

2016 Annual Report
For NC-1202:
Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety

Report Information

- Annual meeting: Dec 3-4, 2016, in Marriott Hotel (Michigan/Michigan State Room), Chicago, IL, in conjunction with the annual CRWAD meeting
- Period the Report Covers: 10/01/2015 to 09/30/2016

Participants:

Peter Johnson, USDA
Margo Holland, USDA
Roy Curtis, Arizona State University
Qijing Zhang, Iowa State University
Frank Blecha, Kansas State University
Weiping Zhang, Kansas State University
Philip Hardwidge, Kansas State University
M.M. Chengappa, Kansas State University
T.G. Nagaraja, Kansas State University
Linda Saif, Ohio State University
Gireesh Rajashekara, Ohio State University
Richard Isaacson, University of Minnesota
Rodney Moxley, University of Nebraska-Lincoln
Jun Lin, University of Tennessee
Bledar Bisha, University of Wyoming
Sheela Ramamamoorthy, North Dakota State University
Radhey Kaushik, South Dakota State University
Joy Scaria, South Dakota State University
Devendra Shah, Washington State University
Jorge A. Vizcarra, Alabama A & M University
Bibian Law, University of Arizona
Raghavendra Amachawadi, Kansas State University
Dongwan Yoo, University of Illinois at UC
Mo Saif, Ohio State University
Heather Wilson, University of Saskatchewan
Yewande Fasina, NC A and T University

Brief Summary of Minutes of Annual Meeting

Day-1: 3rd December 2016

1. Meeting called to order at 8:30 am by Jun Lin, Chair and meeting organizer.
 - Distribution of AES reports: Do we distribute to all (as it is done currently)? Group recommended that report be distributed to the station representatives or station coordinators who will then distribute reports to all participants from their respective stations (including those who may not be able to make it to the meeting but are interested in reading reports). It was decided that an updated specific format for the annual report will be send out next year so that participants can limit their descriptions to the provided word limits.
2. Opening remarks by Frank Blecha
 - Dr. Blecha informed the group that the 5-year renewal application for NC1202 project has been submitted to NIMSS online system and is currently being reviewed. Dr. Blecha thanked all the participants for providing information needed for proposal writing in timely manner and also Dr. Lin for his effort to compile the information.
3. Margaret Holland and Peter Johnson
 - Distributed handout with information on NIFA updates and NIFA factsheet and budget for 2014-2018 and AFRI competitive programs. Invited input on several issues including whether new investigator definition align with NIH or not, whether to exclude the investigators funded by non-AFRI grants? Requested that participants send in their recommendations to NIFA by email. Should program fund more investigators? Participate in stakeholder input webinars to make your voice heard. Informed that a standard report will be posted regarding stakeholder input and priorities in early 2017. Also informed that priorities might get linked to RFA so that the investigators know about the stake-holder input on each of the priorities. USDA may take decisions based on priorities to find gaps. These may have direct impact (eg., federal initiative on microbiome may feed into next generation to assess and make policy decisions)
 - Dual purpose study section will meet early next year (2017). Dual purpose program will continue for two more years (2017 and 2018). These proposals are due in September and the letter of intent is expected approximately a month before. Margaret Holland and Peter will be at the infectious disease study section. Two more deadlines are guaranteed (2017 and 2018), thereafter it is not guaranteed if this program will continue. However, if this program continues, it may be called something different. It cannot be the same program. Multi-state can also recommend that Dual program should continue in some fashion in the email communication on multi-state recommendations.
 - NSF and inter-agency programs. Informed that there is a Functional Annotation of Animal Genome initiative (international consortia) and there is a push for this. NIH, NSF and NIFA have interest. Please provide stakeholder input if multi-state group is interested in inter-agency program, may include this in the email recommendation.
 - Whether AMR and microbiome both could be combined in terms of priorities? There is also interest around microbiome field, but no specific program at this time. There are currently two microbiome projects (turkey and cattle) are ongoing. It may happen in near

future. Please provide stakeholder input. There was a stakeholder webinar on microbiome, please provide stakeholder input for that too. Verbal transcripts are available online. There was a stakeholder webinar on AMR, please provide stakeholder input for that too. Verbal transcripts are available online.

- It is unclear at this point when exactly the next USDA-NIFA RFP will be published. It may be released in February 2017, but this may change because of administrative changes. It is also not clear what the challenge areas will be, likely very different. Lot of uncertainty at this point.

Fourteen (14) Progress Reports were presented from 10:00 am to 5:00 pm on Dec 3, 2016.

Meeting adjourned at 5:00 pm

Day-2: 4th December 2016

Meeting called to an order at 9:00 am by Dr. Jun Lin, Chair of NC1202. NC1202 members discussed following issues:

- NC1202 5-year renewal proposal update: Dr. Jun Lin provided information and update on NC1202 5-year renewal proposal and thanked all participants for providing input in timely manner. Dr. Lin informed that the proposal will be officially submitted on Dec 15th, 2016. Review is expected late March 2017. Additional modifications will be made on June 1st. Before September 2017, it is expected that the new project will be finally approved.
- NC1202 membership and recruitment: A total of 18-19 institutions are currently participating in NC1202. Members emphasized on need to focus on new member recruitment. Dr. Linda Saif proposed that each station representative could promote/recruit more virologists to join and contribute to NC1202 project. Dr. Curtiss proposed that he could recruit Dr. Stephany Cart from Florida. Dr. Zhang also informed that he will request virologists at Iowa State to join and contribute to multi-state NC1202 project. There was consensus that NC1202 group members should make as much effort as possible to recruit more virologists in this project. Group members should reach out to poultry and bovine enteric viral disease specialists. Microbiome was proposed as one of the focus areas of expertise for recruitment of new members to NC1202. Dr. Isaccson mentioned that historically the NC group started as enteric diseases of swine, but over the years the group has expanded to cattle, poultry etc. He also proposed why we don't have joint meetings with other multi-state projects. Dr. Mo Saif suggested that the group should reach out to the PDRC in Athens, Georgia and invite to join the group to strengthen poultry section.
- Meeting scheduling: It was proposed that the presentation time be increased to 30 minutes (20-25 minutes for presentation and 5-10 minutes for Q & A) so that each member gets sufficient time to present new data and then the group can have some time for a fruitful discussion. Depending on the numbers of presentation few presentations can extend to Sunday morning. There was general consensus on increasing time for presentation and discussion. It was proposed that NC1202 group may consider starting

NC meeting on Friday, however concern was that there will be conflict with other meetings on Friday and some members may not be able to make it on Friday due to their scheduling conflicts and/or travel restrictions.

- AMR symposium: This year NC1202 group organized AMR symposium in collaboration with CRWAD. NC1202 members invited Dr. Karen Bush (Professor of Practice in Biotechnology at Indiana University Bloomington) as a keynote speaker for AMR symposium. NC1202 also invited other outside scientists as guest speakers for the AMR symposium, which include Dr. Doug Call (Washington State University), Dr. Morgan Scott (Texas A&M), and Dr. Keyong-Ok Chang (Kansas State University).
- Budget: Dr. Lin presented the NC1202 budget to all members.
 - Dr. Karen Bush (keynote speaker for AMR symposium) was paid \$600 as honorarium from the balance carry over from 2015 NC1202 meeting. To reciprocate NC1202's efforts to organize AMR symposium in collaboration with CRWAD, the CRWAD management waived keynote speaker's registration fee'; however CRWAD did not cover speaker honorarium. It was proposed that the group should have a defined strategy/policy for inviting keynote speakers to future symposiums.
 - Last year's invoice was presented by Dr. Lin. Set up of the presentation screen and cable appears to be very expensive (\$700). Dr. Lin proposed that he could bring the cable so we may not need to pay for cable and save on that expense.
 - \$1155 (carry over from 2015) and received \$1000 in registration fees for 2016. Expenditure for 2016 is \$315.9/day (total \$631.80) and honorarium to the key note speaker (\$600).
- Student Oral and poster presentation awards: NC-1202 offers awards for students to compete in the Pathobiology of Enteric and Foodborne Pathogens Section of CRWAD. Drs. Weiping Zhang (Chair) and Joy Scaria (Co-Chair) are in charge of the student award selection committee. The student awards were funded by the annual registration fee. This year total of 30 abstracts with 9 oral competition and 2 poster competition were submitted to the Pathobiology of Enteric and Foodborne Pathogens Section. This year, a total of two oral (\$250 for first and \$150 for second oral presentation) and one poster (\$200) presentations were offered awards.
 - NC1202 group members raised concerns over few student poster submissions. It was suggested that the award amount needs to be raised to match with awards given by other societies in the range of \$500 to \$1000. Award money appears to be an important factor that students may be using to apply for certain awards. NC1202 needs to be competitive to attract best posters/oral presentations. The budget is a limiting factor. Some questions raised included, what are some potential ways to raise funds from industry? Alternatives were also proposed: May be NC1202 should offer only one poster and only one oral and increase the award amount.
 - This year's funds for oral and poster awards: NC1202 has approximately \$900. The proposal is first oral (\$250), second oral (\$150) and first poster (\$200). This will still leave approximately \$300 as carry over for the next year.

- o Judges for oral: Dr. Moxley, Vizcarra and Fasina.
 - o Judges for poster: Dr. Nagaraja and Amachavadi.
- Dr. Qijing Zhang provided update on discussions on CRWAD council meeting: Several items were discussed including how CRWAD can encourage participation by multi-state projects, members, students and other relevant entities. Currently, keynote speakers are asked to pay registration fees. Council will waive registration fees in future and get some travel support (possibly from Industry). Sections can identify important areas/topics as “THEME” areas to make symposiums more meaningful. Council wants to engage section leaders in organizing CRWAD meeting annually. Council will have telephone conferences with section leaders. Change in title of CRWAD is under consideration. There may be a call for title contest such that it appears more inclusive. There is currently no rotation policy for section chairs at council level. It’s under consideration. In general, there was consensus that the CRWAD need to make its presence more visible internationally. Generally, member agreed that there should be better co-ordination between different groups that participate in CRWAD. Dr. Zhang requested all members to attend the keynote this evening and provide input to make CRWAD better. Dr. Zhang requested all members to fill out the survey regarding location, timing of CRWAD. Consider CRWAD as members write integrated proposals or conference proposal as collaborating body that can support organizing symposiums as part of proposals.
- Symposium topics for the next year (2016):
 - o Following committee was established to organize the symposium in 2017: Dr. Shah, Dr. Curtiss and Dr. Zhang (communication with council)
 - o Funding: Need to reach out to the industry to find funds to support symposium. Members should reach out to the industry contacts.
 - o Dr. Shah proposed a more focused topic for symposium “alternative to antibiotics (new antimicrobials, vaccines, probiotics and other innovative ways)”. Dr. Curtiss proposed that we should also include economics and implications on animal health relevant to alternatives and sustainability of alternatives.
 - o One potential theme that was generally accepted by all members included “alternatives to antibiotics, fate of antimicrobial resistance and sustainability of alternatives in agriculture animal production system.
 - o We need to identify experts in alternatives to antibiotics, economics, epidemiology, sustainability of alternative strategies and experiences (including European) etc.
 - o There was a consensus that we should have symposium next year.

ACCOMPLISHMENT

Objective 1. Focus on emerging diseases: We will identify, characterize and develop improved detection and prevention methods related to newly recognized, novel or emerging causes of zoonotic enteric disease and enteric pathogens of food animals.

A. Campylobacter jejuni

Iowa

Pathogenesis of *Campylobacter jejuni* clone SA. We showed that SNPs in *porA* gene encoding MOMP is associated with hypervirulence of clone SA in guinea pig model. We completed whole genome sequence analysis of 99 *C. jejuni* isolates recovered from abortion cases in sheep and demonstrated that there is a clonal expansion of clone SA with a monophyletic origin, with a reduced recombination rate since its emergence in the early 1970s. We also showed that the jump of *tetO* (encoding tetracycline resistance) from plasmid into the chromosome has facilitated the population expansion of clone SA. We also identified and characterized toxin-antitoxin systems in *C. jejuni*. We demonstrated that a virulence plasmid, pVir plays a key role in maintaining plasmid stability, but it is not required for abortion induction in the guinea pig model.

Michigan

A new murine model of GBS following *C. jejuni* infection to examine differences in pathogenesis of *C. jejuni* strain. We have developed and successfully tested a new murine model (BALB/c IL-10^{-/-}) to study pathogenicity of *C. jejuni*.

B. Salmonella

Washington State

Role of KsgA in *Salmonella* pathogenesis: We previously showed that KsgA contributes to virulence in *S. Enteritidis*. We have now completed *in vitro* genotypic and phenotypic characterization of KsgA-deficient mutant of *S. Enteritidis*. These studies revealed that KsgA deficiency alters outer membrane structure of the *Salmonella*.

Role of SPI-13 in *Salmonella* pathogenesis: We have completed study to investigate role of SPI-13 in *Salmonella* pathogenesis in chickens and mouse model of infection. This study revealed that SPI-13 is a host-specific island that contributes to pathogenicity in mice but not in chickens and may have implications in *Salmonella* virulence. We also demonstrated that SPI-13 is involved in metabolism of tyramine which may suggest role of SPI-13 in nutritional virulence of *Salmonella*.

C. Shiga toxin-producing E. coli (STEC)

Nebraska

Comparison of agar media for detection and quantification of STEC in cattle feces. We evaluated performance of various culture media for detection of STEC and show that CHROMagar STEC performs better than Possé or SHIBAM for detection of STEC in bovine feces. Adjustments in

the concentrations of novobiocin and potassium tellurite in the latter two media result in significant improvements in performance.

Membrane insertion for the detection of lipopolysaccharides: We have developed a membrane insertion assay for serogroup-specific detection of STEC amphiphilic LPS in complex samples such as beef lysates.

Development of 11-plex MOL-PCR assay for the rapid screening of samples for detection of STEC. We developed and validated 11-plex MOL-PCR assay that can be used to screen samples for several important STEC-associated serogroups (O26, O45, O103, O104, O111, O121, O145, and O157) and virulence factors (*eae*, *stx*₁, *stx*₂).

Bacterial community profiling of cattle hides: We completed 16S ribosomal RNA (rRNA)-based bacterial community profiling of cattle hides. Our results show a significant correlation between low bacterial diversity and samples positive for the presence of *E. coli* O157:H7 and/or the non-O157 STEC groups: O26, O111, O103, O121, O45, and O145.

Kansas

Prevalence and concentration of STEC-7 on hides, and indicator organisms on pre-intervention carcasses, of fed and culled cattle harvested within the same plant and time. We completed this study and the results show that all STEC-7 serogroups, except STEC O121, were detected on hides from all cattle types. Overall, O26, O103 and O145 serogroups were most the frequently detected STEC.

Microbial transfer during processing of beef carcasses with and without gut adhesions. We collected carcass sponge samples from the inside and outside of carcasses over nine processing days in a commercial beef processing facility. Non-*E. coli* coliforms were present on carcass surfaces at a higher concentration compared to *E. coli*, and mean log concentration of both indicator organisms was higher for samples collected from the outside of carcasses, irrespective of carcass condition.

Quantification of EHEC on cattle hides and of *E. coli* potential transfer from cattle hides to carcasses in commercial slaughter operations. This study included four large commercial beef processing plants in the Midwestern U.S that were visited three times each in summer, fall, and spring seasons. Carcass surface swabs were collected at each sampling point: hide-on, pre-wash, pre-evisceration, post-evisceration, and post-intervention. Most hide-on samples had high counts of generic *E. coli* in all seasons. In general, the study showed that in-plant interventions were effective at reducing the concentration of *E. coli* on beef carcasses.

Feedlot- and pen-level prevalence of EHEC in feces of commercial feedlot cattle in two major U.S. cattle feeding areas. We studied four commercial feedlots in each of the two major U.S. cattle feeding areas. All study feedlots and 31.0 % of the study pens had at least one non-O157 EHEC positive fecal sample, whereas 62.4 % of pens tested positive for EHEC O157. Within-pen prevalence of EHEC O157 varied significantly by sampling month; similarly within-pen prevalence of non-O157 EHEC varied significantly by month and by the sex composition of the

pen (heifer, steer or mixed). Feedlot management factors, however, were not significantly associated with fecal prevalence of EHEC-7.

D. *Brachyspira hampsonii* and *Lawsonia intracellularis*

Minnesota

Interactions between *Salmonella enterica* and *Lawsonia intracellularis*. We completed a study to determine whether a commercial live vaccine used to prevent disease caused by *L. intracellularis* might also reduce shedding of *S. Typhimurium*. We observed a statistically significant reduction in *S. Typhimurium* shedding in vaccinated pigs also challenged with *L. intracellularis* compared to non-vaccinated pigs. Vaccination did not have any effect on shedding numbers in pigs only challenged with *S. Typhimurium*. In addition to the lower level of *S. Typhimurium* in the feces of the dually infected and vaccinated pigs, the total number of pigs in each group (n=9) also was lower compared to non-vaccinated pigs of vaccinated pigs challenged only with *S. Typhimurium*. Thus, vaccination against *L. intracellularis* might be a step to mitigate *S. Typhimurium* shedding.

Characterization and recognition of *Brachyspira hampsonii* sp. nov., a novel intestinal spirochete that is pathogenic to pigs. We sequenced the genomes of strains NSH-16^T, NSH-24, and P280/1, representing *B. hampsonii* genetic groups I, II, and III, respectively, and compared them with genomes of other valid *Brachyspira* species.

Complete genome sequence of *Brachyspira hyodysenteriae* Type Strain B-78 (ATCC 27164). We sequenced and annotated the complete genome sequence of the type strain B-78 (ATCC 27164) of *Brachyspira hyodysenteriae*, the etiological agent of swine dysentery.

Development of a swine dysentery duplex PCR. We (i) developed a sensitive and specific duplex gel-based PCR to detect *B. hyodysenteriae* and *B. hampsonii* (both clades); (ii) compared the effect of varying storage times and temperature on diagnostic results; (iii) compared the limit of detection of direct fecal duplex PCR, pure culture + MALDI and primary culture + duplex PCR from spiked fecal samples.

E. *Coronavirus*

Ohio

Reverse transcription-PCR assays for the differentiation of various US porcine epidemic diarrhea virus (PEDV) strains. We developed RT-PCR assays for the detection and differentiation of different US PEDV variants. These assays successfully differentiated three PEDV strains: PC22A (the original US virulent), Iowa106 (S-INDEL), and PC177 (S-197DEL). The assays were PEDV-specific and did not amplify porcine deltacoronaviruses or transmissible gastroenteritis viruses.

F. Calicivirus

Ohio

Genetic characterization and classification of human and animal sapoviruses (SaVs). We sequenced the full length genomes of 3 new porcine SaVs belonging to 3 genogroups (GV, GVII and GVIII) and classified human and animal SaVs into 15 genogroups (G) based on the major structural protein VP1 sequences. We also determined the 5'- and 3'-ends of sea lion GV SaV and canine GXIII SaV. Although the complete genomic sequences of GIX-GXII, and GXV SaVs are unavailable, common features of SaV genomes were identified in this study.

G. Rotavirus

Minnesota

Genome sequence of a porcine rotavirus H strain. We sequence the first porcine rotavirus U strains from the United States, which was compared to the available rotavirus H sequences in GenBank to understand the genetic diversity of this pathogen.

A novel diagnostic platform for in situ detection and subtyping of Rotaviruses in pigs. We developed a novel *in situ* hybridization technique in a duplex assay for simultaneously detection Rotaviruses Groups A, B and C. The designed probes were successfully able to differentially detect and in situ subtype RVA, RVB, RVC. The lack of non-specific staining in the negative controls demonstrated the high specificity. This new ISH platform will be useful to determine contribution of each Rotavirus group in neonatal and post-weaning diarrhea in piglets.

H. Antimicrobial Resistance (AMR)

Iowa

C. jejuni: We identified the resistance-enhancing variant (RE-CmeABC) of the predominant efflux pump CmeABC in *C. jejuni*. We also demonstrated that RE-CmeABC is horizontally transferable, shifts antibiotic MIC distribution among clinical isolates, and is increasingly prevalent in *C. jejuni* isolates, suggesting that it may confer a fitness advantage under antimicrobial selection. We also identified a novel plasmid-associated *cfr* variant in *C. jejuni* from feed lot cattle. Cloning and expression of the single *cfr(C)* gene into a susceptible *C. jejuni* isolate conferred resistance to phenicols, lincosamides, oxazolidinones, and pleuromutilins (*Campylobacter* is naturally resistant to streptogramin A) and resulted in 8- to 256- fold increase in the MICs of these antibiotics.

Washington State

Salmonella: We completed AMR profiling of >800 *Salmonella* isolates belonging to 12 most prevalent poultry associated *Salmonella* serotypes (MPPSTs) recovered from the US poultry sources. MPPSTs include Kentucky, Enteritidis, Heidelberg, Typhimurium including its biphasic variant, Montevideo, Infantis, Schwarzengrund, Hadar, Mbandaka, Thompson and Senftenberg.

Generic *E. coli*: We completed several clinical and observational studies describing AMR patterns and its ecology on dairy farms. We have worked closely with producers, workers, and veterinarians to develop strategies to mitigate resistance when it is high and to support management systems when resistance is low. We are in the middle of population survey studies on the ecology of AMR on dairy farms. Preliminary data suggest that there are strong farm influences on resistance ecology and patterns of resistance are farm dependent and some patterns are management driven. There is little evidence that resistance patterns on a farm are correlated.

Other Gram negative organisms: We have expanded our AMR surveillance efforts and isolated >180 Gram negative organisms from 35 different backyard poultry flocks of the WA state and completed AMR profiling of these isolates. Majority of Gram negative isolates show MDR (resistance to >3 antibiotic classes).

Michigan

Antimicrobial Susceptibility Profiles of Human *C. jejuni* Isolates and Association with Phylogenetic Lineages. We characterized 94 *C. jejuni* isolates collected from patients at four Michigan hospitals in 2011 and 2012. A high proportion of antibiotic resistant isolates were classified as multilocus sequence type (ST)-464, a fluoroquinolone-resistant lineage that recently emerged in Europe. A significantly higher prevalence of tetracycline-resistant *C. jejuni* was also found in Michigan and resistant isolates were more likely to represent ST-982, which has been previously recovered from ruminants and the environment in the U.S. Notably, patients with tetracycline-resistant *C. jejuni* infections were more likely to have contact with cattle.

Wyoming

Role of wildlife in the dissemination of AMR to livestock operations. We have characterized a subset of approximately 1,370 isolates of AMR *E. coli* and *Enterococcus* spp. for resistance to 18 and 13 major antibiotics, respectively. These isolates originated from 40 concentrated animal feeding (CAFOs) facilities, associated wildlife (mammalian and avian), and environment (feed and water).

Objective 2. Focus on preventions and interventions: We will develop and improve preventative measures and interventions to reduce the incidence and prevalence of infections of food animals with enteric and foodborne and waterborne pathogens.

A. *Campylobacter jejuni*

Arizona

C. jejuni vaccine development. We have revised recombinant attenuated *Salmonella* vaccines (RASV) to stably express protective *Campylobacter* antigens at high levels.

Tennessee

Enterobactin (Ent)-mediated iron acquisition for *Campylobacter* infection. We optimized conditions to conjugate Ent to two carrier proteins (KLH and BSA) and successfully raised Ent specific rabbit antibodies for testing their antibacterial activity against *C. jejuni*.

Regulatory mechanisms of beta-lactamase expression in *Campylobacter jejuni*. We have obtained compelling evidence showing that a lytic transglycosylase (**LT**), which cleaves peptidoglycan and generates muropeptide signal, plays a critical role in β -lactam resistance by inducing β -lactamase production in *C. jejuni*. We have successfully determined a preliminary 2.16Å resolution crystal structure of the LT, which is critical for future in-silico screening to identify LT inhibitors for mitigation of beta-lactam resistance.

Development of bile salt hydrolase (BSH) inhibitors, a novel alternative to antibiotic growth promoters (AGP). We evaluated the effects of three BSH inhibitors using chicken model and demonstrated enhanced body weight gain and feed efficiency. Metabolomics analysis linked the growth promotion to the changes in intestinal bile acid profile in response to BSH inhibitor treatment. We also identified residues critical for catalysis and substrate specificity using site-directed mutagenesis, which will be used for future translational research by developing potent BSH inhibitors.

Ohio

C. jejuni and AMR. We completed a study which revealed that the average number of *C. jejuni*-positive in organically grown hens was lower in comparison to conventionally grown hens. The isolates from organically grown hens on two farms exhibited significantly lower resistance to ciprofloxacin, erythromycin, and tylosin.

Effect of on-farm practices on litter as a source of *C. jejuni*. We selected 4 broiler farms that used the same approach to treat litter. We sampled litter (n = 384) and ceca (n = 192) from 6 houses on each farm. We showed that the litter treatment reduced the moisture of the litter, but the treatment did not result in reduction in *Campylobacter* in the flocks particularly in the high prevalence farms.

Effect of probiotic EN on *C. jejuni* invasion in enteric cells. We showed that the pre-treatment of HT-29 human colonic cells with the probiotic EcN results in significant reduction (~ 2.5 log) in *C. jejuni*. The results suggest that EcN impedes *C. jejuni* invasion and intracellular survival by affecting HT-29 cells barrier function and tight junction integrity.

High-throughput chemical screens to identify small compounds for control of *C. jejuni*. We screened 4182 compounds for anti-*C. jejuni* activity. A total of 12 compounds were selected to which *C. jejuni* showed no resistance when cultured with lethal or sub-lethal concentrations. A total of 10 compounds had an anti-*C. jejuni* effect in Caco-2 cells at concentrations as low as 25 μ M.

B. *STEC* and *ETEC*

Nebraska

Effect of distiller's grains or fiber on EHEC in steers. We recently completed a feeding study which showed decreased prevalence for shedding of O111 in steers fed increased distillers grains at higher fiber level compared to steers fed corn fiber isolate diets.

Efficacy of urtoxazumab (TMA-15 humanized monoclonal antibody specific for Shiga toxin-2) against post-diarrheal neurological sequelae caused by *EHEC* O157:H7 infection in the neonatal gnotobiotic piglet model. We completed this study to show that urtoxazumab may be effective for prevention or reduction of extraintestinal vascular and ischemic sequelae attributable to Stx in human patients acutely infected with EHEC.

Kansas

Prevalence of STEC-8 in house flies in the urban environment. We collected 224 house flies from the select restaurant areas of a town in the northeastern Kansas. The most common STEC serogroup was O103 (42 isolates from 24 individual flies), followed by O26 (16/7), and O45 (4/3). Serogroups O104 and O121 were found in very low numbers (3/2 and 3/3, respectively) whereas serogroups O145, O111, and O157 were not found. No STEC specific virulence genes were detected from any isolates.

C. *Salmonella* and *E. coli*

Washington

Identification and characterization of immunomodulatory CpG motifs from *Salmonella* pan-genome: We completed screening of 256 newly identified CpG motifs from *Salmonella* pan-genome and identified 8 CpG motifs that induce production of pro-inflammatory cytokines (Il-1 beta) and nitric oxide in avian macrophages. We are testing the ability of these CpG motifs to induce innate immune response in chickens and to induce protection against colonization by *Salmonella* and Avian pathogenic *E. coli*.

D. *Brachyspira*

Minnesota

Antimicrobial susceptibility patterns of *Brachyspira* sp isolated from the US swine herds. This study showed that *B. hampsonii* have high susceptibility to most commonly used antimicrobials. In general, US origin *B. hyodysenteriae* isolates were more susceptible to antimicrobials than isolates originating from other countries.

E. *Coronavirus* and *Norovirus*

Kansas

Small molecule protease inhibitors against coronaviruses. Using feline coronavirus as a model organism, we identified small molecule protease inhibitors with anti-coronavirus activity, and one lead molecule effectively reversed the fatal infection of coronavirus in the natural host (cats).

Norovirus antivirals. We reported the structure-based design of the first class of permeable, oxadiazole- and triazole-based macrocyclic inhibitors of norovirus 3C-like protease, as well as pertinent X-ray crystallographic, biochemical, spectroscopic, and antiviral studies.

Ohio

Effect of in vitro passage on PEDV strain PC22A. We completed *in vitro* growth kinetics of PEDV in vero cells, virulence in neonatal pigs and the whole genomic sequences of selected passages [P95C13, P100C4, P100C6, P100C4+P10, P120, P140 and P160]. Our results suggest that PEDV attenuation can go through various molecular mechanisms.

Pathogenicity of the tissue culture (TC)-adapted PEDV strain PC177. We completed testing of pathogenicity of PC177 and TC-PC177 strain in piglets. TC-PC177 caused mild diarrhea with no mortality in piglets, whereas PC22A/PC21A caused severe clinical signs and 100% mortality. Deletion of 197 aa-fragment in spike protein can attenuate a highly virulent PEDV.

North Dakota

Develop rapid response vaccines and diagnostics for PEDV, which can be broadly applied to other emerging RNA viruses. We have optimized methods for culturing and quantifying PEDV and to inactivate PEDV for the development of first generation vaccine. The developed vaccine candidates are currently being tested in a swine challenge model. To develop rapid response serological assays, a panel of PEDV-specific field sera was obtained from a collaborating institution. To assess cross-reactivity, anti-sera specific to TGEV and PRSV were also obtained from NVSL or other researchers. Eleven computationally predicted targets were commercially synthesized and screened against the panel of sera. Two promising targets were generated by in-vitro transcription and translation. Methods for the optimization of the computationally designed ELISA were optimized and published.

F. Rotavirus and calicivirus

Ohio

Development of Gn piglet model. We developed a Gn piglet model for HRV disease. In this model, use of broad-spectrum antibiotics in therapeutic doses resulted in more severe HRV disease and was associated with complex modulation of the microbial population and intestinal epithelial cell dynamics. Probiotic *E. coli* Nissle (EcN) supplementation partially reversed the negative effects of Cipro on HRV diarrhea and microbial population. The probiotic EcN protected the intestinal epithelium from damage by upregulation of genes in the intestinal cells.

Establishment of germ-free (GF) piglets. We established germ free (GF) piglets transplanted with human infant fecal microbiota (HIFM pigs) as a clinically relevant model to investigate the effects of dietary insufficiency and enteric pathogens on the intestinal microbiota.

Establishment of porcine small intestinal enteroids: We have established porcine small intestinal (jejunal and ileal) enteroids derived from neonatal piglets and currently are optimizing culture media composition and IE propagation/expansion regimens. Additionally, we are testing various cell lines that produce different growth factors required for IE propagation/differentiation.

Behavior of HuNoVs and calicivirus surrogates inside lettuce and spinach plants. We showed that the HuNoV GII.4 can be internalized through the roots of lettuce and spinach plants and viral RNA can be detected in their leaves for at least up to 6 days post inoculation. Our data also

suggested that TV and SaV maybe more restricted than HuNoVs to the roots (or are more susceptible to degradation inside the leaves). We also demonstrated that H type HBGA-like carbohydrates are present in the cell walls of lettuce, and the virus-like particles (VLPs) of GII.4/HS194 strain bind to α -L-fucose residues in lettuce.

Wyoming

We have developed MALDI-TOF MS-based method for rapid identification and characterization of AMR bacteria. Using this method, predictive biomarkers for *E. coli* were identified for ampicillin, amoxicillin, aztreonam, ciprofloxacin, and tetracycline. For enterococci, predictive biomarkers were identified for nitrofurantoin, quinupristin-dalfoprisin, rifampin, chloramphenicol, and doxycycline among others.

H. Microbiome-Host Interactions

Minnesota

Identification of metabolites associated with usage of the antibiotic growth promoter tylosin. We employed HPLC to identify metabolites in feces that are differentially present in pigs that received tylosin compared to non-treated pigs. We showed that certain bile acids are increased in pigs receiving tylosin suggests a potential role for bile acids in growth promotion.

Objective 3. Focus on disseminating knowledge: We will provide training or continuing education to disseminate new information to students, producers, veterinarians, diagnostic labs and others to implement interventions and preventative measures.

The PIs and Graduate Students involved in the project have been continuing to give presentations and updates enteric diseases and food safety at various scientific, veterinary, and diagnostic meetings in the previous year. They have effectively disseminated new information, reagents, and procedures to producers, industries, veterinary diagnostic laboratories and veterinarians. They also have generated many high impact peer-reviewed journal articles. The members have actively organized various outreach/education activities. For instance, through several venue Kansas State have provided educational opportunities to professional and graduate students as well as continuing education to veterinarians on enteric pathogens in livestock production systems. The majority of the shared information has focused on the impacts of Shiga toxin-producing *Escherichia coli*, *Salmonella*, *Campylobacter*, and antimicrobial use/resistance in the beef industry, the conclusions that can be reached based on recent research, and the potential opportunities to reduce these pathogens in beef production systems.

Objective 4. Group interaction: The group will interact in a variety of ways to facilitate progress including direct collaborations with joint publications, sharing of resources (pathogen strains, gene sequences, statistical analysis, bioinformatics information/expertise), and friendly feedback and facilitation for all research efforts at annual meetings.

- NC 1202 organized a Mini-Symposium focused on antimicrobial resistance on Dec 5, 2016. Six experts including one keynote speakers on AMR were invited by NC1202 to present their research work at CRWAD.
- NC-1202 members and their students presented their work in numerous national and international meetings. We held annual NC1202 meetings in Dec of 2016, and also sponsored a total of 3 student awards for best oral (two awards) and poster (one award) presentations at the CRWAD.
- NC-1202 members have established active collaborations, partly reflected by joint grant/publications. Following are three active integrated food safety projects in which our NC1202 members are project directors and involve several NC1202 members at the participating institutions as co-project directors:
 - 1) Zhang, Q. (PD, Iowa State Univ.) and other Co-PDs who are NC1202 members: Jun Lin (Univ. of Tennessee), Gireesh Rajashekara (Ohio State Univ). USDA NIFA Food Safety Challenge Grant, Novel Approaches for Mitigation of *Campylobacter* in Poultry. \$2,500,000. Award period: 07/01/2012 – 06/30/2017.
 - 2) Law, B. (PD, Univ. of Arizona) and other Co-PIs who are NC1202 members: Roy Curtiss III is a scientific advisor, and Ken Roland from the Curtiss lab is the Co-PI. USDA NIFA Food Safety Challenge Grant, The Development of an Efficacious Vaccine to Reduce *Campylobacter* in Chickens. \$2,500,000. Award period: 8/1/2012 – 7/31/2017.
 - 3) Moxley, R. (PD, Univ. of Nebraska) and T.G. Nagaraja and David Renter (Co-PDs, Kansas State Univ.) USDA NIFA Coordinated Agricultural Program Award, Shiga-toxigenic *Escherichia coli* (STEC) in the Beef Chain: Assessing and Mitigating the Risk by Translational Science, Education and Outreach. Total funding: \$25,000,000 for 16 institutions, 51 collaborators. Award period: 1/1/2012 – 12/31/2016

IMPACT STATEMENT

Campylobacter:

- Understanding pathogenesis of *C. jejuni* clone SA will provide key information needed for developing vaccines and other interventions to control ruminant abortion.
- Development of effective *Campylobacter* vaccine for poultry would reduce *Campylobacter* load in poultry, consequently reducing human Campylobacteriosis.
- The reduction of *Campylobacter* in general and antimicrobial resistant *Campylobacter* in particular will benefit public health. This will benefit stakeholders by enhancing their capabilities to control this pathogen in their products, which would increase the competitiveness of chicken farming.
- Overall, our efforts have resulted in several novel approaches to control *Campylobacter* in the host and farm environment.

Salmonella

- We have shown that KsgA-deficiency in *Salmonella* alters outer membrane of this important food borne pathogen and also reduces pathogenicity. Future studies are directed towards developing a model to manipulate OM of *Salmonella* to reduce pathogenicity and its potential use as a vaccine for use in food animals including poultry.
- The 8 newly identified CpG motifs from *Salmonella* pan-genome that induce production of pro-inflammatory cytokines (Il-1 beta) and nitric oxide in avian macrophages have a potential to be used as immunomodulating agents to reduce colonization by *Salmonella* and other food-borne pathogens in chickens. We are currently testing the ability of these CpG motifs to induce innate immune response in chickens and to induce protection against colonization by *Salmonella* and Avian pathogenic *E. coli*.
- We have shown that SPI-13 contributes to tyramine utilization in *Salmonella*. This new information will help in further investigations on role of tyramine in *Salmonella* colonization and strategies to target this mechanism to reduce *Salmonella* loads in reservoirs of *Salmonella* (eg., rodents) in poultry production.

Lawsonia intracellularis

- Our observation that there is an interaction between *S. Typhimurium* and *L. intracellularis* provides a potential intervention target. The live attenuated vaccine is an effective means to prevent proliferative enteritis. Our data demonstrates that this vaccine also may have a practical place in reducing food borne illnesses associated with swine by also reducing levels of *S. enterica*.

Brachyspira hyodysenteriae and hamppsonii:

- We provided support for position of *Brachyspira hamppsonii* sp. nov., as a valid species by providing key information on its whole-genome sequences, genomic relatedness to other *Brachyspira* species and ultrastructural morphology and most importantly our studies show that this new species is pathogenic to pigs.
- We provided complete genome sequence of *Brachyspira hyodysenteriae* Type Strain B-78 (ATCC 27164) isolated from North America.
- The newly developed method of a duplex PCR of primary culture plates is expected to reduce client report time for cases of suspected swine dysentery caused by *Brachyspira* spp. to one-third of its current duration.

Shiga toxin producing *E. coli* (STEC) and Enterotoxigenic *E. coli* (ETEC):

- The development of new and improved methods for detection of USDA, FSIS-adulterant EHEC in pre- and post-harvest samples from cattle will increase food safety.
- Risk factors and prevalence estimates for STEC and virulence genes, as well as indicator organisms of fecal origin, in feces, hides, and carcasses of commercial fed and cull cattle are critical to populate risk assessments and identify the distribution, intervention opportunities, and potential risks of human illness.

- The determination of the effects of feeding distillers grains and fiber from distillers grains on the prevalence of FSIS-adulterant EHEC in the feces of feedlot beef cattle will help in the development of dietary strategies aimed at reducing these organisms in cattle.
- The assessment of prevalence and concentration of FSIS-adulterant EHEC in cattle feces, hides and dehided carcasses will provide an important source of data for quantitative microbial risk assessment model with the goal of reducing the occurrence and public health risks of these organisms in beef.
- The determination of the efficacy of a humanized monoclonal antibody against Stx2 may assist in ultimate approval of a therapy against lethal post-diarrheal sequelae of EHEC infection in human patients.

Antibiotic Resistance:

- We have completed AMR surveillance of >800 isolates of MPPSTs. The data has been published and new data is now available. We are continuing to isolate, archive and monitor AMR of *Salmonella* isolates recovered from poultry and poultry products. The continued monitoring and studying trends in field isolates encompassing major serotypes will help in designing strategies to reduce prevalence of MDR *Salmonella*.
- We have expanded our AMR surveillance efforts to include other gram negative organisms from poultry in WA State. To this end, we have isolated >180 Gram negative organisms from 35 different backyard poultry flocks of the WA state. Further studies are ongoing to characterize the AMR traits of these isolates. This information will be helpful to understand the ecology and epidemiology of AMR in backyard poultry.
- Studies aimed to understand epidemiology and mechanisms of antibiotic resistance in *Campylobacter* will facilitate the development of mitigation strategies for controlling antimicrobial resistance in poultry and ruminants.
- The recent reemergence of swine dysentery in North America, the emergence of *B. hampsonii*, and the reports of *Brachyspira* strains with reduced susceptibility to multiple antimicrobials all highlight the need to monitor pathogens and their susceptibility to commonly used antimicrobials, in order to control and to treat *Brachyspira*-related diseases.
- We have developed MALDI-TOF MS protocols for identification and typing of antimicrobial resistant bacteria.
- Identification of metabolites associated with AGP usage in swine will contribute to our understanding of how these compounds increase animal production. By understanding these processes, alternate strategies to improve animal growth may be possible by supplementing feeds with these compounds or by identifying important microbes that promote these metabolic activities and can be novel feed supplements.
- Research on the development of alternatives to antibiotic growth promoters will lead to novel 'One Health' measures for enhanced animal production, food safety, and human health.
- We have continued to focus on antimicrobial resistant bacteria with special emphasis on the role of wildlife in the dissemination of AMR to livestock operations. These studies may open new avenues for treatment and prevention of resistant foodborne pathogens important in animal health and food safety.

Enteric Viruses:

- Small molecule inhibitors of coronaviruses have been developed.
- A new diagnostic platform for in situ detection and subtyping of rotaviruses in pigs will provide a better understanding of contribution of each rotavirus group in neonatal and post-weaning diarrhea in piglets. This information is crucial for driving decisions regarding control strategies in the field because the commercially available vaccine only contains rotavirus group A and the cross-protection among the other groups is questionable.
- Our findings will contribute to identification of optimal probiotic treatments and regimens applicable to neonates that compensate for the antibiotic-associated negative effects on neonatal immunity, intestinal epithelial cells and the commensal communities, thereby moderating rotaviral disease, reducing mortality and improving neonatal health.
- We developed RT-PCR assays that allow the specific detection and differentiation of all major types of US PEDV variants. These assays can be used in molecular epidemiological studies to monitor the prevalence of PEDV variants in swine farms.
- The emerging, highly virulent PEDV causes up to 100% mortality in nursing piglets, poor growth and reproductive performance in weaned pigs and sows, respectively. New diagnostic methods and assays will indirectly promote the control and prevention of PEDV, thus contribute to pig health and help maintain a sustainable pork industry.
- Our studies will generate live attenuated PEDV vaccine candidates, and expand the knowledge on immunology, pathology and molecular biology of PEDV infection in pigs.
- Protocols for the quick generation of computationally designed targets and use of the targets in the PEDV anti-body detection assay were optimized.
- Intellectual property disclosure was filed with the NDSU technology transfer office for the rapid-response vaccines against PEDV.
- Our results suggest that several animal SaVs have genetic similarities to human SaVs. However, the ability of SaVs to be transmitted between humans and animals is uncertain.
- Available complete genome sequences for animal SaVs are still limited compared to human SaVs. Therefore, our results provide basic information for better understanding SaV evolution, and potential interspecies transmission of SaVs between human and animals. Based on the sequence data, better diagnostic assays can be developed for SaVs.
- Our newly established HIFM transplanted neonatal Gn pig model of malnutrition will allow increased knowledge of the interactions among the commensal microbiota, enteric pathogens, immunity and diet and to test future potential interventions to restore the affected immunity, intestinal barrier and absorptive functions and microbial structure.
- We demonstrated that EcN can be used as a potent and safe probiotic that alleviates HRV disease severity and moderates antibiotic-associated negative effects in neonatal hosts. Understanding the mechanisms of EcN immunomodulation will allow development of optimal interventional strategies to decrease diarrheal disease burden in humans and animals, thereby improving the overall health in circumstances when vaccines are not available/not effective or antibiotic treatment is unavoidable.

- The HIFM transplanted Gn pig provides a highly relevant model to comprehensively evaluate the interactions among enteric pathogens, diet and host factors (commensal bacteria and immunity) and to test potential interventions.
- Established porcine small intestinal enteroids (IE) to use them as an ex vivo preclinical platform to study the interaction between human and porcine enteric pathogens, commensal and probiotic bacteria and macro-/micronutrients.
- Human norovirus (HuNoV) is the leading pathogen causing food- or water-borne gastroenteritis. Knowledge generated from our studies will help prevent and control foodborne diseases, thus enhance human health.

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Peer-reviewed journal articles

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