Project Number: NC1206
Project Title: Antibiotic Resistance
Period Covered: October 1 2017 –May 14, 2018
Date of Annual Report: August 24, 2018
Annual Meeting Dates: May 14-15, 2018 (Minneapolis, MN)

Participant Attendance at Annual Meeting:
Bo Norby - Michigan State University; George Smith - Michigan State University (Administrative Advisor); Kollanoor Johny Anup – University of Minnesota; Michelle Soupir – Iowa State University; Peter Davies – University of Minnesota; Renata Ivanek - Cornell University (chair); Sophia Kathariou – North Carolina State University; Terry Lehenbauer – University of California- Davis; Timothy Johnson – Purdue University (secretary); Torey Looft – ARS USDA; Yrjo Grohn - Cornell University; Raghavendra Amachawadi - Kansa State University; Max Teplitski – NIFA USDA

Absent from the meeting
Alan Mathew - Purdue University; Bing Wang – University of Nebraska-Lincoln; Carlos Vilalta - University of Minnesota; Catherine Logue – Iowa State University; Fabio Lima - University of Illinois; Francis Welcome - Cornell University; Gireesh Rajashekara The Ohio State University; Jennifer Granick - University of Minnesota; Jeongmin Song - Cornell University; Johann Coetzee - Kansas State University; Joy Scaria - South Dakota State University; Ludek Zurek- Kansas State University; Paolo Moroni - Cornell University; Paul Plummer – Iowa State University; Qijing Zhang – Iowa State University; Randall Singer - University of Minnesota; Richard Pereira V. – University of California- Davis; Roderick Mackie – University of Illinois; Sandra Godden - University of Minnesota; Silva del Rio Noelia University of California-Davis; Sung Woo Kim - North Carolina State University; Timothy Goldsmith - University of Minnesota; Timothy Johnson - University of Minnesota; Yung-Fu Chang - Cornell University

Minutes of Annual Meeting
Max Teplinski NIFA update

- Explanatory Notes
- NIFA listens
- Budget requests
- The above could help us know where the funding might come and in what areas while we are waiting for the RFA to come out
- Sustainable Agriculture Systems (SAS) replaced challenge areas and is now live.
- Federal AMR funding landscape
- Current funding focuses around
    - Food processing/ safety
    - Cattle
    - Poultry
    - Resistance mechanism
    - Management of pests
    - Water contamination
    - Virulence
    - Soil management systems
    - AMR resistant bacteria
    - Pathogenic E coli

- AMR fits into all the categories of NIFA so put yourself in there
- Agencies: NIH, NIFA, ARS
- Directives
  - SAS – AMR is a big topic here.
  - Organic Ag
  - Fellowship programs came out last week. Administered through "workforce development"
- Examples:
  - Collateral benefits of vaccines on reducing food safety path
  - Surfaces prep to reduce biofilms
  - Probiotics and prebiotics to reduce pathogens
  - Engineered water nanostructures
  - AMR reduction in cattle
  - Campy vaccine in chickens from Univ AZ
- Funding projections for AFRI
  - Increased every year
  - To keep this up we need to communicate impacts
- NIFA needs to know impacts
  - Because people higher up are asking about how the funding has made a difference.
  - The only way NIFA gets info is for us to communicate to them.
- Impact can come in 3 flavors
  - Change in knowledge
  - Change in behavior*
  - Change in condition for society**
  - Feedback
- Products, outputs
  - Events
  - Services
  - Publication
  - Graduates
  - Patents
- Impacts
  - Productivity
  - Economic
  - Environmental
  - Social
  - Health and Well-being
- How to demonstrate change
  - Society learned something
  - People changed their ag approach
- What is the value to the taxpayer?

Annual Meeting next year: Same format as this year, May 13-14, 2019

Location: Atlanta to interact with CDC and invite a speaker. Kerry and Megan, Kathy Louge at CDC

Grohn Cornell Veterinary

Impact of interventions on resistance

- Did the VFD decrease resistance in farms?
- How big of a sampling area do we need to try to see impacts from meat to people

AMR in beef and cattle

- Pharmacodynamics, pharmacokinetics of antibiotics in vivo
- Transmission of resistance genes

Sustainable AM use in animal agriculture and public health impacts

- What would be the cost of removing all antibiotics from dairy production?
- o We do not really know the actual efficiency of antibiotics because there is no control.
- o About $63 per cow/year
- o What is the value of animal well-being?
- Living with resistance Project

What is the economic and agricultural optimum in management?

Terry Lehenbauer UC Davis Veterinary Medicine in Tulare, CA

AVMA: Definition of Antimicrobial Stewardship
Over the counter antimicrobials in vet med.
State bill passed in Oct 2015 Livestock use of antimicrobial drugs
Created a new program called antimicrobial use and …
Halted the sale of OTC drugs important to human medicine

AMR and drug use in dairy calves

- Survey of farmers an identifying resistance in calves from birth to weaning

Reduce AB use in dry cows

Dry cows are routinely given AB until lactation

BRD treatment feedback loop
Probiotics and Prebiotics in preweaned calves to reduce antibiotic use.

For extension: The leaders know what is recommended for AB use, but do the actual farm workers know what should happen? They are tracking what is actually happening on the ground.

We still have a big gap in the knowledge in actual antibiotic use on the ground. We are only using sales data to determine how much antibiotics are used in farms.

Sophia Kathariou NC State Listeria and Campylobacter in Turkeys

Campylobacter in commercial turkey flocks
Testing non-standard turkey breeds, e.g. "Bronze" for colonization rate
Studying for a long time a multiresistant and C. jejuni strain widespread in the industry in North Carolina
Campy is generally pan "resistant to tetracycline, frequently together with kanamycin
Observe multidrug resistant C coli in turkeys not fed antibiotics when flock was in close proximity to conventional turkey flocks
The resistant C jejuni are the most prevalent and potentially specific to some turkey breeds
Flies as a vector for campy transmission.
Competition experiments for resistant vs susceptible campy.

Peter Davies UMN College of Vet Med
6 new positions in Vet Med in microbiome and AB use and resistance
Tim Johnson plasmid biologist, microbiome
Noelle Noyes (beef) microbiome AMU/AMR
Randy Singer (poultry/env) AMU/AMR Epidemiology, NAE in multiple species
Kim Vanderwaal (epidemiology) AMR in wildlife
Peter Davies (swine) epidemiology MRSA/ S aureus, AMU
Sandra Godden (dairy) mastitis
Tim Goldsmith (cattle) medicine, stewardship
Jeff Bender (companion animals) epidemiology, stewardship
Jen Granick (companion animals) medicine, stewardship
Connie Gebhart, Maria Pieters

Reporting ABU in poultry seems to be a niche market over swine because it is easier to get away with. Some producers see that transparency will eventually be required and progressive people are trying to get ahead of the game. In addition, they will see that a ballot issue will be more gregarious than if they can come up with their own plan.
How can we get good data from producers about their actual AMU? Barriers to data collection? Depends on species for one.
Study on the behavior of veterinarians on prescribing ABx.

Raghu KSU Vet Med
A large group across Vet Med and college of Ag interested in AMR

Raghu's research specifically
Do AB alternatives select for AMR genes?
Addition of Cu selects for plasmids that encode resistance to Cu. These plasmids also encode resistance to antibiotics. They found this in both E coli and …

Also, saw increase in resistance in Staph aureus due to Zn in cattle

Looked for resistance genes in probiotics and found many resistance genes in probiotic products. However, not clear if they are transferable. Now looking at feed trials of probiotics w/ or w/o AMR genes to see impact in microbiome and pathogens AMR

Also looking at route of administration of tylosin. Water, feed or injected. To assess AMR in the gut

Medium chain fatty acids
Sorghum additives

If probiotics, essential oils and everything else selects for AMR what else is left? This is unclear, but probably more regulations or standards are required.

Michelle Soupir ISU
ISU has an AMR initiative to build teams in AMR research
Her research focuses on manure to edge of field fate of AMR. Are the practices focused on nutrient capture also sufficient for AMR capture in fields?
Project 1: ionophore addition to swine manure pits

See increase in ermB when manure containing tylosin is added to microcosms
Resistance in field, resistance levels return to within 6-12 months in a corn soybean rotation
With continuous corn plots, receiving manure had higher ermB and ermF in the drainage water
Now testing different manure application timing to try to get the manure application closer to the time of crop need, and the effect of AMR run-off
Prairie strips for surface run-off "treatment" water uptake by plants
In addition, water shed scale of ARG fate, they are seeing higher ARG levels in water shed scale than in plot scale.
Now comparing watersheds with differing levels of water management/conservation practices.

Torey Looft USDA ARS NADC
Food Safety
BMD project
Plasmid capture in Enterococcus in litter
Plasmid capture in Salmonella in birds
Egg shell as an intervention point to add probiotics
Gnotobiotic system for germ-free chickens

Bo Norby MSU Vet Med
Shannon Manning STEC
Linda Mansfield Campy
Jim Tiedje and co.
Wei Zhang env fate
Hui Li env fate

Bo's work is primarily phenotypic detection of resistance
Intervention strategies in ceftiofur in cattle
Effect of housing location on antimicrobial resistance in dairy cows
Cows that stay in the home pen had lower level of resistance than those that stay in hospital setting.
Giving ceftiofur in udder reduced coliforms in the GI tract. So seems there is transfer of drug from udder to GIT.
Commercial direct fed microbial on AR in preweaned calves increased ceftiofur resistance in E coli
Came up with a mixture of 10 non-resistant E coli to feed for competitive exclusion, this reduced ceftiofur resistant E coli by about 10-fold.

Anup Johny UMN Animal Science
Phytobiotics
Tested the translocation of Salmonella Heidelberg within the chicken. They saw high cecal colonization and some low presence in thigh, drumstick and breast in 14 day old poults.
However, in adult challenge the Salmonella did not translocate.
Essential oil application during processing prevented Salmonella Heidelberg attachment to turkey skin
Pre and probiotic combination reduced Salmonella colonization much better than either alone.

Renata Ivanek Cornell
Perceptions of producers
Qualitative and quantitative approaches to farmer surveys and interviews to understand their understanding of AMU and AMR
Themes that emerged from interviews with farmers:

1.	Unclear AMR means something that the animal immune system is
2.	They think their use is already very low and that animal health would suffer if use were reduced.
3.	Interest in prevention rather than treatment
4.	AMU decisions are informed by a variety of sources including their *own knowledge* and veterinarians.
5.	Consumer misconceptions about products from conventional farming

Veterinarian perspective:
1.	Farmers are trying to do what's best but might lack the set up (facilities) to change

Public:
1.	Don't know what antibiotics do
2.	Willing to pay more for NAE milk
3.	ABU in dairies is a threat?
4.	Think organic animals are treated better.

Mathematical modeling of resistance in cattle

Merla Merant NPL on AMR and the SAS program.

Possible future funding discussion:
Benchmarking for AMR in the different animal systems
Social aspects of perspectives. Consumer behavior.
Uniformity in the One-Health research
Data sharing between groups and how to do that? What databases do we need and how do we manage them?

Accomplishments:

Objective 1. Enhance surveillance and monitoring of antibiotic resistance and develop improved diagnostic tests.
California
-	Publication of two articles, currently in press (accepted for publication), focused on monitoring *Salmonella* antimicrobial resistance in cattle in CA; prevalence of drug residues in non-saleable milk (waste milk) in dairy farms in CA.
Nebraska
-	Bovine respiratory disease (BRD) is the most significant and economically costly diseases of cattle production. BRD is a multi-factorial disease with viral and bacterial pathogen components. *Mannheimia haemolytica* is one of the most frequently isolated bacterial pathogens that causes BRD. Antimicrobial resistance is an emerging concern as pan-resistance among *Mannheimia haemolytica and* other BRD pathogens has recently been reported. Resistance in *M. haemolytica* can be conferred through acquisition of mobile genetic elements (ICE) which can possess and/or acquire multiple genes conferring resistance. A real time multiplex PCR assay (rtPCR) was designed that detects four genes conferring macrolide *erm(42)*, *mph(E)*, *msr(E)*) or tetracycline (*tet*H) resistance. The assay was developed and validated using *M. haemolytica* isolates that had been subjected to whole genome sequencing and that were positive for an ICE containing one or more of the fours resistance genes. A comparative analysis was undertaken with this assay to a

classical culture and MIC testing approach. Lung tissues from 136 animals submitted to the Nebraska Veterinary Diagnostic Center for bovine respiratory disease diagnosis were included in the analysis. The tissues were subjected to isolate culture and susceptibility testing using routine methods. Additionally, DNA was extracted directly from lung tissue using a high-throughput DNA extraction method and then subjected to the rtPCR assay. Resistance or susceptibility of isolated *M. haemolytica* was determined by application of CLSI approved breakpoints for tilmicosin, tulathromycin and oxytetracycline. Optimal Cq cutoff values that maximized sensitivity and specificity with isolation of a macrolide or tetracycline resistant *M. haemolytica* were calculated using Receiving Operating Characteristic (ROC) curve analysis. Following are the primary findings: (i) The optimal Cq cutoff value for testing macrolide resistant *M. haemolytica* ranged from 28.9-32.5, with Kappa statistic (K) showing the agreement between the rtPCR and culture-based methods ranging from 0.66-0.79 with Sensitivity (Se) 80-100% and Specificity (Sp) 80-90%; (ii) For tetracycline resistance Cq cutoffs were calculated to be 31, with Se of 87.5 and Sp of 86.2 and a Kappa of 0.72; (iii) Overall detection of resistance genes by rtPCR had mostly moderate levels of agreement with isolation of resistant M. haemolytica from lung tissues. The presence of other ICE containing organisms within lung may have increase the level of disagreement between the two assays.

New York
- Continued to analyze the NARMS data to understand its usefulness as antimicrobial monitoring tool. Currently working on multidrug resistance over-dispersion, which was observed in our previous paper (Zawack et al., Preventive veterinary medicine 152, 81-88).

Objective 2. Determine the ecology and mechanisms involved in resistance and transmission of resistance.

California
- Publication of one article focused on effect of feeding waste milk on enteric microbiota.

Michigan
- A study was conducted to compare the response to two treatments with ceftiofur crystalline free acid (CCFA; Excede®) in dairy cows with metritis in cows remaining in their fresh pen and cows moved to the Hospital pen. The working hypothesis was that dairy cows treated with ceftiofur for acute metritis will have higher levels of resistance to ceftiofur if they are placed in the hospital as compared to staying in their home pen. Forty-five cows were included in this study, 22 and 23 cows remained in their fresh pen or were moved to the hospital pen, respectively. Fecal grab samples were collected on day zero and every three day until day 30. Total coliform counts and phenotypic resistance to ceftiofur in coliform bacteria were assessed phenotypically in every fecal sample. Additionally, 16S rRNA gene sequencing was conducted, and antibiotic resistance genes were targeted via 144 primer sets in selected samples. Results: (i) Treatment with CCFA significantly decreased coliform counts compared to baseline (day 0); (ii) Treatment with CCFA significantly increased ceftiofur-resistant coliform counts compared to baseline (day 0); (iii) For 2nd and higher lactation cows, cows in placed in the hospital pen had a significantly higher count of ceftiofur-resistant bacteria compared to cows remaining in their fresh pen; (iv) The number of previous lactation cycles and potential exposures to antibiotic shaped the relative abundance of ARGs in the gut microbiome prior to and immediately after treatment; (v) The *blaCMY* genes, typically associated with ceftiofur resistance and approximately 20 additional co-selected mobile genetic elements and ARGs increased following treatment; (vi) Resistant bacterial populations were more persistent in cows that had previously undergone multiple lactation cycles; (vii) Susceptible populations that initially decreased 3-9 days following injection, varied in 30 day recovery due to separation of cows into hospital pens.

Indiana

- Characterizing antibiotic resistant plasmids that were horizontally acquired by Enterococcus faecalis JH2-2 in poultry litter. Horizontal gene transfer is an important mechanism by which bacteria can become resistant to one or more antibiotics be acquiring the needed genes from their bacterial neighbors. We found that *Enterococcus faecalis* JH2-2 usually acquires two resistance gene at a time.
- Study of the effects of bacitracin methylene disalicylate (BMD), an in-feed antibiotic that continues to be used for feed efficiency as well as for disease treatment on turkey intestinal microbial communities over 14 weeks. Two-hundred-forty turkeys were divided into three treatment groups (no antibiotic control, sub therapeutic BMD (50 g/ton), and therapeutic BMD (200 g/ton)). Cecal contents were collected to characterize microbial population shifts using high-throughput 16S rRNA gene amplicon and shotgun metagenomic analysis. Both concentrations of BMD had immediate and lasting impacts on the microbiota structure, reducing species richness in the BMD-treated turkeys through the end of the study. Members of the microbiome that were enriched due to BMD included members of *Lachnospiraceae* and *Ruminococcaceae* families, while members of the TM7 phylum and *Lactobacillus* were eliminated or decreased due to BMD. Antibiotic resistance genes (ARGs) (n= 155) were annotated in the assembled metagenomic contigs of which, 96 contigs contained 2 to 6 resistance genes. Some contigs contained multiple classes of antibiotic resistance (i.e. aminoglycoside and streptothricin resistance genes) or a resistance gene(s) with a mobile genetic element. Normalized counts of mapped reads demonstrated that therapeutically fed birds had 18, 77, and 106 ARGs enriched (q < 0.05) on day 7, 35, and 78, respectively. Enriched resistance genes potentially confer resistance to beta-lactams, aminoglycosides, tetracyclines, vancomycin, and macrolides. Complete type G vancomycin resistance operons (vanRSHGXY) were detected together on contigs and was enriched due to BMD. Vancomycin-resistant Enterococcus (VRE) is a significant issue in poultry production, and factors that increase vancomycin resistance should be further identified. Furthermore, it appears that BMD co-selects for many types of ARGs, including many important for human medicine.

Nebraska
- Antimicrobial resistant bacteria and genes may be present in cattle production environments, persist through manure storage and its application on crop lands, and contaminate environment causing risks for human health. Three antibiotic treatments (Control – no antibiotic use, Tylan and CTC) were introduced to beef cattle in feed and the prevalence and concentration of resistant bacteria including *E. coli, Salmonella* and *Enterococcus* harboring antimicrobial resistance genes were determined in feces, hides of the cattle and pen surface materials during beef cattle production, in manure stockpiles post production and in manure amended soils. In addition, bioaerosol samples were collected in the upwind and downwind environment of the beef cattle pens, manure stockpiles and manure application sites to evaluate the transfer of antimicrobial resistant bacteria through air and/or dust. The major findings are: (i) *Salmonella* was not detected on beef cattle feedlot, manure stockpiles or amended soil throughout the project; (ii) There is no significant difference in the level of bacteria or resistant bacteria in feces and on hides collected at the feedlot among three antibiotic treatment groups including cattle fed with no antibiotics, with Tylan and chlortetracycline; (iii) During a 3-month manure stockpiling, the concentration of generic *E. coli* and *Enterococcus* in stockpiled manure dropped more than 100 times. By the end of the storage period there was no resistant bacteria detected or enumerated in almost all samples; (iv) Resistant *E.coli*, *Salmonella*, or *Enterococcus* in amended soils were all below the level of quantification in this study; (v) Generic *E.coli* and *Enterococcus* can be transmitted in air over a distance of up to 80m at feedlot, manure stockpiles and land application sites (greater distance was not evaluated in this study), but the resistant bacteria was only detected at the manure storage sites at a very low level.

Objective 3. Develop and evaluate interventions (including alternatives to antibiotics) that reduce antimicrobial resistance in food production systems.

New York

- We have found an extremely promising intervention alternative to antibiotics, a tomato protein with an outstanding bactericidal effect on multidrug-resistant *Salmonella* in an acidic environment. Of note, *Salmonella* is an intracellular pathogen, which alters host cells to an acidic environment that is favorable for its intracellular survival and replication. As we do not know the identity of this protein yet (although we know that this protein possesses superior bactericidal activities), an immediate goal is to identify this protein via standard biochemical approaches in conjunction with mass spectrometry analysis.
- Ongoing project to evaluate: (i) the socio-economic impact of public policies implemented at the national level to fight against AMR; and (ii) the need to compensate farmers for the hypothetical losses associated with a decrease in AMU.

North Carolina

- Investigated nutritional strategies to enhance intestinal health status of newly weaned pigs fed diets without antimicrobial supplements in this year. Outcomes are to provide basis of intestinal complications due to weaning stress without dietary supplementation of antimicrobials. Key outcomes are: (i) Weaning process causes intestinal complications to pigs including inflammation and oxidative stress in the small intestine; (ii) Increased inflammation and oxidative stress to intestinal mucosal tissues cause gut leaking; (iii) Increased gut leaking with intestinal complications cause damages to mucosal structures (villi); and (iv) Pigs' ability for nutrient digestion and absorption is impaired causing reduced growth efficiency.

Nebraska

- Concerns about pharmacologic antibiotic use in beef cattle production arise due to the perceived possibility of the emergence and transmission of AMR bacteria to the beef supply. However, it is not fully known if AMR bacteria strains have enhanced ability to resist the bactericidal efforts imposed by commercial antimicrobial interventions (AMIs) used in beef processing. The objective of this study was to compare the efficacy of different AMIs on reducing AMR and non-AMR *Salmonella* contamination on fresh beef. Two approaches were employed. In approach 1, a laboratory experiment was designed to determine whether AMI alone or sequential treatments equally affect inoculated AMR and non-AMR *Salmonella*. In approach 2, systematic review and meta-analysis were conducted to summarize what has been known for the impacts of commercially available AMIs on beef-associated AMR and non-AMR *Salmonella* strains. Experiment results showed there was no significant difference in the concentration reduction between AMR and non-AMR *Salmonella* on the fresh beef surface treated with the same antimicrobial. The order of decontamination efficacy is lactic acid (LA) = LA + peracetic acid (PAA) > PAA, where LA + PAA is for the combination of LA and PAA in sequence. From the systematic review, nine citations with eligible data were used for meta-analysis, from which acid, oxidizer, physical, thermal and other interventions were evaluated. Efficacy ratio for a particular intervention was computed as the ratio of the efficacy of eliminating AMR strains to the one for non-AMR strains. Findings showed most of the efficacy ratios were around 1.0. In general, most antimicrobial interventions employed at the beef processing can exert similar bactericidal effects regardless of the antibiotic resistance profile

Objective 4. Quantify animal health, public health, social, economic, and environmental impacts of antimicrobial interventions in food production systems.

New York

- Used a mixed-methods (qualitative and quantitate) approach to elucidate the attitudes, beliefs and behaviors of dairy farmers and veterinarians in New York (NY) regarding antibiotic use and resistance and the knowledge, willingness and abilities amongst them to amend antibiotic use, particularly antibiotics of medical importance. Completed semi-structured in-person interviews with 12 conventional dairy farmers and 6 bovine veterinarians in NY. Preliminary findings indicate that conventional dairy farmers believe they are already using antibiotics judiciously on their farms. They are concerned that additional reductions in antibiotic use would compromise animal welfare, increase production costs, and increase the price of dairy products. Conventional farmers expressed frustration with consumers' perceived lack of knowledge and misconceptions about the dairy industry, particularly regarding antibiotic use.
- Completed phone-based national level survey of the general public involving 1,000 US adults and preliminary statistical analysis. Preliminary results indicate that (i) generally, more educated members of the population perceive antibiotic use in cattle as a public health threat and are willing to pay more for milk produced by cows raised without antibiotics; (ii) half of the participants did not know that antibiotics only kill bacteria and those who did know tended to be more educated and more favorable to organic farming; and (iii) almost 50% of participants perceived treatment of cattle on organic farms as better than on conventional farms. These people tend to be younger and willing to pay much more for milk from cows raised without antibiotics.
- Ongoing mathematical modeling study to explore potential reductions in antibiotic resistance on feedlots and test a novel hypothesis that nutrient-enrichment and environmental conditions modulate the effect of antibiotic use on antibiotic resistance. Preliminary results suggest that increased nutrients from deposited manure may support the proliferation of antibiotic resistance irrespective of antibiotic use.

Nebraska
- Antibiotic usage in animal primary production is considered as one of factors determining the selection and spread of antimicrobial resistant determinants and pathogens to humans. Meat animal products, including beef products, may contribute to the occurrence of antimicrobial resistant bacterial infections in humans, as ABR pathogens harbored in these livestock can potentially escape the primary production, enter the food supply chain, survive interventions during meat processing, and be transferred to consumers. In this project, a quantitative microbial risk assessment model was established to evaluate the impacts of antibiotic usage on feedlot on the occurrences of generic or β-lactam resistant *E. coli* in three different beef products: intact and non-intact (i.e. tenderized and ground) beef. The major findings were: (i) From the baseline model, the number of overall generic *E. coli* (all *E. coli* regardless of resistant profile), as well as the number of β-lactam resistant *E. coli* were estimated as the model outputs. In general, contamination level of general *E. coli* is much higher than β-lactam resistant *E. coli* (10^4-10^6 times higher), but both are at very low level. For both types of *E.coli*, the contamination is predicted highest in ground beef, followed by tenderized beef cuts and then intact beef cuts; (ii) two alternative antibiotic use scenarios were compared with the baseline scenario. In the baseline scenario, the ratio between beef cattle raised on conventional farm (CONV) vs without antibiotics (RWA) was 9:1. In alternative scenarios, the ratios were changed to 5:5 and 1:9, in which less and less antibiotics are simulated to be used during the primary production of beef cattle. The scenario analysis showed that with less antibiotic uses on feedlot the contamination of generic *E. coli* in beef product serving would either stay at a similar level or with slight increase, while the β-lactam resistant *E. coli* contamination would with either stay a similar level or with slight increase. However, the absolute changes are very small, considering the risk of contamination of on various beef products is predicted to be at a very low level from the baseline.

Objective 5. Create and deliver programs on antibiotic stewardship in food production systems through education and outreach.

Indiana
- Online courses will be developed at Purdue University (PU) to communicate findings from AMR studies to people working in food production systems. A current online course at PU will be used as the foundation for formatting and logistics. The course will be divided into modules based on production type. For example, one module will focus on the milk industry and include the findings from the current study on selective dry cow therapy at the University of Illinois.
- PU Extension will expand upon the Purdue Food Animal Education Network program, which uses web-based media and experiential learning to provide research-based information to consumers who have little to no connection to livestock production. Information regarding AMR will be a key element to this informational resource.

Impacts:
- A promising intervention alternative to antibiotics, which has the potential to be implemented in food production systems. The implementation of this new organic molecule in the food industry, in the long-run, will lower the amount of antibiotics used in food production systems and hence reduce the development of drug resistance in enteric bacteria.
- Ban of antimicrobial use as growth promoting supplements (AGP) in fact challenged pig producers with keeping good intestinal health of pigs after weaning. Understanding process of intestinal complications upon weaning provides ways to nutritional strategies for alternatives to the use of AGP in pig diets.
- Obtained insight into temporal chances in antimicrobial susceptibility of *Salmonella* isolated from cattle in a Veterinary Hospital. *Salmonella* is a important human (zoonotic) and animal pathogen, and this study provide information relevant to better evaluate how different factors may affect prevalence of AMR in this pathogen over time.
- Evaluated milk parameters and farms practices that could affect the presence of antibiotic residues in waste milk at commercial farms. This information will be important to estimate the hazard that waste milk may represent for selection of antimicrobial resistance; it also provide information to generate interventions that target the main drug of relevance to animal and human health.
- Evaluated the longitudinal impacts of feeding pre-weaned calves milk containing drug residues on the fecal microbial function. . Antibiotic residues resulted in a significant difference in relative abundance of microbial cell functions between control and treatment group, especially with genes linked with stress response, regulation and cell signaling, and nitrogen metabolism. These changes could directly impacts selection and dissemination of virulence and antimicrobial. Findings from this study support the hypothesis that drug residues, even at very low concentrations, impact the gut microbiota of calves and result in changes in the functional profile of microbial populations.
- Identified potential control points that can influence ARG dissemination following therapeutic treatment of dairy cows. Aggregating cows when they are treated with antibiotics might increase antibiotic resistance genes in these cows as compared to leaving them with their original group-mates.
- Improved understanding of perceptions of dairy farmers about antibiotic use are expected to aid the design of effective and targeted social marketing campaigns to promote judicious antibiotic use in agriculture.
- Improved understanding of the knowledge and perceptions of US consumers support that consumer preferences for organic products may be impacting farmers' antibiotic use behaviors, and thus may impact human health and animal health and welfare.

- Improved understanding of the effect of manure management and environmental factors on the dynamics of antibiotic resistance in a feedlot pen may lead to improved management of antibiotic resistance with positive economic and health impacts.
- Although bacitracin is not critical to human medicine, its potential co-selection of ARGs needs to be considered when used in animal production.
- To date, the anchor website (www.purdueFAEN.com) has over 22 informational pages and the site has been visited over 200,000 times with over 75,000 unique visitors. Educational videos are hosted on its corresponding YouTube channel (viewed over 6,000 times). Much of the information is a direct extension of Purdue Extension faculty research with topics including the role of antibiotics in livestock production, foodborne illness trends, emerging diseases, and environmental impact of livestock production.
- The data analysis pipeline is established to support a faster development of AMR rapid detection methods. Potential audience of the approach are primarily veterinary diagnostic laboratories in Nebraska and other regions, as well as in human clinical settings, because genes conferring AMR in pathogens from different hosts (such as food-producing animals and humans) are nearly identical.
- The implementation of AMR rapid detection methods will facilitate a real-time individual-based AMR diagnosis, which can guide effective antibiotic treatment with higher success rate and promote judicious antibiotic use in veterinary and human clinical settings. In addition, the availability of AMR rapid detection method will enable a large-scale surveillance that is considered as the cornerstone in understanding and mitigating AMR spread.
- Improved understanding of the link between antibiotics use and resistance development, and characteristics and transfer of AMR bacteria and their determinants in the environment. This information will facilitate a science-based risk management process of minimizing the levels of antibiotic uses and AMR bacteria/determinants introduced into the environment and subsequently decreasing the spread to humans.
- Determined which AMR bacteria are less sensitive to most antimicrobials currently used and which antimicrobial is the most effective in reducing AMR bacterial contamination determining a potential Standard Operating Procedure (SOP) for the use in processor Hazard Analysis and Critical Control Points (HACCP) plan development and/or reassessment.
- The application of QMRA approach can facilitate a risk-based process to determine if with the reduction of antibiotic uses during the primary production stage, the risk of human exposure to resistant contamination through beef consumption can be significantly reduced.
- Findings from the QMRA research can potentially support cattle businesses to better communicate with and address the concerns from affected individuals who may have perceptions on potential negative impacts arising from the development and transfer of ARB/ARG due to the use of antibiotics in their operations.

Publications:
- Chen, H., S. Zhang, I. Park, and S. W. Kim. 2017. [Invited] Effects of supplemental protease on growth performance, nutrient digestibility, and gut health in nursery pigs fed diets with corn or sorghum. Animal Nutrition 3:359-365 (https://doi.org/10.1016/j.aninu.2017.09.005).
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Presentations:
- Beauvais, W., Jennings, R., Arthur T., **Ivanek, R**. A mathematical model to explore potential reductions in antibiotic resistance on feedlots. December 4-5, 2017, CRWAD 2017. Oral presentation.
- Wemette, M., Beauvais, W. and **Ivanek, R**. Antibiotic use and resistance: perceptions of dairy farmers and epidemiological modeling of a farm. The Pew Charitable Trusts, Washington DC, June 26, 2017. Oral presentation.
- Wemette, M., Beauvais, W., Ceres, K., Greiner Safi, A., Shapiro, M., Moroni, P., Welcome, F.L., **Ivanek, R.** Understanding New York State Dairy Farmers' Perceptions Regarding Antibiotic Use in Cattle and Antibiotic Resistance. Merial-NIH Veterinary Scholars Symposium, NIH Bethesda, MD, August 3-5, 2017. Poster presentation.
- Wemette, M., Beauvais, W., Greiner Safi, A., Shapiro, M., Moroni, P., Welcome, F.L., **Ivanek, R.** A qualitative study of New York State dairy farmers' perceptions regarding antibiotic use and resistance in dairy cattle. December 4-5, 2017, CRWAD 2017. Oral presentation.
- Yangjunna Zhang, Sapna Chitlapilly Dass, Tommy Wheeler, Nor Kalchayanand, Bing Wang. Comparison of the Effectiveness of Antimicrobial Interventions on Reducing Antibiotic Resistant and Susceptible Beef-Associated *Salmonella*" (Poster presentation: ID# 17711, P3-168) at International Association for Food Protection Annual Meeting 2018 in Salt Lake City, Utah

Report:
- Bing Wang. Ecology and Transfer of Antimicrobial Resistant Bacteria and Genes in the Feed-yard and Land-application Environment When Cattle are Fed Different Antibiotics.

Research Project Summary Outline - Final Report to National Cattlemen's Beef Association and Nebraska Beef Council. April 30, 2018.