

NC1202 Annual Accomplishments Report

Project/Activity Number: NC1202

Project/Activity Title: Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety

Period Covered: 10/01/2020 to 09/30/2021

Date of Report: 12/20/2021

Annual Meeting Dates: 12/04/2021

Annual Meeting Participants (total n=33):

On-site attendee (n=19): Qihong Wang (wang.655@osu.edu), Orhan Sahin (osahin@iastate.edu), Weiping Zhang (wpzhang@illinois.edu), Devendra Shah (dshah@wsu.edu), Zhengguo Xiao (xiao0028@umd.edu), Gireesh Rajashekara (rajashekara.2@osu.edu), Jun Lin (jlin6@utk.edu), Glenn Zhang (glenn.zhang@okstate.edu), Raghu Amachawadi (agrachav@vet.k-state.edu), Bledar Bisha (bbisha@uwyo.edu), Milena Saqui-Salces (msaquisa@umn.edu), TG Nagaraja (tnagaraj@vet.k-state.edu), Joy Scaria (joy.scaria@sdstate.edu), Radhey Kaushik (Radhey.kaushik@sdstate.edu), Qijing Zhang (zhang123@iastate.edu), Phil Hardwidge (hardwidg@vet.k-state.edu), Kush Yadav (Yadav.94@isu.edu), Scott Kenney (kenney.157@osu.edu), and Yung-Fu Chang (yc42@cornell.edu).

Virtual meeting attendee (n=14): Frank Blecha (blecha@vet.k-state.edu), Rodney Moxley (rmoxley1@unl.edu), Roy Curtiss (rcurtiss@ufl.edu), Connie Gebhart (gebha001@umn.edu), Hua Wang (wang.707@osu.edu), Jay Reddy (nreddy2@unl.edu), Xiaolun Sun (xiaolun@uark.edu), Getahun Agga (getahun.agga@usda.gov), Linda Saif (saif.2@osu.edu), Mo Saif (saif.1@osu.edu), Yuhua Farnell (yfarnell@tamu.edu), Yanhong Liu (yahliu@ucdavis.edu), Kathe Bjork (kathe.e.bjork@usda.gov), Anastasia Vlasova (vlasova.1@osu.edu).

Brief Summary of Minutes of Annual Meeting from 8:30 am – 5:30 pm on 12/04/2021:

- Opening welcome by Dr. Qihong Wang (Chair)
- Opening remarks - Dr. Frank Blecha, NC1202 Administrative Advisor, Kansas State University
- Dr. Kathe Bjork, National Program Leader, USDA NIFA gave a presentation on funding and perspectives.
- Self-introduction and current collaboration status with other members
- Business meeting: Discussed budget, student awards, future meeting format, new proposal submitted, logo ideas for NC1202, and updating membership; Elected new secretary
- Voluntary presentations (n=7, on-site and virtual) of research followed by active discussions (4 hr). Several members expressed that they got good ideas for their own projects from other member's work and suggestions and may seek new collaborations among members.

Accomplishment Summaries (focus on novel discoveries and collaboration efforts):

1. We quantified the frequency, distribution, and variability of fecal shedding and super-shedding of STEC in feedlot cattle over time and determined the effects of dietary fiber from distillers grains on the prevalence STEC in feedlot cattle.
2. We determined the efficacy of a humanized monoclonal antibody specific for Shiga toxin-2 to protect against post-diarrheal brain infarction in gnotobiotic piglets infected with *E. coli* O157:H7.
3. We developed an infrared radiation (IR) spectroscopy rapid detection method for MDR *Salmonella enterica*.
4. We studied the prevalence of *Salmonella* in backyard poultry in WA State. We completed antibiograms and whole genome sequence assembly and annotation, followed by antimicrobial resistance gene analysis of a set of bovine *S. enterica* isolates, performed the global transcriptomic analysis of tyramine and D-glucuronic acid metabolic pathways in *Salmonella*, reported the genomic organization and role of *Salmonella* pathogenicity island 13 in nutritional fitness of *Salmonella*, and identified common highly expressed genes of *S. Enteritidis*.
5. We investigated the effect of different fluoroquinolone treatments (enrofloxacin and danofloxacin) on the development of fluoroquinolone resistance in *Campylobacter jejuni* in calves.
6. We evaluated the potential of a novel approach to combat fluoroquinolone resistant *Campylobacter* by inhibition of expression of the MDR efflux gene *cmeABC* via antisense peptide nucleic acid (PNA) and

identified two novel narrow spectrum *Campylobacter* specific small molecule growth inhibitors, which reduced *C. jejuni* burden in broiler chicken's ceca and had minimal impact on the cecal microbiota.

7. We showed that experimental evolution of *Campylobacter jejuni* leads to loss of motility, *rpoN* (σ 54) deletion, and genome reduction.
8. We have successfully infected the enteroids with *Lawsonia intracellularis*, thus providing a swine-specific *in vitro* model for the study of proliferative enteropathy.
9. We identified and characterized a restriction-modification enzyme reducing conjugation efficiency in *Campylobacter jejuni*.
10. We found that decreased population of European starlings was not associated with corresponding reductions in bovine fecal prevalence of ciprofloxacin-resistant *E. coli*.
11. We have successfully produced enterobactin (Ent) conjugate vaccine candidates that are promising to control enteric pathogens, such as *E. coli*, *C. jejuni*, and *S. enterica*, in food animals.
12. We performed multiple chicken trials to test three bacterial bile salt hydrolase (BSH, a promising antimicrobial target) inhibitors and investigated the biological basis of the antibacterial phenomenon using state-of-the-art metabolomics, metagenomics, and computational approaches.
13. We successfully developed innovative and cost-effective encapsulation technologies to improve viability and stability of powdered probiotics for controlling enteric diseases.
14. We evaluated *Campylobacter* colonization in chickens vaccinated with several recombinant attenuated *Salmonella* Vaccines expressing *Campylobacter* surface antigens and found highly promising results.
15. Our studies have identified small molecule inhibitors, probiotic bacteria and their derived peptides having a promising effect in reducing *Salmonella* load in chickens.
16. we developed mRNA and adenoviral vaccine candidates and monoclonal antibodies against *C. jejuni* and *Clostridium perfringens* infections.
17. We profiled the ileal microbiomes of chickens following an experimental induction of necrotic enteritis (NE) and correlated them with disease severity.
18. We developed transparency-based electrochemical and paper-based colorimetric analytic detection platforms to detect indicators of fecal contamination *E. coli* and *Enterococcus* species in a single assay.
19. We established that Porcine epidemic diarrhea virus (PEDV) vaccination of sows in the second trimester optimally stimulated the gut-MG-sIgA axis resulting in greatly improved lactogenic immune protection in suckling piglets.
20. Using our newly developed virus-like particle (VLP)-based ELISA, we demonstrated that gilts (first parity animals) with diarrhetic litters had significantly lower rotavirus C (RVC) antibody titers in milk (but not in serum) compared with those of sows with healthy litters.
21. Our comparative pathogenesis studies demonstrated that current vs. historic RVC strains can cause more severe clinical disease and higher virus replication.
22. We discovered novel probiotics for the swine industry to combat rotavirus diarrhea.
23. We demonstrated that protein-calorie malnutrition induced an immune-deficiency state in gnotobiotic pigs aggravating rotavirus disease and reducing rotavirus vaccine efficacy.
24. We have demonstrated that *E. coli* Nissle 1917 modulates rotavirus immunity and it can be successfully formulated to increase its stability and simplify its administration.
25. We established a reverse genetics system for porcine deltacoronavirus (PDCoV) and investigated whether PDCoV evolved directly from sparrow deltacoronavirus.
26. We found that bovine neutrophils form extracellular traps and produce IL-10 in response to *Ostertagia ostertagi*, a gastrointestinal parasite of cattle.
27. We showed that inhibitors for *Cryptosporidium parvum*'s unique bacterial-type lactate dehydrogenase (CpLDH) and plant-like pyruvate kinase (CpPyK) enzymes of the glycolytic pathway can stop the growth of this parasite and prevent disease development in infected mice models.
28. We recently described our vaccine to control *C. perfringens*-induced necrotic enteritis. This vaccine was licensed by APHIS and commenced to be marketed by Huvepharma as AVERT.

Impacts: Enteric pathogens of food animals not only cause diseases in animals but some are also zoonotic pathogens and often cause sporadic cases and outbreaks of gastroenteritis in humans. The collaborative efforts of NC1202 multistate group focus on both applied and basic research, including the identification and characterization of emerging enteric pathogens, surveillance of the mutants of existing pathogens, development of improved diagnostic assays and prevention methods (e.g., vaccines and antibiotic alternatives) to control and prevent enteric infections of food animals and humans under the One Health concept. New knowledge is timely published and distributed among producers and shareholders.

Publications of Collaborations:

1. Hyesuk Seo, Carolina Garcia, Xiaosai Ruan, Qiangde Duan, David A Sack, **Weiping Zhang**. 2021. Preclinical characterization of immunogenicity and efficacy against diarrhea from MecVax, a multivalent enterotoxigenic *E. coli* vaccine candidate. *Infect Immun.* 89:e0010621.
2. Atsushi Hinenoya, Xing-Ping Li, Ximin Zeng, **Orhan Sahin, Rodney A Moxley**, Catherine M Logue, Barbara Gillespie, Shinji Yamasaki, **Jun Lin**. 2021. Isolation and characterization of *Escherichia albertii* in poultry at the pre-harvest level. *Zoonoses Public Health.* 68:213-225.
3. Guo Y, Candelero-Rueda RA, **Saif LJ, Vlasova AN**. 2021. Infection of porcine small intestinal enteroids with human and pig rotavirus A strains reveals contrasting roles for histo-blood group antigens and terminal sialic acids. *PLoS Pathog.* 17(1):e1009237. doi: 10.1371/journal.ppat.1009237.
4. Wenjing Geng, Sarah L Long, Yun-Juan Chang, Arnold M Saxton, Susan A Joyce, **Jun Lin**. 2020. Evaluation of *in vivo* efficacy of bile salt hydrolase inhibitors using chicken model system. *Scientific Reports.* 10:4941
5. Stephanie N Langel, **Qihong Wang, Anastasia N Vlasova, Linda J Saif**. 2020. Host Factors Affecting Generation of Immunity Against Porcine Epidemic Diarrhea Virus in Pregnant and Lactating Swine and Passive Protection of Neonates. *Pathogens* 18;9(2):130.
6. Xiaoyu Niu, Yixuan J Hou, Kwonil Jung, Fanzhi Kong, **Linda J Saif, Qihong Wang**. 2021. Chimeric Porcine Deltacoronaviruses with Sparrow Coronavirus Spike Protein or the Receptor-Binding Domain Infect Pigs but Lose Virulence and Intestinal Tropism. *Viruses* 13:122.
7. Patricia A. Boley, Moyasar A. Alhamo, Geoffrey Lossie, Kush Kumar Yadav, Marcia Vasquez-Lee, **Linda J. Saif, and Scott P. Kenney**. 2020. Porcine Deltacoronavirus Infection and Transmission in Poultry, United States. *Emerg Infect Dis.* 26:255-265.
8. Shifeng Wang, Charles L. Hofacre, Soo-Young Wanda, Jingyu Zhou, Richard A. Callum, Bob Nordgren, Roy Curtiss III. 2022. A triple-sugar regulated *Salmonella* vaccine protects against *Clostridium perfringens*-induced necrotic enteritis in broiler chickens. *Poultry Science* 101:101592.

Fund Leveraging via collaborative grants between stations and members.

1. Dr. Jun Lin at The University of Tennessee has an active collaboration with Dr. Glenn Zhang at Oklahoma State University for the ongoing NIFA Food Safety Challenge Grant (NIFA 2018-68003-27462). Novel non-antibiotic approaches for mitigation of antimicrobial resistance in poultry.
2. Zhang, W (Illinois) collaborates with Moxley, R. (Nebraska), Cernicchiaro, N. (Kansas) on USDA NIFA project (2017-67015-31471) entitled “A broadly protective vaccine against porcine post-weaning diarrhea (PWD)”. 2020-2025.
3. R.A. Moxley and J.D. Loy (University of Nebraska-Lincoln) collaborate on a project entitled “Genetic Analysis and Rapid Detection of Multidrug-Resistant *Salmonella enterica* Isolates from Cattle”. Nebraska Beef Council and National Cattlemen’s Beef Association. 10/1/2020-9/30/2021.
4. Y. Sang (Tennessee) has collaborated with Drs. Wenjun Ma and Frank Blecha (Kansas) on the antiviral potency and functional novelty of porcine interferon-omega subtype (USDA NIFA 2018-67016-28313).
5. Zhang, Q and Sahin, O (Iowa) collaborate with J.F. Coetzee and Z. Lin (Kansas) on USDA NIFA integrated project (2017-68003-26499): Mitigation of fluoroquinolone-resistant *Campylobacter* in cattle. 2017-2022.
6. R. Curtiss (Florida) and G. Rajashekara (Ohio) have collaborated on the development of a food safety vaccine to control *Salmonella* Enteritidis and reduce *Campylobacter* in poultry (USDA NIFA 2017-67017-26179).
7. Kenney, Saif and Wang (Ohio) collaborate on NIFA, USDA 2020-67015-31618. Title: Functional genomics approach in livestock to delineate host factors critical for emerging virus replication.

8. Saif, Vlasova, and Wang (Ohio) collaborate on NIH-USDA Dual Purpose with Dual Benefit (NICHD R01HD095881): Research in Biomedicine and Agriculture. Title: The impact of vitamin A on the gut-mammary gland-secretory IgA axis during enteric viral infections.
9. Drs. Kenney and Wang (Ohio) collaborate on OSU Seeds grant entitled “Interspecies transmission mechanisms of deltacoronavirus from birds to pigs”.