Necrotic enteritis (NE), in acute or subclinical forms, is a concern for the poultry industry, particularly in antibiotic-free poultry production. The causative agent of NE is the Clostridium perfringens, and one of the predisposing factors is the Eimeria spp. colonization. The efficacy of a specific composition* of 1-monoglycerides of short- and medium-chain fatty acids (1-MG), in reducing C. perfringens infection and Eimeria spp. colonization in broiler chickens was investigated. Ninety female one-day-old Ross 308 broiler chicks, not vaccinated against coccidiosis, were randomly housed in poultry isolators and allotted to 3 Treatments. Birds were fed diets supplemented or not with 1-MG (Control diet, C) 0% in all periods; Treatment 1, G1: 0.5% from d 1 to d 10 and 0.25% from d 11 to d 21; Treatment 2, G2: 0.5% from d 1 to d 10 and 0.25% from d 11 to d 21). Each bird was orally infected at d 5 of life with 3,000 sporulated oocysts of Eimeria acervulina, maxima and tenella, and at d 11–12 with 10^6 cfu of C. perfringens. The Clostridium strain belonged to toxin type A, producing α-toxin in vitro and carrying the netB gene. On d 16, 21 and 35 of age 10 birds from each treatment were weighted and sacrificed to collect intestinal samples. A 4-point lesion scoring system according to Johnson and Reid (1970) was applied for each Eimeria species. Macroscopic gut lesions due to C. perfringens were evaluated according to the procedure of Keyburn et al. (2006). Results showed that C. perfringens and E. tenella intestinal lesion score in birds of Treatments G1 and G2 were significantly lower than in Control group (P < 0.01 and P < 0.001 respectively, according Chi-squared test, GraphPad Software); no mortality was recorded in treated groups G1 and G2 while the mortality in Control group was 3.3%. Weight gain in treated groups was significantly higher compared with Control group (P < 0.001 - MIXED procedure of SAS). Results suggest that the specific 1-MG composition may prevent NE, decrease Eimeria spp. colonization, maintain high-standard weight gain and represent an alternative to enteric antibiotics in broiler feed programs. *The 1-MG composition was supplied by SILO S.p.A. (Firenze)

**Key Words:** antibiotic-free, Clostridium perfringens, Eimeria spp., monoglycerides, necrotic enteritis

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**337** Efficacy of a specific composition of short- and medium chain 1-monoglycerides in controlling Clostridium perfringens induced necrotic enteritis in broiler chickens. Manuela Parini*1,2, Giovanni Tosii1,2, Guido Coelho1,3, Arianna Bucchioni2,2, and Alessio Paoli2,1, BASF Corporation, Humble, TX, 2Company SILO, Firenze, Italy, 3Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna, Forlì, Italy, 4Università degli studi di Firenze, Firenze, Italy.

Necrotic enteritis (NE), in acute or subclinical forms, is a concern for the poultry industry, particularly in antibiotic-free poultry production. The causative agent of NE is the Clostridium perfringens, and one of the predisposing factors is the Eimeria spp. colonization. The efficacy of a specific composition* of 1-monoglycerides of short- and medium-chain fatty acids (1-MG), in reducing C. perfringens infection and Eimeria spp. colonization in broiler chickens was investigated. Ninety female one-day old Ross 308 broiler chicks, not vaccinated against coccidiosis, were randomly housed in poultry isolators and allotted to 3 Treatments. Birds were fed diets supplemented or not with 1-MG (Control diet, C) 0% in all periods; Treatment 1, G1: 0.5% from d 1 to d 10 and 0.25% from d 11 to d 21; Treatment 2, G2: 0.5% from d 1 to d 10 and 0.25% from d 11 to d 21). Each bird was orally infected at d 5 of life with 3,000 sporulated oocysts of Eimeria acervulina, maxima and tenella, and at d 11–12 with 10^6 cfu of C. perfringens. The Clostridium strain belonged to toxin type A, producing α-toxin in vitro and carrying the netB gene.

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**Key Words:** antibiotic-free, Clostridium perfringens, Eimeria spp., monoglycerides, necrotic enteritis

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**339** Characterization of Clostridium perfringens recovered from broiler chicken affected by necrotic enteritis. Samuel Mwangi*,1, Jennifer Timmons1, Steve Fitz-Coy2, and Salina Parveen1, 1University Of Maryland Eastern Shore, Princess Anne, MD, 2Merck Animal health, Millsboro, DE.

Necrotic enteritis (NE) caused by Clostridium perfringens (Cp) has emerged as an important disease associated with major economic losses in the poultry industry worldwide. The ban and voluntary withdrawal of antimicrobial growth promoters used to control NE has resulted in resurgence of NE. Moreover, consumer demand for antibiotic-free poultry product has continued to grow. The presence of the NetB gene in broiler chicken does not automatically result in death but other factors such as health of the bird before infection, presence of Cp with NetB gene in broiler chickens does not automatically result in death but other factors such as health of the bird before infection, presence of Cp with NetB gene in broiler chickens does not automatically result in death but other factors such as health of the bird before infection, presence of Cp with NetB gene may be critical for development of NE.
Heat stress and *Eimeria* oocyst infection affect immune tissue histology in broiler chickens. Bryan Aguanta*, Albert Fuller, Susan Williams, Marie Milford, Romdhane Rekaya, and Samuel Aggrey, University of Georgia, Athens, GA.

Heat stress and *Eimeria* infection are stressors that have been shown to affect performance in meat-type chickens, but the biological mechanisms that underlie these stressors are not fully elucidated. We designed an experiment to investigate the histological changes in broiler chickens under heat stress and/or *Eimeria* infection. Fourteen-day-old Cobb500 chickens were assigned randomly in a 2 × 2 × 3 factorial design experiment with 2 temperatures (25°C or 35°C), 2 infection levels (infection with 2.5 × 10^5 *Eimeria* oocysts or non-infection), and 3 anticoccidial treatments (no coccidiatost, 100g monensin/1,000 kg of feed, or 113.5g nicarbazin/1,000 kg of feed). There were 12 treatments, 5 replicates per treatment and 8 birds per replicate. Coccidiatost administration began at 14 d of age, and *Eimeria* infection took place at 15 d of age. Heat treatment began at 15 d of age and ended at 28 d of age. Five birds per treatment were euthanized at 15, 21, and 28 d of age. The spleen, thymus, bursa and liver tissues were collected and fixed in 10% buffered formalin. Following fixation, samples were trimmed, routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Slides were examined by light microscopy. Tissues were scored on a scale of 0 to 6 based on lesion type and severity, with the exception of hypereosinophilic fibers in skeletal muscle tissue, which were scored on a scale of 0 to 4. Compared with the control birds, heat-stressed birds developed significantly more lymphoid depletion in the spleen and thymus at 21 and 28 d of age. The histology data also show that *Eimeria* were found in the duodenums of infected birds raised at 25°C in significantly higher numbers than in infected birds raised at 35°C. These results point to the existence of a network of interactions between heat stress and the broiler immune system, warranting continued investigation of these interactions at the histologic, immunologic, and molecular levels.

**Key Words:** *Eimeria* oocysts, heat stress, lymphoid depletion, immunosuppression, histology

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341 Live *Salmonella* vaccination of broilers: Pen trials to the field. Charles Hofacre*, 1 Roy Berghaus 1, Gregory Mathis 2, and Robert Evans 1, 1The University of Georgia, Athens, GA, 2Southern Poultry Research Group, Athens, GA, 3Elanco Animal Health, Harrisonburg, VA.

*Salmonella* foodborne illness continues to be a significant public health concern. Vaccination of broiler chickens with live attenuated *Salmonella* vaccines has been successfully demonstrated in laboratory challenge studies. Twenty-four floor pens (50 birds/pen) were vaccinated twice with a commercial live *Salmonella typhimurium* vaccine at one day (spray) and 14 d (drinking water). Four days post vaccination, 50% of each pen were orally gavaged with 2.5 × 10^5 *Salmonella* vaccine per bird. At 42 d, 10 horizontal challenged (not tagged) birds were selected from each pen, humanly euthanized, and ceca aseptically collected. Ceca were cultured for prevalence by tetrathionate/XLT-4 agar containing S.H. enumeration was by the Most Probable Number (MPN) method of Berghaus. Ceca prevalence and MPN’s were compared using generalized estimating equations logistic and linear models (P < 0.05). MPN values were log-transformed before statistical analysis. The live S.T. vaccine significantly lowered S.H. prevalence (16% control vs. 0% vaccinated) and lowered the number of S.H. (1.4 cfu/g control vs. 0.0 cfu/g vaccine) in positive ceca. A U.S. broiler company with a *Salmonella* Group D chose to test this vaccine. Prior to vaccination, 3 randomly selected broiler farms per week for 4 weeks were sampled by collecting 6 ceca/farm at ~28 d of age. Vaccination began at one day (spray) and 14 d (drinking water). Ceca were cultured in same manner as pen trial ceca. The live vaccine did not significantly lower overall *Salmonella* prevalence (39% pre vaccination vs. 28% post vaccination). However, the vaccine significantly reduced Group D *Salmonella* prevalence (17% pre vaccination vs. 3% post). Also, positive ceca from vaccinated broilers had significantly less Group D *Salmonella* (15.7 cfu-MPN/g pre vaccination vs. 1.0 cfu-MPN/g post vaccination). Study results demonstrated first by floor pen data and confirmed by field data, that live *Salmonella* vaccine, given twice, can be a valuable tool in reducing potential risks of *Salmonella* human foodborne illness originating in broiler chickens.

**Key Words:** *Salmonella*, broilers, group D, farm study

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342 Genomic traits of a strain of *Salmonella Heidelberg* isolated in broilers in Brazil and related phenotypic tolerance to organic acids and antibiotics. Elizabeth Santin*, 1 Ricardo Hayashi 1, Mariana Camargo Lorenco 1, Raquel Bighetti Araujo 2, Ricardo Gonzalez-Esqueru 2, Marcelo Falsarella Carazzolle 1, Cáo César de Melo Freire 2, Paulo Sergio Monzani 1, and Anderson Ferreira da Cunha 1, 1Universidade Federal do Paraná, Curitiba, Paraná, Brazil, 2Novus International Inc., Indiana, USA, 3Brazil, 4Unidade Estadual de Campinas, Campinas, SP, Brazil, 5Universidade Federal de São Carlos, São Carlos, SP, Brazil, 6Universidade de São Paulo, Pirassununga, SP, Brazil.

*Salmonella enterica* serovar Heidelberg is found in broilers worldwide with isolates from Brazil (*SH*BR) increased since 2011 and showing greater tolerance to control measures. A previous trial showed that a probiotic composed of 3 *Bacillus subtilis* strains was effective vs. *SH*BR in broilers. Herein, we aimed at sequencing the genome of *SH*BR and relate genomic differences with *SH*BR tolerance to some organic acids (OA), antibiotics (AB); and clinical signs in broilers. Two trials used 1d old chicks housed for 21d in 8 sterilized isolated negative pressure rooms with 4 battery cages (reps) of 12 birds each. In both trials, birds were challenged or not with 10^7 cfu/bird of *SH*BR orally, and exposed, or not, to OA in a factorial 2x2 design. Challenge to *SH*BR occurred at 1 or 7d of age; and OA tested consisted of either formic + propionic acids in drinking water at 0.05% from 1 to 7d and 15–21d; or calcium butyrate fed at 2kg/ton of feed from 1 to 7d in trial 1 or 2, respectively. Nine AB were titrated in an in vitro MIC model using Mueller-Hinton agar as in CLSI(MIC) guidelines, to test *SH*BR, AB tolerance. *SH*BR DNA was sent to the High Throughput Sequencing Facility (University of North Carolina). The library was prepared using PacBio 20Kb template prep protocol PN 100–286–000–06, a size-selected range of 8000bp - 50,000bp, and the PacBio native pipeline for De novo assembly. The genome was deposited at NCBI genome database (No. CP020101) and compared with *SH*SL476 strain. Performance and immune response traits were unaffected by *SH*BR (*P > 0.05). The use of OA did not reduce *Salmonella* counts found in cecum and liver of challenged birds (*P > 0.05). *SH*BR was susceptible to amoxicillin-clavulanic acid, cephalosporin, ciprofloxacin, enrofloxacin, penicillin and trimethoprim-sulfamethoxazole and tetracyclin with mild resistance to gentamycin and cefotiofur. Several DNA fragments were missing in the *SH*BR genome which were associated with the codification of proteins involved with cell cycle regulation, virulence, drug resistance, cell adhesion, salt efflux, and various transposases and integrases that may relate to those deletions. These genomic findings relate to the phenotypic observations of low pathogenicity, OA tolerance and AB susceptibility of *SH*BR.

**Key Words:** Brazilian *Salmonella* Heidelberg, antibiotics, organic acids, resistance, comparative genomics
Pathomicrobial and immunohistochemical findings in broiler chickens naturally infected with *Salmonella enterica* serotype *gallinarum* biotype *gallinarum*. Gulbeena Saleem*1, Umar Farooq1, Asim Aslam1, Tariq Javed2, Muhammad Younas3, and Iram Liaquat4, 1University of Veterinary and Animal Sciences, Lahore, Lahore, Punjab, Pakistan, 2University of Agriculture, Faisalabad, Faisalabad, Pakistan, 3University of Veterinary and Animal Sciences, Lahore, Jhang, Pakistan, 4Government College University, Lahore, Pakistan.

A study was conducted to investigate pathomicrobial and immunohistochemical findings in broiler chickens naturally infected with *Salmonella enterica* serotype *gallinarum* biotype *gallinarum* (*S. gallinarum*). A total of 150 broiler chickens were selected from 6 outbreaks of Salmonellosis from different commercial poultry farms. Twenty-five birds were selected from each flock showing typical signs of disease including high fever, bright yellow to mucoid green yellow droppings. Major findings at the time of post-mortem were hemorrhagic, enlarged liver with bronze discoloration, splenomegaly, enlarged kidneys, enteritis, and enlarged ceca with cheesy material. Following postmortem examination samples from different organs (liver, spleen, lungs, intestine, kidney, ceca and heart) were initially cultured, followed by biochemical and serological confirmation. Histopathology and immunohistochemistry was also done on these samples. Microbiological investigations showed that 12% of broiler chickens were positive for *S. gallinarum*. *S. gallinarum* was recovered from liver, intestine, ceca, kidney, lung heart and spleen. None of the birds had *S. gallinarum* in all of the organs investigated. Liver had highest counts (*P* < 0.001) of *S. gallinarum* (2 × 10^{10} cfu/g) followed by spleen (1 × 10^{10} cfu/g), lungs (9 × 10^{9} cfu/g), intestine (7 × 10^{9} cfu/g), and ceca (5 × 10^{9} cfu/g) while count was least in kidney (3 × 10^{8} cfu/g) and heart (9 × 10^{8} cfu/g). Histopathological findings showed sinusoidal congestion and hepatic cord necrosis in liver, interstitial nephritis and tubular necrosis in kidneys. Microscopically lungs showed congestion and intestinal sections showed degeneration along with necrosis. Immunohistochemical findings showed localization of bacteria except heart where no immunoreactivities were observed. It can be concluded from the present study that *S. gallinarum* may not be isolated from all broiler chickens showing signs of *S. gallinarum* infection furthermore not all the birds positive for *S. gallinarum* have bacteria in all the organs investigated in the present study. Liver was the major organ that harbour highest number of *S. gallinarum*. It is further concluded that immunohistochemistry was found precise and sensible tool to locate *S. gallinarum* in body organs.

**Key Words:** *S. gallinarum*, pathomicrobiology, postmortem, immunohistochemistry