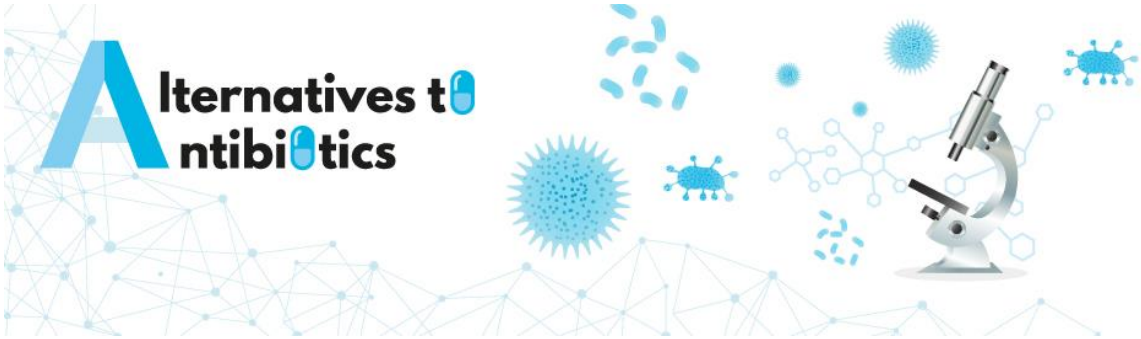

Research Gap Analysis Workshop

National Animal Disease Center
Ames, Iowa
April 5-7, 2016

Poultry Working Group Report

The workshop was organized at the National Animal Disease Center, Ames, Iowa from April 5-7, 2016 with the support of the Center for Food Security and Public Health (Iowa State University), PROCINORTE (Inter-American Institute for Cooperation on Agriculture), USDA Agricultural Research Service (ARS) and National Institute of Food and Agriculture (NIFA). The workshop included representatives from government and academic research organizations, food animal and plant commodity groups, and regulatory and funding agencies (FDA, APHIS-CVB, and USDA-NIFA).

Objective

The objective of the workshop was to identify strategic research initiatives for agricultural animals, plants, and food safety that will enable the development of alternatives to antibiotics that could reduce the use of medically important antimicrobials. A thorough research gap analysis was conducted to identify diseases in animal and plant production that will be most affected by the reduction of antibiotic usage in agriculture; either because they no longer work due to antimicrobial resistance, or because of regulatory restrictions on their use to safeguard medically important antibiotics for veterinary and human use. The workshop provided a forum to discuss and identify alternative antimicrobial strategies and their mechanisms of action for disease prevention, treatment, and agricultural production.

Opening

The workshop started with a welcome note from Dr. Marcus Kehrli, Director of National Animal Disease Center, Ames, Iowa. Dr. Gary Sherman (NIFA) presented an overview of USDA's research support efforts through the agency's extramural investments in sciences supporting the development of alternatives to antimicrobials in livestock. Dr. Cyril Gay (ARS) addressed the participants by outlining the workshop objectives and the expected outcomes.

Workshop Sessions

The workshop was divided into 8 sessions with specific goals and each session was followed by a panel discussion. The details of the sessions and their objectives are presented below.

Session1: Industry perspective
The aim of this session was to identify issues that are affecting agriculture due to the loss of antibiotics and identify needs and expectations for alternatives to antibiotics from the agricultural community. Representatives from various animal and plant industries described the ongoing disease problems and identified research or antibiotic alternative needs.

Session2: Alternatives to Antibiotics: Vaccines that could reduce the use of medically important antibiotics
The aim of this discussion was to identify gaps in vaccines for production diseases. The overall goal was to establish need for vaccines that could reduce the use of medically important antibiotics and pinpoint potential research solutions. Researchers presented the current challenges in development of vaccines to diseases like post-weaning diarrhea in pigs and bovine respiratory disease complex in beef cattle. Scientists from plant and poultry fields presented their research on the use of beneficial bacteria and phytochemicals as potential antibiotic alternative strategies to prevent and treat diseases.

Session3: Alternatives to Antibiotics: Lessons from nature
The aim of this session was to address novel biocontrol approaches for preventing and/or treating bacterial pathogens (and where applicable viral and parasitic pathogens) in food animal production. These strategies may include: antimicrobial peptides from nature, probiotics and prebiotics, bacteriophages and lysins, naturally occurring antibacterial lytic enzymes such as bacteriocins that share an added 'no resistance confidence factor' by having co-evolved with their target hosts, recombinant or hyperimmune therapeutic antibodies, siRNAs, or other novel biotherapeutic alternatives in the pipeline, including demonstrated synergistic approaches that could both reduce costs and increase efficacy while reducing the risk of drug resistance development.

Session4: Altering innate defense mechanisms to enhance disease resistance and treat animal infections

This session addressed novel drug-free alternative host directed strategies to enhance innate defense mechanisms by modulation of innate immune pathways or activation of conserved innate immune sensing molecules of the host immune system. This session focused on topics such as regulators of innate signaling derived from natural host defense peptides, and novel bio therapeutics and anti-infectives targeting conserved innate sensing receptors such as TLR, NLR, and RLR as means to activate effector mechanisms against infectious diseases where current therapeutic drug therapy is not working. Research on the improvement of host innate immunity through upregulation of endogenous production of antimicrobial peptides as a novel alternative strategy was presented. Industry representatives provided practical examples of new technologies that have reached commercialization emphasizing the path taken towards the successful development of antibiotic alternatives.

Session5: The gut microbiome and immune development, health and disease

Recent advances are demonstrating that the microbiota plays a key role in health and disease. Sequencing and microbiome analysis have enabled a much more precise description of microbial communities in gut ecosystems than has hitherto been possible. The challenge now is to relate description to function, particularly as it relates to health and disease in animals and humans. This session attempted to capture state-of-the-art results in farm animals and humans to assess how microbiome analysis is helping to solve disease problems. Changes in the microbiome can lead to changes in the host animal's immune response, affecting inflammation. Understanding the microbiome changes that accompany, and possibly cause, inflammation should lead to new methods to medicate disease and improve the health of animals in animal production. Scientists from swine and poultry research groups have presented gut microbiota research and their established role in health and development.

Session6: Alternatives to antibiotics to promote growth in livestock, poultry and aquaculture production

The aim of this session was to explore novel approaches that can be used as alternatives for antibiotic growth promoters in poultry, swine, ruminant, and aquaculture production. Although data on field performances are of importance, a key aim of this session was to improve knowledge on mechanisms of action of the growth-promoting characteristics of the proposed approaches. Research presentations on the mechanism of action of various antibiotic growth promoters were given and possible mechanisms were discussed in the panel discussion.

Session7: Regulatory pathways to enable the licensing of alternatives to antibiotics

This session reviewed the regulatory pathways in the United States to license alternatives to antibiotics. The particular regulatory challenges that are faced in taking new molecules from discovery to commercial production were addressed. This session also covered procedures to seek approval for labelling claims that are new with specific focus on claims that a product is able to reduce the use of antibiotics. Representatives from FDA and APHIS provided insight into the regulatory pathways and answered panel questions concerning regulatory approval of antibiotic alternative products.

Session8: Breakout sessions- Gaps and Solutions
At the end of the workshop, a gap analysis was conducted for each species and various disease problems were prioritized and the research needs and gaps were identified.

Poultry diseases-Gap analysis report

The breakout session for research gap analysis of poultry diseases was chaired by Dr. Dave Hermes (Perdue Foods LLC), Ms. Gretta Irwin (Turkey Federation), Dr. Hyun Lillehoj (ARS, USDA) and Dr. Brad Bearson (ARS, USDA). The poultry group was represented by various scientists from ARS, universities and poultry industry. The group actively discussed the ongoing disease problems in both broiler and turkey industry and identified various research gaps and desired alternatives for each type of disease. The moderators presented a formal report separately for broilers (Dave Hermes) and turkeys (Gretta Irwin) at the end of the session highlighting the problem diseases in priority order along with their identified research needs and possible limitations.

Broilers

The group concluded that the two most important diseases in broiler production that needs immediate attention in developing alternatives for prevention and treatment include Necrotic Enteritis (NE) and coccidiosis. Other less important disease problems listed include systemic secondary *E. coli* infections, Infectious Bronchitis, food borne pathogens (*Salmonella*, *Campylobacter*).

Necrotic Enteritis

NE, caused by *Clostridium perfringens* (CP), is a widespread infectious disease in the US and throughout the world causing great economic impairment to the poultry industry by compromising the integrity of the intestinal mucosa and thus performance. The losses caused due to NE were estimated to be $2 billion dollars globally, but recent reports states that the true costs of NE come close to $6 billion dollars annually. Subclinical NE infections can incur significant economic losses due to reduced growth, increased feed conversion ratio, which were estimated to be 12% and 10.9% respectively compared to healthy birds, and contributes to the major portion of economic losses caused by NE. The mortality in clinical outbreaks can reach up to 50% before effective control measures were introduced. Over the past several decades, prophylactic supplementation of in-feed antibiotics is used as the major strategy for controlling NE. With increasing regulations on the use of antibiotics in animal and poultry feed, NE outbreaks increased in occurrence and severity over the past several years.

There are no reliable prevention strategies for NE and thus the disease continues to remain as an issue for poultry production especially for those raised on antibiotic or drug-free program. Toxoid vaccines are in use for immunizing breeders but the immunity induced is not long lasting and the protection conferred to progeny is partial and highly variable. During the discussion,

the group agreed that this is the **number one disease (top priority)** requiring immediate attention in the development of drug-free alternate measures. The group identified the following alternative strategies that needs to be developed as a prophylactic or therapeutic tool against this disease.

1. Phytonutrients
   Plant phytochemicals with efficacy against CP should be developed as an antibiotic alternative strategy to control NE. Research should focus on characterizing the mechanism of action of various phytonutrients as there is a clear lack of knowledge in this aspect. The effects observed with phytochemicals in field conditions are variable owing to differences in doses, purity of the extracts used. Further research should be carried out on optimizing the dose and stability of the compounds and improve delivery techniques for more uniform and sustained *in vivo* effects.

2. Prebiotics/Probiotics
   Novel probiotics, prebiotics or their combinations should be developed as a drug-free strategy to control NE. Research focusing on identification and selection of suitable candidates, methods to improve delivery to ensure uniformity and sustained *in vivo* effects should be conducted.

3. Hyperimmune IgY
   Oral delivery of pathogen-specific egg yolk IgY should be developed as an antibiotic alternative strategy that can be adapted to commercial application in poultry diets. Novel oral delivery methods like microencapsulation should be tested to increase the *in vivo* efficacy of orally administered IgY and reduce inactivation of the antibodies in the acidic gastric environment. Improved delivery techniques will also help reduce the dose of antibodies required to confer protective efficacy compared to traditional administration.

4. Antimicrobial peptides
   Further research for identifying and characterizing novel antimicrobial peptides with efficacy against NE is desired. The group identified cost and delivery as one of the most important limitation associated with using AMPs. Methods to produce large quantities of peptides on a commercial scale at cheaper costs should be developed. *In vivo* efficacy of the peptides should be improved thorough improved delivery techniques and peptide modifications. Research to identify compounds that can increase the endogenous production of AMPs in birds should also be undertaken as a novel measure to increase the bird's inherent ability to combat pathogenic microorganisms thus eliminating or reducing the use of antibiotics.

5. Broad spectrum toxin binders
   Broad spectrum toxin binders should be developed for use against toxin producing enteric pathogens like CP. The panel agreed that the major limitation with this group of alternatives is that they are limited for use against enteric pathogens only.

6. Bacteriophages
   Bacteriophages with efficacy against CP should be identified and tested. The major limitations identified by the panel for research with bacteriophages include lack of *in vivo* challenge models and inconsistency in *in vivo* effects. Further research is warranted in these areas.

Coccidiosis

Avian coccidiosis is one of the most widespread infectious diseases of chickens. The etiologic agent of avian coccidiosis is *Eimeria*, a genus of eukaryotic obligate intracellular parasites belonging to the phylum Apicomplexa. These parasites infect the intestinal tract and are transmitted between chickens though a fecal-to-oral route. Clinical manifestations of infection include damage to the intestinal epithelium, decreased nutrient absorption, inefficient feed utilization, and impaired growth rate, which, in severe cases, may lead to mortality. The disease is also seen in subclinical form in the field without any overt clinical signs and causes reduced body weight gain and decreases feed efficiency leading to economic losses to the industry. Worldwide, the annual cost of coccidiosis in chickens is estimated to be more than $3 billion dollars.

Over the past few decades, in-feed anticoccidial drugs (ionophores) have been used to control the disease by interrupting the fecal-oral infection cycle. The regulations on the use of antibiotics in poultry feed also extends to ionophores making them unavailable for coccidiosis control in the near future. Several coccidiosis vaccines that contain live parasite mixtures of different *Eimeria* species are commercially available. While the use of *Eimeria* vaccines has been valuable in reducing the need for in-feed medication, it is also associated with risk of clinical outbreaks and economic losses due to early diminution in chicken growth. Experimental coccidiosis vaccines based upon recombinant *Eimeria* genes and proteins have been developed and shown to be effective in model systems of experimental infection, but are yet to be commercially marketed on a widespread basis.

The panel reviewed this disease as the second most important disease in need of antibiotic alternatives after NE in poultry. Coccidiosis is also one of the most important predisposing factor for the pathogenesis of NE and therefore development of optimal alternative control strategies against it would also help reduce the incidence of NE. In addition to the above mentioned alternatives that could also be developed against coccidiosis, the panel reviewed the following strategies as the main research needs.

1. Vaccines

Current coccidiosis control relies on the use of vaccines containing live oocysts. Birds are vaccinated at day of hatch and the development of immunity depends on the recycling of vaccinal oocysts in the litter and re-exposure of birds to live oocysts. The disadvantages of existing live vaccines include clinical outbreaks due to irregular cycling of the vaccinal oocysts. Birds that do not develop immunity either because of improper delivery or poor viability of the

vaccinal oocysts pose a greater risk for development of NE. The shelf life of the existing vaccines is very low. Research should focus on improving the vaccine delivery for uniform applications and improving the shelf-life. Research on development of attenuated vaccines should be conducted to reduce the risk of clinical outbreaks associated with live vaccine use.

2. Saponins

Saponins with demonstrated anticoccidial activity should be identified. Research should be undertaken to improve the efficacy of the developed products.

3. Essential oils

Novel compounds should be identified and research should focus on characterizing their mechanism of action. The major limitation with the use of essential oils is the reduced palatability of the feed. Methods to improve delivery of the products without affecting the palatability of feed should be developed.

E. coli *infections*

Secondary systemic infections of *E. coli in* immunosuppressed birds (viral disease or stress) pose a constant threat to poultry industry. Commercial vaccines exist but the efficacy is not uniform because of the occurrence of several strains and serotypes. There is a need for development of broad spectrum vaccine or vectored vaccines to improve the efficacy. Research should also focus on the development of novel therapeutics.

Infectious Bronchitis

The existing vaccine is not efficacious and the virus constantly mutates and evolves into a pathogenic strain. Research on development of vectored vaccines using a more conserved epitope should be considered. An up to database with all the sequence information of the new field strains (that evolve pathogenic as a result of mutations) should be maintained to predict the trends in mutagenesis and use that information towards vaccine development.

Foodborne agents

Salmonella spp. and *Campylobacter* are identified as the two most important foodborne pathogens. Vaccines against Salmonella are not effective owing to the vast number of serotypes (2500). Research on development of novel vaccines, antimicrobial peptides and phytochemicals should be undertaken. The panel addressed the issue of how to use immune modulation strategies to direct host immunity against commensal organisms like Campylobacter. Further research should also be done on targeted delivery of the products to the required region of the intestine. For example, to reduce *Campylobacter*, methods to deliver compounds directly to cecum with minimal loss in bioactivity should be developed.

Turkeys

The two most important diseases identified by the panel that needs immediate prophylactic or therapeutic measures include Clostridial Dermatitis/Cellulitis and Histomoniasis. In addition to these two diseases, other pathogens/diseases identified as important in turkeys include

coccidiosis, *E. coli* infections, *Salmonella*, *O*. *rhinotracheale* (ORT), *Bordetella avium*, cholera, *Campylobacter*, *Mycoplasma* and *Listeria monocytogenes*.

Clostridial *Dermatitis/Cellulitis*

There is no established disease model for this disease and research efforts should be directed towards development of a disease model. Similar to the pathogenesis of NE in chickens, pathogenesis of cellulitis involves a number of predisposing factors and most of them are not clearly characterized. Research on characterizing the etiology of this disease should be undertaken to identify potential preventive measures. Novel therapeutics should be developed.

Histomoniasis (Black Head)

The disease is caused by protozoan parasite *Histomonas meleagridis*. The disease is fatal in turkeys causing significant mortalities. Clinical signs include depression, inappetence, poor growth, increased thirst, mustard-yellow diarrhea, listlessness and dry, ruffled feathers. The drugs (arsenicals) once used for the treatment of Histomoniasis are no longer approved for use and currently there is no treatment available for the disease. Research should focus on the development of novel therapeutics against this disease.

Overall, the panel discussed the research path for better control of poultry (broilers and turkeys) pathogens using drug-free strategies. They identified major research gaps in poultry antibiotic alternative development plan and outlined the following major research needs.

1. Identify antibiotic-alternative products that allow animals to reach their genetic potential
2. Define symbiosis parameters for poultry gut microbiome. Identify microbial alteration trends associated with health and disease and characterize its relation with phenotypic expression. Identify specific compounds/metabolites produced by microbes that affect host's physiological/metabolic pathways in a positive way.
3. Better understand immunity of poultry and develop immune reagents and cell lines. Availability of reagents helps improve or develop diagnostics for poultry diseases especially subclinical infections without overt clinical signs
4. Develop novel antibiotic-alternative therapeutics
5. Improve delivery methods for developed alternatives to increase in vivo efficacy and for site targeted delivery.