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

# Project Data for Accession Number 1032749

## Project Title

Vaccine to protect against Clostridium septicum-induced necrotic dermatitis and C. perfringens-induced necrotic enteritis 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>2024 |
| **Grant No.**<br>2024-77045-43081 | **Cumulative Award Amt.**<br>$462,480.00 |
| **Proposal No.**<br>2023-06323 | **Multistate No.**<br>(N/A) |
| **Project Start Date**<br>Sep 01, 2024 | **Project End Date**<br>Aug 31, 2027 |
| **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

Clostridial dermatitis (CD), also known as gangrenous dermatitis (GD), is a major problem for the turkey industry in the United States, causing significant financial losses. This disease is mainly caused by two bacteria: C. septicum and C. perfringens. C. septicum can cause high death rates in turkeys, while C. perfringens not only causes CD but also leads to a deadly intestinal disease called necrotic enteritis (NE), which costs the poultry industry $6 billion annually. Although we developed the first vaccine targeting C. perfringens-induced NE in 2020, there is still no effective way to control C. septicum. To solve the problem of CD, we need to target both bacteria. This project aims to help not only the turkey and layer industry but also the entire poultry industry.We will use innovative techniques to tackle C. septicum. Our licensed vaccine contains two components that target C. perfringens. We have also found one component that protects against both C. perfringens and C. septicum, and two additional components that target C.

septicum. We will use these five components to develop new vaccines that target both bacteria. These vaccines will be delivered orally or through a spray using a harmless form of Salmonella to protect turkeys and layers from CD and NE. This project will benefit turkey and layer farmers by improving the efficiency and sustainability of poultry production. It will also provide valuable data that could help protect against other diseases not covered by this project. Additionally, the vaccine may reduce the spread of Salmonella, improving food safety. By preventing CD and NE, we can significantly reduce the need for antibiotics in agriculture. This reduction in antibiotic use helps to prevent the development and spread of antibiotic-resistant bacteria, which is a major concern for both animal and human health. This aligns with USDA goals to support a prosperous agricultural system and provide safe, nutritious food. The project is important for the economy, community, environment, and overall agricultural health.

## Animal Health Component

Animal Health Component: 100%

## Research Effort Categories

| | |
|---|---|
| Basic | 10% |
| Applied | 90% |
| Developmental | 0% |

## Classification

| Knowledge Area (KA) | Subject of Investigation (SOI) | Field of Science (FOS) | Percent |
|---|---|---|---|
| 311 | 3210 | 1090 | 50% |
| 311 | 3230 | 1040 | 50% |

## Knowledge Area

[311] Animal Diseases

## Subject Of Investigation

[3210] Egg-type chicken, live animal

[3230] Turkey, live animal

## Field Of Science

[1090] Immunology

[1040] Molecular biology

## Keywords

Salmonella, broiler, laying hen, turkey, vaccine, Clostridial dermatitis, Clostridium septicum

## Goals / Objectives

We will modify and improve a recently developed Protective Immunity Enhanced Salmonella Vaccine (PIESV) synthesizing and delivering the PlcC and NetB protective antigens to protect poultry from Clostridium perfringens-

induced necrotic enteritis (NE) to now synthesize and deliver 5 protective antigens including the PlcC and NetB antigens in our current vaccine, a protective antigen that is very homologous in both C. perfringens and C. septicum and then two additional non-toxic derivatives of C. septicum toxins to induce high-level protective immunity against C. septicum-induced necrotic dermatitis (DN) as well as C. perfringens-induced NE in turkeys and layers. We will evaluate a new improved PIESV vector strain compared to the vector strain used in our previously constructed vaccine against NE in layers. Both of these strains have genetic alterations to enhance their immunogenicity and preclude biofilm formation while exhibiting regulated delayed attenuation and regulated delayed antigen synthesis in vivo with a regulated delayed lysis phenotype precluding long-term persistence in vivo and no survival if excreted into the environment. We will also use a new regulated delayed lysis plasmid vector to encode and independently regulate synthesis with or without secretion for delivery of all five protective antigens. The final vaccine will have complete sensitivity to all antibiotics and will lack any vestiges of antibiotic-resistance elements. The vaccine is designed for production in a fermenter followed by concentration and lyophilization. The vaccine will be administered either by course spray in the hatchery or in drinking water in the production facility.The objectives are:1) Aim 1. Construct regulated delayed lysis plasmid vector encoding C. perfringens-encoded PlcC, NetB and Fba and non-toxic derivatives of C. septicum-encoded Csa and CstA using a newly constructed plasmid vector that enables synthesis with and without delivery by secretion of five or seven protective antigens. 2) Aim 2: Evaluate PIESV constructs to synthesize and deliver protective antigens of C. septicum and C. perfringens, validate their efficacy when mucosally administered to turkey poults and layer chicks to prevent CD and NE and preserve feed conversion efficiency and growth performance.3) Aim 3: Evaluate the bi-valent PIESV-C. septicum-C. perfringens to diminish colonization of layer chicks with Salmonella enterica serotypes prevalently colonizing layers and transmitted through the food chain to humans.

## Project Methods

A. General Materials and MethodsA.1 Bacterial strains, media and bacterial growth. LB broth and agar with 0.1% arabinose, 0.1% mannose and 0.1% rhamnose will be used as complex media for propagation and plating of PIESV strains. SS agar and Brilliant Green agar with above supplements will be used to enumerate Salmonella from chicken and turkey tissues or feces. Rappaport-Vassiliadis broth with supplements will be used to enrich for PIESVs from cecal and intestinal contents, bursa, liver and spleen. Bacterial growth is monitored by plating for colony counts and spectrophotometrically. C. perfringens CP4, Plc+, NetB+ and TpeL+, will be grown in cooked meat broth and fluid thioglycolate broth. Tryptose-sulfite-cycloserine agar containing egg yolk emulsion will be used to enumerate C. perfringens from tissues and fecal samples. C. septicum clinical isolates will be culture in brain heart infusion (BHI) broth A.2. Molecular and genetic procedures. Methods for DNA isolation, restriction enzyme digestion, DNA cloning and use of PCR for construction and verification of vectors are standard.. Plasmids will be evaluated by DNA sequencing performed commercially and ability to specify synthesis of proteins using gel electrophoresis and western blot analyses.A.3 PIESV strain characterization. PIESVs with antigen expression plasmids will be compared with vector-control strains for stability of plasmid maintenance, integrity and protein synthesis ability when PIESVs are grown in the presence of arabinose, rhamnose, mannose and DAP for 50 generations. Molecular genetic attributes are confirmed by PCR with appropriate primers. Measurement of LPS core and O-antigen will be performed after electrophoresis using silver-stained gels. We will evaluate final PIESVs for bile sensitivity, acid tolerance and ability to survive in sera with and without complement and for sensitivity to antibiotics used to treat Salmonella infections.A.4 Hemolytic activity and cytolytic assay of the candidate antigens will evaluated using RBS, SUP-T1 and Vero cells according to the estatlished methods B. Monitoring of induced immune responses. B.1. Antigen preparation: Protective antigens specified by PIESVs will be synthesized as His-tagged proteins in recombinant E. coli. Salmonella LPS O-antigens are obtained commercially. These antigens will be used as controls in western blots and for immunoassays as described below. Their toxicity will be tested as described in A4. B.2. ELISA: Blood and intestinal scraping samples will be collected. IgY responses in serum and IgA responses in intestinal scrapings will be measured by indirect ELISA as previously described.B.3. Neutralizing antibody analyses. The experiment is carrying out as described in A4.2 except protein will be incubated with serially diluted serum for 30 min before adding to cells.B.4. Animal experiments in turkeys in SPRG. Studies on PIESV-C. septicum-C. perfringens vaccines will be conducted in 1-

day-old layers and 3-days-old Nicholas turkey poults (unsexed) obtained from a local commercial hatchery by SPRG. Chicks will be orally immunized with 108 CFU candidate PIESVs vaccines. Layers will be challenged with C. perfringens as described. Poults will challenged with C. septicum. Serum samples will be collected.B.5. Animal experiments for Salmonella protection. Newly hatched chicks will be immunized orally with PIESV doses of 108 CFU, after which food and water will be provided ad libitum. For challenge with Salmonella serotypes, birds will be housed in isolators under ABSL-2 containment.