an oral "probiotic-based vaccine" to control CD in turkeys. To achieve this, our methodology will involve a three-step approach. First, using an established laboratory screening model, at least two Lactobacillus bacterial strains that possess excellent probiotic anti-inflammatory property will be selected from a pool of lactobacilli isolated from the turkey gut. Second, the gene for ntATX-D2 (vaccine candidate) will be inserted into the genome (chromosome) of the two selected Lactobacillus strains to develop probiotic-based molecular vaccines. Lastly, the efficacy of these two vaccines in providing robust long-lasting immunity against CD disease while promoting gut health in turkeys will be evaluated. One other unique aspect of this approach is that it involves insertion of vaccine candidate gene directly into the Lactobacillus bacterial genome such that the live probiotic-based vaccines developed in this work will not need to carry antibiotic resistance genes in them to survive within the gut. This attribute will ensure that these vaccines will not pose any potential environmental risk related to the spread of AMR, and thus become most ideal for food animal application. Therefore, this work will generate significant industry value by providing timely help to the turkey industry to control CD disease without dependence on antibiotics, and thus enhancing its sustainability and profitability. Future applications can also include devising novel probiotic vaccine-based strategies for the control of many other diseases affecting poultry and perhaps other food animals.

## Animal Health Component

Animal Health Component: 100%

## Research Effort Categories

| | |
|---|---|
| Basic | 50% |
| Applied | 25% |
| Developmental | 25% |

## Classification

| Knowledge Area (KA) | Subject of Investigation (SOI) | Field of Science (FOS) | Percent |
|---|---|---|---|
| 311 | 3230 | 1040 | 50% |
| 311 | 3230 | 1100 | 25% |
| 311 | 3230 | 1090 | 25% |

## Knowledge Area

[311] Animal Diseases

**United States Department of Agriculture**
**Project Initiation**

## Subject Of Investigation

[3230] Turkey, live animal

## Field Of Science

[1040] Molecular biology

[1100] Bacteriology

[1090] Immunology

## Keywords

probiotic, turkeys, vaccine, Clostridial dermatitis, and immunity

## Goals / Objectives

Major Goal:To develop a viable and efficacious oral vaccine to prevent Clostridial dermatitis disease in turkeys. Specific objectives:Screening and selection of Lactobacillus strains (turkey origin) possessing superior anti-inflammatory properties in vitro.Using an established immune cell-based in vitro model, several Lactobacillus strains isolated from the intestines of healthy turkeys will be screened for their immunological properties. From these experiments, at least two Lactobacillus strains that exhibit excellent anti-inflammatory property will be chosen for vaccine construction work.Cloning and expression of Clostridium septicum ntATX-D2 antigen into the selected Lactobacillus strains to develop a recombinant probiotic-based vaccine.A non-toxic domain of C. septicum alpha toxin, referred to as ntATX-D2, has been recently identified by the PD lab as an excellent vaccine candidate for protecting Clostridial dermatitis in turkeys. Therefore, the gene sequence encoding ntATX-D2 will be cloned into the chromosome of the two selected (from objective 1) Lactobacillus strains to construct a recombinant probiotic-based vaccine expressing ntATX-D2 antigen. The vaccine construction and expression of ntATX-D2 protein in vitro will be assessed using molecular techniques, including conventional and quantitative real-time PCR, and Western blot analysis using antibodies raised against ntATX-D2 protein.Oral vaccination of turkeys with the recombinant Lactobacillus vaccines and assessment of clinical protection against Clostridial dermatitis as well as evaluation of immune protection mechanisms.In order to assess the protective efficacy of the two recombinant Lactobacillus-based probiotic vaccines, turkeys will be vaccinated via oral route using a prime-boost immunization strategy. The two vaccines will be adminsitered in single as well as in combination formulations and the protection against a disease challenge will be assessed based on the following parameters related to clinical behaviour, pathology and immunology: a. Monitoring of clinical signs and mortality.b. Gross and histopathology scoring of tissues (skin, muscle, spleen and cecal tonsils).c. Immune gene expression in various tissues via real-time PCR technique.Cellular immunophenotyping of blood using Flow cytometry technique.

## Project Methods

Methods below are described in 3 parts in relation to the proposed objectives of this project.Screening and selection of Lactobacillus strains (turkey origin) possessing superior anti-inflammatory properties in vitro.Efforts: An in-house established in vitro avian macrophage-based cellular model will be used to characterize the immunological properties of Lactobacillus strains isolated from the turkey intestines.Efforts: The macrophage cells have been previously validated to produce certain immune signaling cytokines such as interleukin (IL)-6 and IL-1? (represent signature pro-inflammatory cytokines) and IL-10 and transforming growth factor (TGF)? (represent hallmark cytokines for anti-inflammatory/ immunomodulatory responses).Evaluation: About 20 Lactobacillus strains will be used to interact with macrophages and the cellular gene expression of these cytokines will be measured using quantitative real-time PCR method to characterize each strain into one of the three categories of i) pro-inflammatory, ii) anti-inflammatory, and iii) no change. Thus, at least two Lactobacillus strains exhibiting anti-inflammatory property are anticipated to be selected for the next part (objective 2).Cloning and expression of Clostridium septicum ntATX-D2 antigen into the selected

**United States Department of Agriculture**
**Project Initiation**

Lactobacillus strains to develop a recombinant probiotic-based vaccine.Efforts: A protective immunogen of C. septicum, namely the 'ntATX-D2', identified previously in Kulkarni laboratory will be used as a vaccine candidate gene for cloning into the selected two Lactobacillus strains to develop a recombinant probiotic-based vaccine for controlling Clostrdidial dermatitis in turkeys. For this purpose, an efficient, antibiotic marker-less, double-crossover homologous recombination-based gene replacement system in Lactobacilli, which has been previously established by us and used successfully for generating recombinant strains with chromosomal gene deletions or insertions, will be used. This system uses chromosomal insertion and expression of Clostridium genes, and thus, ntATX-D2 cloning cassette will be chromosomally inserted downstream of a highly expressed housekeeping gene (enolase or other) to achieve stable and high expression level of ntATX-D2 protein antigen bypassing the requirement for having an antibiotic resistance gene for plasmid maintenance.Evaluation: The confirmation of expression of ntATX-D2 by the recombinant Lactobacillus strains will be determined at two levels: i) Transcription (mRNA production) of ntATX-D2 gene, and ii) Western blot assays will be performed to confirm detection of the ntATX-D2 protein.Oral vaccination of turkeys with the recombinant Lactobacillus vaccines and assessment of clinical protection against Clostridial dermatitis as well as evaluation of immune protection mechanisms.Efforts: A 'prime-boost' immunization strategy will be used to administer turkeys, via oral gavage route, with recombinant Lactobacillus vaccines on the day-of-hatch (prime), followed by at 4wks of age (boost) and challenge with virulent C. septicum at 11-12 weeks of age to allow effective strain colonization and induction of antigen-specific immunity against Clostridial dermatitis.Evaluation: The protection assessment will be based on the following:Mortality, and gross and histopathology lesion scores, as well as % body weight gain (pre-challenge to termination).The immunological evaluation will include expression of immune genes (IL-6, IL-1?, IFNÝ, IL-10, IL-4, TGF?) in the ileum/cecum, skin, muscle, spleen and cecal tonsil tissues, and cellular immunophenotyping of CD4+, CD8+, MHC-II+ and IgM+ cells in the blood, spleen and cecal tonsils. Furthermore, serology will be performed on the samples collected at pre- and post-challenge time points to assess ntATX-D2-specific binding as well as neutralizing antibodies, as an indicator of protection mechanism and as an indirect confirmation of a stable expression of ntATX-D2 by the vaccine vectors in vivo.The microbial analysis will include MiSeq sequencing of ileocecal contents to determine the changes in the gut microbiota populations.