An ACPV Workshop

Enterococcus: an Old Dog With New Tricks

March 12, 2023
Sacramento, CA

Algis Martinez
Program Chair

Alexandra Reilley
Fundraising Chair

Speakers

Dr. Luke Borst
Dr. Martha Pulido
Dr. Douglas Rhoads
Dr. Arne Jung
Dr. Grace Mountainspring
Dr. Kevin Kessler

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2023 ACPV Workshop

Enterococcus: An Old Dog with New Tricks

SUNDAY, MARCH 12, 2023		
7:00 AM	**Continental Breakfast**	**Sponsored by:** HUVEPHARMA
SESSION 1 Moderator: Dr. Scott Gustin		
8:00 AM	**Welcome**	Dr. Algis Martinez, Program Chair
8:10 AM	Overview of EC Infections in Poultry	Dr. Luke Borst
9:10 AM	Dynamics of EC infections in Broiler Production	Dr. Martha Pulido
10:00 AM	**Coffee Break**	**Sponsored by:** Cobb
10:15 AM	*Enterococcus cecorum* Sepsis: Polyphyletic Origin	Dr. Douglas Rhoads
11:00 AM	Q&A SESSION	Drs. Borst, Pulido, and Rhoads
11:30 AM	European Perspective of EC infections	Dr. Arne Jung
12:15 PM	**LUNCH (ON YOUR OWN)**	
FIELD EXPERIENCES Moderator: Dr. Phil Stayer		
1:15 PM		Dr. Grace Ricci
2:00 PM		Dr. Kevin Kessler
2:45 PM	Q&A SESSION	Drs. Jung, Ricci, Kessler
3:30 PM	**CLOSING REMARKS AND ADJOURN**	Dr. Algis Martinez, Program Chair

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Dear Colleagues,

On behalf of the American College of Poultry Veterinarians (ACPV), I welcome you to our upcoming workshop titled “Enterococcus cecorum. An old dog with new tricks”. As we all know this bacterial condition has emerged as one of the main causes of lameness and mortality in broilers in the US, and I am confident that this workshop will allow us poultry health professionals learn more about the condition itself. The workshop will be held in Sacramento on Sunday March 12, 2023, prior to the Western Poultry Disease Conference (WPDC).

This year, thanks to the outstanding support of our sponsors, we have a very select group of speakers consisting of highly recognized industry veterinarians, and University researchers. I am sure they will provide us with valuable information that will be useful to our attendees, and beneficial to our industry. My special gratitude goes to these distinguished poultry health professionals for accepting our invitation to share their knowledge, experiences, and perspectives with our diplomates.

As we all get ready to get together and learn more about this costly bacterial disease, this effort reminds me of Carl Sandburg’s famous quote “Everybody is cleverer than anybody”. Therefore, on behalf of the college, I would like to express my sincere appreciation to all the sponsors, the speakers, the fundraising committee members, the ACPV office, and everyone involved with the development, logistics, and organization of this workshop.

I trust that the knowledge we gain out of the workshop will help us find the tools to preventing and controlling this emerging challenge.

See you all in Sacramento!
Algis

Algis Martinez, DVM, ACPV Diplomate
Program Chair, ACPV Workshop (2023)
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Overview of EC Infections in Poultry
8:10 AM

Dr. Luke Borst
North Carolina State University

Biography
Dr. Luke Borst is Assistant Director of Anatomic Pathology for the West Coast for Antech Diagnostics. Dr. Borst attended the University of Illinois from 2000-2009 where he received his BS in chemistry, BS in veterinary medicine, DVM, DACVP, and PhD.

For the last 13 years, he has been a faculty member at NC State College of Veterinary Medicine rising to level of Associate Professor. There he enjoyed leadership roles as faculty liaison to the histopathology laboratory and service chief for the clinical and anatomic pathology groups. In that position he split his time teaching, researching important bacterial infections of veterinary species, and serving the college and surrounding community as a diagnostic pathologist. In his role as a diagnostic pathologist, Dr. Borst handled a diverse caseload of routine and specialty cases from a wide variety of species. Dr. Borst and his team has produced important advances in understanding of the genetic drivers of virulence for pathogenic Enterococcus cecorum and synergies between Enterococci and E.coli during extraintestinal infection. Borst's interest in the diagnosis and pathogenesis of neoplastic and infectious disease processes, with a special focus in poultry pathology, is reflected in his contributions to over 80 peer reviewed publications on these topics. Dr. Borst resides outside of Raleigh, NC with his wife, 2 kids, 2 horses, 4 dogs, 6 cats, 2 budgies, and 1 turtle.

Abstract
Pathogenic strains of Enterococcus cecorum emerged globally in the early 2000s as an important cause of mortality in broilers. In broilers, pathogenic E. cecorum infection results in symmetrical hind-limb paralysis which peaks at weeks 5-7. This paralytic disease presentation, often called 'Kinky-back', is the result of compression of the spinal cord due to infection of the free thoracic vertebra. The free thoracic vertebra is susceptible to infection due to the presence of osteochodrosis (OCD) lesions in the articular cartilage. These OCD lesions are very common in broilers and occur independently of a strong predisposition for strain, sex, or growth-potential. Recently, producers have reported a new presentation of acute pericarditis and septicemia that peaks around 2-3 weeks. This fatal septicemic disease has been associated with moderate to high mortality.

Early epidemiologic investigations of emerging strains of E. cecorum using pulsed-field gel electrophoresis and whole genome sequencing demonstrated that emergent virulent E. cecorum strains had a high level of genetic similarity (clonality). This finding was surprising given the global vertically integrated poultry production systems. The presence of genetically similar isolates on geographically distant farms receiving broiler chicks from the same hatchery suggested the possibility of vertical transmission of pathogenic strains. However, direct observation of vertical transmission remained elusive due to difficulty in sensitively detecting virulent strains at the hatchery. More recently, improved selection and enrichment culture protocols coupled with the development of a pathogenic E. cecorum specific PCRs have allowed for the detection of pathogenic E. cecorum in egg slurries at transfer. Isolates collected using this method were determined to be genetically related to isolates from disease outbreaks in progeny broilers. These methods may be useful in the timely and accurate prediction of outbreaks and represent a significant advance in the detection pathogenic E. cecorum. Embryos experimentally infected with pathogenic E. cecorum often die in ovo; however, a proportion of

infected embryos hatch and are the likely source of infection. Apparently, a very low frequency of infected chicks can cause a flock-wide outbreak as E. cecorum spreads rapidly within flocks via fecal-oral transmission. The organism has limited survival in the environment.

The cause for the sudden development of virulence in this organism has been the topic of much investigation. Pathogenic E. cecorum is an outlier among the enterococci which are typically benign enteric commensals. In fact, prior to 2002, the entire role of E. cecorum was as a dominant member of the normal enteric microbiota of adult chickens. However, emergent pathogenic strains have developed an armament of virulence factors that allow these strains to 1) colonize the gut of birds in the early life period; 2) escape the gut niche; 3) spread systemically while evading the immune system; and 4) colonize the damaged cartilage of the free thoracic vertebra. Comparative genetic analysis revealed gene cassettes encoding an enterococcal polysaccharide antigen (epa) locus and a polysaccharide capsule (cps) locus were enriched in pathogenic strains. These regions may have arisen from recombination events with human pathogens as the epa locus shares significant homology to the epa locus observed in hospital acquired E. faecalis and the capsule (cps) locus resembles that of E. faecium and Streptococcus pneumoniae. These regions which encode cell surface carbohydrates were found to be important for virulence. Gene deletion mutations disabling core regions of the capsule synthesis operon resulted in complete attenuation of pathogenic strains. These studies demonstrate that cell surface associated carbohydrate is important for virulence. As such these genetic loci and the carbohydrate surface decorations they produce represent an important target for further study which may yield important applications for both poultry and human health.

Dynamics of EC infections in Broiler Production

9:10 AM

Dr. Martha Pulido
Mississippi State University

Biography

Dr. Martha Pulido is an Associate Clinical Professor of Avian Medicine at Mississippi State University and Poultry Research and Diagnostic Laboratory.

Abstract

Dynamics of Enterococcus cecorum infections in Broiler Production
Martha Pulido-Landínez DVM, MS, PhD, DACPV.

Associate Clinical Professor. Poultry Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University.

Bacteria from the genus Enterococcus (E.) are considered normal inhabitants of the gastrointestinal tract of chickens. These are facultatively anaerobic, gram-positive cocci. Among the most isolated from poultry are E. faecalis, E. faecium, E. hirae, E. durans, and E. cecorum. Although considered part of chickens' normal microbiota, over the past two decades pathogenic strains of E. cecorum have emerged within the commercial poultry industry causing mainly enterococcal spondylitis (vertebral osteomyelitis - VOA), and more recently, severe systemic disease in young broilers.

In recent studies, we made a retrospective analysis of an emerging E. cecorum outbreak in a Southern US broiler integration where the main lesions were septicemia. 100 broiler chicken cases received at the Poultry Research and Diagnostic Laboratory of Mississippi State University from April to December of 2021 were analyzed. The peak in cases was observed from June to August. The average age of broilers was approximately 21 days with a range of 15-31 days. Most of these cases were related to systemic disease and leg problems with gross lesions including characteristic white pericarditis along with perihepatitis, and arthritis. In six of the 100 cases, E cecorum was isolated from the free thoracic vertebrae with the remaining being recovered from various other locations including the liver, hock/joints, femoral head/bone marrow, and pericardium (Dunnam et al, 2022). E. cecorum identification was performed by using Vitek matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Vitek – MS -MALDI-TOF). Now, in 2022, other cases are being analyzed and in additional to the previously mentioned sites, this bacterium has been isolated from the medial thigh muscles area (flexor cruris medialis, adductor profundus, and the femoro-cruralis) causing marked myositis and in severe cases, muscle rupture. This muscle damage along with the commonly observed femoral head necrosis and hock joint arthritis is contributing to the frequent report of leg problems. So, these findings arose multiple questions related to the origin and behavior of these bacteria in chickens younger than 4 weeks.

The emergence of E. cecorum systemic disease must be considered important because it can negatively impact the productive performance of the affected chickens. An infection with this bacterium would cause high mortality and high morbidity. Its tropism for cartilages results in marked leg problems because of the presentation of femoral head necrosis and synovitis. Additionally, the presence of Enterococcaceae family

members in chickens must be considered important in terms of antimicrobial resistance and its potential implication on public health.

The characteristics of Enterococcus spp isolates recovered from breeders, hatcheries, and broilers of vertical integrations in the Southern US have been analyzed. E. cecorum has been isolated from pullets. E. cecorum, E. faecium, and E. faecalis from adult breeders and their environment (litter). At the hatchery, E. faecalis, E. faecium, and E. gallinarum have been isolated from newborn chickens and the hatchery environment. Related to broiler samples, E. hirae were isolated from broilers 6 days old, and E. cecorum was mainly isolated from broiler chickens older than 2 weeks. These results suggested Enterococus spp presence varies according to the age of the birds, and the type of sample. Since E. cecorum was not isolated from newborn chickens, hatchery, and chickens younger than three weeks, these results suggest that the presence of Enterococcaceae family members in chickens presence varies according to the age of the birds, and the type of sample.

It is necessary to recognize the dynamics of E. cecorum in vertical integrations from its identification and characterization when isolated from breeders, hatcheries, and broilers youngest than 4 weeks with systemic disease, and chickens older than 4 weeks with VOA. A better understanding of the behavior of this emerging broiler chicken systemic bacterium, the knowledge of their survival time in poultry materials and temperatures, and their resistance to disinfectants will be valuable tools in terms of control.

Enterococcus cecorum Sepsis: Polyphyletic Origin

10:15 AM

Dr. Douglas Rhoads
University of Arkansas

Biography

Douglas Rhoads is a University Professor of Biological Sciences, affiliated faculty member in the Center of Excellence for Poultry Science, and director of the interdisciplinary graduate program in Cell and Molecular Biology at the University of Arkansas (UA). His Masters training was in genetics of human pathogenic yeasts, followed by PhD and Postdoctoral training in first hamster and then human molecular genetics. He joined the faculty at UA in 1990, to establish molecular genetics for the campus. His work has included genomics in human and plant pathogenic yeasts/fungi, tomatoes viral resistance, black bears, Caribbean todies, rattlesnakes, and scorpions. His main focus since 1993 has been genomics in poultry including male fertility, pulmonary arterial hypertension (ascites), and bacterial diseases. He has 71 peer-reviewed publications, 12 industry reports, and 177 presentations/seminars. His funding has come from NIH, NSF, USDA, and corporations ($2.2M as PI and $11.8M as coPI). Rhoads has served as vice chair and chair of the department of Biological Sciences. He led efforts to establish the Cell and Molecular Biology program for the campus and has been the director of that program since 2006. The program now includes 120+ faculty in 17 departments and 4 colleges. The program is one of the two largest PhD programs on campus.

Abstract

Phylogenomics suggest a polyphyletic origin of *Enterococcus cecorum* sepsis.

Douglas Rhoads[1,3], Adnan Alrubaye[1,2]; [1]Cell and Molecular Biology, [2]Poultry Science, [3]Biological Sciences, University of Arkansas

We have been characterizing the genomic diversity of *Enterococcus cecorum* colonizing poultry to better define the origins and solutions to the recent outbreaks of sepsis. Previously, this species had been defined as a commensal of the gastrointestinal tract, but could also contribute to lameness through infection of the free thoracic vertebrae in vertebral osteomyelitis, a form of bacterial chondronecrosis with osteomyelitis (BCO). Our analyses of other bacterial species contributing to BCO has identified mobile genetic elements that are associated with either host specificity or pathogenesis. Genome assemblies for 3 BCO isolates and 32 sepsis isolates were assembled into a phylogenomic tree with the 88 *E. cecorum* genomes from NCBI. Although some of the sepsis isolates cluster in the tree, sepsis isolates appear in more than 4 different branches of the tree and most cluster with BCO isolates. Our conclusion is that the sepsis outbreak arises from existing BCO pathogens and possibly from commensals through acquisition of new mutations or mobile element. Others had identified an 8 gene region, possibly related to capsule formation, that appeared to distinguish BCO isolates from commensals. There is also a second region that also is found in most BCO isolates. These genes are not shared in all of the sepsis isolates, but we did identify a 10 gene mobile genetic element (MGE) that is found in all the sepsis isolates and most of the BCO isolates. This MGE contains a ABC transporter cassette, and metabolic enzymes but how this MGE might contribute too pathogenesis is not clear. SNP analysis of the core genome from all available isolates suggests a missense mutation in a nucleotide scavenging pathway is essential for transition to sepsis pathogen. Isolates were shared with Chr Hansen which has identified commercial probiotic strains that are highly inhibitory in *in vitro* assays. Current work is on determining means of t*r*ansmission with air sampling, and embryo microbiology.

European Perspective of EC infections

11:30 AM

Dr. Arne Jung

University of Veterinary Medicine Hannover, Germany

Biography

Dr. Arne Jung is working as a researcher at the Clinic for Poultry of the University of Veterinary Medicine Hannover, Germany since 2005. He received his veterinary license in 2004, his doctoral degree in 2006 and finished his habilitation in 2022. Until now, he has published more than 40 articles in peer reviewed scientific journals and has given more than 20 public talks at scientific conferences. His main research interest is bacterial diseases of poultry in general and Enterococcus cecorum (EC) associated disease in meat type chickens in particular. Currently he is working on projects dealing with EC immune answer and chicken genotype associated differences in susceptibility to EC. Beside his main job, Arne has experience as a consultant for companies working in the poultry field. In his free time, he enjoys travelling with his family in his camper van and brewing beer with his friends.

Abstract

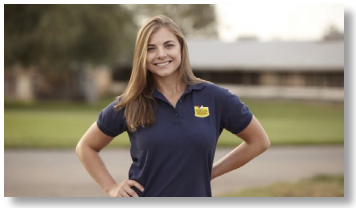
European Perspective of EC infections

Arne Jung[1]

[1]Clinic for Poultry, University of Veterinary Medicine Hannover, Hannover, Germany

European broiler production differs in some aspects from the US production as used broiler genetics, weight at slaughter and management practices. However, Europe is also affected by *Enterococcus cecorum* (EC) infection in broiler type birds. EC associated disease was first detected in the Netherlands and Scotland in 2002. Since that time virulent EC strains have spread around the world and have caused outbreaks in many different countries. Whole genome sequencing and phylogenetic analysis reveal that virulent strains form a phylogenetic group while commensal strains are more diverse. Virulent strains also seem to be able to survive longer in the environment, especially at lower temperatures. Beside hygiene management, vaccination is an important method to combat bacterial diseases. However, even basic data about the broiler immune answer against EC is lacking. Therefore I like to close with some immunology data obtained from EC infection experiments in broilers.

Field Experiences
1:15 PM



Dr. Grace Ricci
Foster Farms

Biography
I attended Oregon State University and obtained my bachelor of sciences from the animal science department in 2011 and earned my doctor of veterinary medicine degree in 2015. I started my career in the poultry industry after graduating from veterinary school with Foster Farms. During my time as a production veterinarian, I completed the Master of Avian Health and Medicine program through the University of Georgia. As a production veterinarian, I am responsible for the overall welfare, health, and protection of our broiler and turkey production systems throughout California, as well as our PNW and Farmerville, LA complexes, including breeders, hatchery, field, and processing facilities.

Abstract
Effects Of Enterococcus Infections from Chick Placement to Processing in Broiler Production

Background:
Enterococci spp are gram positive bacteria that are commonly found within the gastrointestinal tract of many animals, including poultry. Some strains of this bacteria, in particular E. cecorum and E. faecalis, are considered opportunistic pathogens, however, they can cause lingering disease concerns within a production system that affects overall broiler performance. Infections with Enterococci spp. in poultry are typically associated with bacterial chondronecrosis with osteomyelitis as well as polyserositis and septicemia. These conditions often result in high uniformity variances, lameness, mortality, and culls.

Field Experiences:
Bacterial infections emerging from the hatchery can result in a high percentage of late dead embryos, cull chicks, and high first week mortality. Beyond chick quality concerns, enterococci infections can linger throughout the production cycle and increase overall mortality, reduce daily gains, increase feed conversions, and increase condemnation at the processing facility. Investigating the source of enterococci in a production system is challenging and once established in a flock continues to persist and reduce overall flock performance. In a case study of a flock infected with E. cercorum, first week mortality was in target range, at 1.13%. However, by three weeks of age, several houses from the same breeder source began experiencing an increase in cull birds and mortality. Field reports included lameness, variable sized birds, airsacculitis, and pericarditis. At 31 days of age birds were submitted to the state laboratory for bacteriology and additional analyses. E. cercorum was isolated from multiple tissues, including joints, liver, and heart. By market age at 42 days, the affected houses had a total flock mortality ranging from 12.57-14.26%. Compared to flocks processing in the same week, this particular flock as a whole had lower average daily gain and a higher feed conversion ratio. This is one example of how Enterococci can have detrimental effects throughout the life of a flock, leading to overall reduced productivity. This case highlights the importance of maintaining hatchery sanitation as well as having optimal field management and biosecurity practices in a production system that does not utilize antibiotics.

Field Experiences
2:00 PM

Dr. Kevin Kessler
Tyson Foods

Biography
Graduated from NC St CVM, completed poultry internship at NCSU CVM. Internal and technical services for ISA Breeders. Last 23 years as a veterinarian for Tyson Foods.

Abstract
Enterococcus cecorum (Ec)has been established as the principal causative agent in vertebral osteoarthritis (VOA). Recently, Ec has been causing pathology at an earlier age and lesions are femoral head necrosis (FHN) and pericarditis. These conditions tend to recur on farms.

Strategies to combat VOA have included use of penicillin and increased dark time. The penicillin to reduce the load of Ec on the farm and the increased dark time to allow for better bone formation. The proposed route of infection is microfractures occurring in the Free Thoracic vertebrae while the birds are septic with Ec. Strategies to combat the FHN and pericarditis are still being studied. With an increase in No Antibiotics Ever programs, an effective replacement for penicillin needs to be found to limit the load of Ec. Different probiotics are being studied.

Ec is normally found in the gastrointestinal tract and is believed to need assistance to escape the gastrointestinal tract. There is likely not a single agent that allows this. An attaching effacing E coli has been proposed as one factor. Heat stress and "leaky gut" is also proposed as an environmental factor which allows escape from the gut.

Enterococcus cecorum is emerging with a different pathogenesis from its history and production veterinarians are having to emerge with new control strategies.

2023 ACPV CONTINUING EDUCATION COMMITTEE

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