M83 Bacillus Subtilis PB6 based-probiotic (CloSTATTM) improves intestinal morphology and microbiological status of broiler chickens under Clostridium Perfringens challenge

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The aim of the present study was to investigate the influence of a commercial Bacillus subtilis PB6 based-probiotic, CloSTATTM, as an alternative to in-feed antibiotic, Enramycine, on growth parameters, intestinal morphology and ileal bacterial count of broilers during pre- and post-challenge with Clostridium Perfringens (C. Perfringens) challenge. 100, 1-d-old male Ross 308 broilers were allocated in five equal groups each with three replicates. The trial groups included; (1) unchallenged - untreated, (2) challenged with mixed Eimeria oocysts - untreated, (3 and 4) challenged with mixed Eimeria species and were given feed probiotic, and (5) challenged with mixed Eimeria species and treated with the anticoccidial lasalocid at 75 mg/kg. Each experimental group was given the corresponding diet throughout the trial period (42 days). Body weight, feed intake, feed conversion ratio, lesion score, bloody diarrhoea and oocysts count were recorded and calculated. At the end of the experiment, duodenum, jejunum and ileum samples were subjected to morphological evaluation.

The results of the study showed that probiotics supplementation exerted a coccidiostatic effect against Eimeria species, reflected on birds’ performance that was similar to lasalocid (P<0.05). Probiotic groups showed less oocyst numbers, lesion score values and bloody faeces than the control infected group but higher than the lasalocid group (P<0.05). Probiotic groups gave the highest values of villous height and villous count due to CS supplementation. The results from this study indicated that CS under the condition of this trial had a positive influence on broilers performance.

Key Words: Broiler, CloSTAT, intestinal mucosa morphometrics, performance

M84 Effect of Probiotics on Growth Performance, Oocysts Shedding and Gut Morphology of Broiler Chickens after Experimental Infection with Eimeria acervulina, Eimeria maxima and Eimeria tenella Oocysts

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Avian coccidiosis is considered one of the major diseases that have a negative economic impact on the poultry industry worldwide. There is an increasing body of literature indicating the relationship between using antimicrobials, including anticoccidial drugs, and the emergence of pathogens resistance to it. In addition, the increasing awareness and demand of consumers for chemical-free animal products enhances the necessity to identify and investigate alternative coccidia control strategies, such as probiotics which would also have the advantage to be closer to environmentally friendly farming practices.

The objective of this trial was to investigate the potential effect of feed probiotics (PoultryStar® - BIOMIN Holding GmbH) on broilers’ performance and gut health experimentally challenged with sporulated oocysts of Eimeria acervulina, Eimeria maxima and Eimeria tenella. A total of 150 Day-old Ross 308 male broiler chicks were divided into five equal groups each with three replicates. The trial groups included; (1) unchallenged - untreated, (2) challenged with mixed Eimeria oocysts - untreated, (3 and 4) challenged with mixed Eimeria species and were given feed probiotic, and (5) challenged with mixed Eimeria species and treated with the anticoccidial lasalocid at 75 mg/kg. Each experimental group was given the corresponding diet throughout the trial period (42 days). Body weight, feed intake, feed conversion ratio, lesion score, bloody diarrhoea and oocysts count were recorded and calculated. At the end of the experiment, duodenum, jejunum and ileum samples were subjected to morphological evaluation.

The results of the study showed that probiotics supplementation exerted a coccidiostatic effect against Eimeria species, reflected on birds’ performance that was similar to lasalocid (P<0.05). Probiotic groups showed less oocyst numbers, lesion score values and bloody faeces than the control infected group but higher than the lasalocid group (P<0.05). Probiotic groups gave the highest values of villous height and villous height to crypt depth ratios in comparison to all other groups (P<0.05).

In conclusion, probiotics gave substantial improvement in both growth performance and intestinal health in birds challenged with Eimeria species, in comparison to control infected birds and similar improvement to that exhibited by lasalocid.

Key Words: Probiotics, intestinal health, performance, broilers, coccidia

SCAD

T85 Outbreaks of tenosynovitis in broilers caused by a novel avian reovirus

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Avian reoviruses are ubiquitous in poultry and usually not a major concern for poultry producers. While reoviruses can be isolated from healthy birds, they have been associated with several diseases including malabsorption syndrome, running and stunting syndrome although their role in these diseases is not always clear. In contrast, the association of some reoviruses with clinical cases of viral arthritis (VA)/tenosynovitis is quite clear especially when virus isolation is made from the tendons of affected birds. Vaccination of breeders with live and inactivated vaccines is common and live attenuated vaccines are available to use in broilers at day of hatch. S1133 is a common strain of reovirus used in commercial vaccines for VA in the U.S.

From December 2011 until present, tendons from numerous clinical cases of tenosynovitis/lameness in broilers, ranging in age from 2.5-6 weeks, were submitted to the Poultry Diagnostic and Research Center. Reoviruses were isolated from the tendons and characterized using RT-PCR/sequencing (genotyping) of the sigma C and virus neutralization assays using reovirus S1133 and 2408 antisera. Genotypic analysis of the reovirus field isolates revealed two novel genotypes to date that were unrelated to current vaccine strains S1133, 2408 and 1733.
Infectious bronchitis (IB) is an acute and highly contagious disease in
commercial broilers using representative strains from one of the novel
genotypes. Tenosynovitis was reproduced in both studies along with
lower body weights and hydropericardium in reovirus field isolate chal-
enged groups. Novel reoviruses have been isolated in recent cases of
tenosynovitis from multiple broiler companies in several states. The
preparation of autogenous vaccines is under consideration by several of
the companies with affected flocks.

Key Words: Tenosynovitis, Novel Avian Reovirus, Lameness

T86 Adjuvant formulations designed to improve poultry vaccine stability Robert Parker*, Juliette Ben Arous, Sebastien Deville, Jerome Gaucheron, Laurent Dupuis SEPPIC Inc., Fairfield, NJ

Vaccine adjuvants are a key parameter in modern vaccine formulation. Most adjuvants are composed of synthetic components with immuno-
modulator properties combined to create a galenic antigen presentation. However, multivalent vaccine antigens often have properties which de-
stabilize vaccine formulations. We have been working on innovative
adjuvants that allow the formulation of vaccines able to resist to very
destabilizing antigenic media and conditions while keeping safety pa-
rameters and efficacy at requested levels.

First, bacterial vaccines were prepared by using Montanide™ stan-
dard and resisting adjuvants for poultry vaccines and were compared for
emulsion stability over time. The safety of formulations based on
resisting adjuvant Montanide™ ISA 71R VG was then tested by intra-
muscular injection of a double dose (1mL) of formulated trivalent viral
vaccine in chickens. Finally, safety and efficacy properties of a Riemel-
rella vaccine were tested in geese in a 17 weeks trial. 20 animals per
group received a subcutaneous injection of 0.3ml of vaccine. Behavior
of the animals, body weight gain, local reactions at the injection site
(during trial and at slaughter) were assessed during the trial. Specific
antibody titers were measured by ELISA titration at D0 and at 6, 10,
14 and 17 weeks.

We could show that slight adjuvant composition modifications can al-
low the formulation of stable vaccines able to pass severe stress tests. In
chickens and geese trials, both resisting and standard formulations showed comparable acceptable safety levels. Results in the goose mo-
del showed that there were no efficacy differences between standard and
resisting adjuvants, and that one injection of vaccine conferred stable
antibody titers over 17 weeks.

We have shown that new Montanide™ adjuvants developed to resist
to destabilizing antigenic media maintain high antibody levels and an
acceptable safety profile in poultry, even combined with reactogenic
Gram negative bacterial antigens. This new line of adjuvant will help to
increase long term stability of poultry vaccines which are based on
destabilizing antigens or stored in stressing conditions.

Key Words: Vaccine, Adjuvant, Goose, Vaccine stability

T87 Genotypic analysis of GA07 nephropathic field isolates of infectious bronchitis viruses Vijay Durairaj*, Erich Limemann, Vanessa Gauthiersloan, Susan M. Williams, Holly S. Sellers Poultry Di-
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Infectious bronchitis (IB) is an acute and highly contagious disease in
chickens caused by infectious bronchitis virus (IBV). IBV is an envel-
oped, single-stranded RNA virus which primarily affects the respira-
tory and reproductive tract in chickens. Additionally, some viruses have
a predilection towards kidneys and are known as nephropathic strains of
IBV. Nephropathic IB viruses can cause flushing in chickens along
with varying levels of mortality with or without respiratory symptoms.
The cell tropism and pathogenesis of IBV is primarily dependent on
the spike (S) glycoprotein. Several immunogenic regions are located
within the S1 subunit of the spike glycoprotein which is responsible for
protective immunity. The main objective of this study was to analyze
genetic changes within the S1 subunit from previously isolated variant
nephropathic isolates of IBV classified as GA07. The GA07 variant vi-
ruses were isolated from clinical cases of flushing on commercial poul-
try farms during 2007-2012. IBV was isolated from 61 submissions and
genotyped based on S1 spike glycoprotein sequence. The nucleotide
sequence analysis was performed using molecular and bioinformatics
tools and was in silico translated to corresponding amino acids. Phylo-
genetic analysis of the nucleotide sequences from the field isolates were
studied with the common vaccine strains. We compared the amino acid
sequences of GA07 isolates from 2007-2012 and observed multiple
amino acid mutations in the S1 subunit. These mutations were not only
restricted to the hypervariable regions but also seen in the intervariable
regions.

Key Words: Nephropathic IBV, Spike Protein, Genotyping, Phyloge-
netic analysis, Flushing

T88 Assessment of live Newcastle disease virus VG/GA strain (Avinew®) subcutaneous vaccination. Francisco Perozo1, Rosmar
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The Villegas-Glisson/University of Georgia (VG/GA) strain of New-
castle disease virus (NDV) is used worldwide for Newcastle disease
disease and has been tested for spray, drinking water and in ovo applica-
tion. Hatchery vaccination provides a controlled and clean environment
for poultry vaccination, currently day-1 NDV vaccination in most en-
demic countries includes live coarse spray and killed subcutaneous
applications. This work aims to assess the efficacy of including NDV live
VG/GA strain subcutaneous vaccination at the hatchery. Four groups of
10 1-day-old commercial broilers were used (three replicates). Group 1:
one-day-old dual (live/killed) vaccination with two field boosts
(eight and 18 days). For groups 2 and 3, subcutaneous VG/GA strain
was included with or without field revaccination. Group 4 remained as
unvaccinated control. Percentage of survival, serological response and
viral shedding were used as efficacy criteria. All birds where challenged
28 days with a lethal dose of a genotype VII NDV. The control group
died within five days after challenge. All vaccinated birds survived the
challenge, including the group with no field revaccination. No adverse
effects were observed after subcutaneous vaccination. Adequate protec-
tion, plus differences in serological responses and viral shedding sug-
gest the suitability of VG/GA strain (Avinew®) subcutaneous vaccina-
tion and are discussed.

Avinew® is a registered trademark of Merial in the United States of
America and elsewhere.

Key Words: Newcastle Disease, VG/GA strain, subcutaneous vaccina-
tion

T89 The impact of mergers on the history of the poultry industry John Donahoe* Industry Consultant, Flowery Branch, GA

Industry cycles tend to follow a temporal pattern of aging, consolida-
tion, and mergers. This drives implementation of economies of scale.
Early in these cycles, you see numerous businesses founded by entre-
prise.
prenurial technical people with knowledge of the industry and a clear vision of a short term future built on perceived customer needs. Successful startups experience growth which attracts the attention of investors and/or investment bankers. Increased business acumen follows as does a desire to protect the “asset” with regards to growth, ideas, and proprietary knowledge. Rapid innovation is rarely sustained over the long term so economies of scale increase in importance. This drives a process of mergers and acquisitions, the success of which varies. In an era of multinational publically traded companies, the former startups may be involved in merger and acquisition activities but be at some distance from driving the process.

The poultry vaccine industry has experienced this industrial aging cycle as has its main customer bases, the breeder and poultry production industries. What is the impact of this process on poultry biologics customers? The pros and cons of the impact will be discussed as will visions of how innovation and entrepreneurship will be maintained.

Key Words: Poultry Industry, Consolidation

T90  The antigenic characteristics of very virulent infectious bursal disease viruses. Darrell Jackowski1, Simone Stoute  The Ohio State University/OARDC, Wooster, OH

The very virulent infectious bursal disease virus (vvIBDV) strains have long been considered to be antigenically similar to classic IBDV. The suspected reason for the early infection of broilers despite strong maternal immunity to classic IBDV has been their high virulence. Studies were conducted using reassorted IBDV strains with a vvIBDV genome segment A and a classic virus genome segment B. These viruses contained VP2 antigens identical to the vvIBDV but the presence of genome segment B from classic viruses reduced the virulence to that of a non-vvIBDV. Although their pathogenicity was reduced, these viruses still broke through maternal immunity earlier than the classic and variant IBDV controls. These results and our previous studies on amino acids contributing to antigenic drift in IBDV, indicate the vvIBDV may be antigenically distinct from classic viruses. The data also suggest a vaccine specific for vvIBDV may be more efficacious than the classic vaccines currently used to control these highly pathogenic strains.

Key Words: vvIBDV, Antigenicity, Maternal Immunity, Vaccines

T91 Evaluation of inactivated avian influenza H7 vaccines for protection of chickens against a highly pathogenic avian influenza virus H7N3 isolated from chickens in Jalisco, Mexico, during 2012. Darrell Kapczynski1*, Mary Pantin-Jackwood1, Yadira Ricardoz2, Sofia Guzman2, Erica Stackman1, David Suarez1, David Swayne1 1USDA-ARS-SEPRL, Athens, GA 2SENASICA, Cuajimalpa, Mexico

A recent outbreak of highly pathogenic avian influenza (HPAI) H7N3 was reported poultry in Jalisco, Mexico, beginning in June of 2012. To date more than 11 million birds have died or been slaughtered in an effort to stop the spread of disease. In response to the outbreak, vaccine efficacy trials were recently performed to determine if U.S.- and Mexican-origin inactivated H7 vaccine would protect birds from clinical disease and shedding of virus. In the first set of experiments, four phylogenetically-related U.S. low pathogenic avian influenza (LPAI) isolates, either H7N2 or H7N3, were formulated into inactivated emulsion vaccines and injected into 7 week old SPF birds. These isolates contained between 92-97 amino acid similarity to the hemagglutinin gene of the challenge virus (A/chicken/Jalisco/CPA1/2012 H7N3). The APHIS-approved H7 vaccine antigens were included into this experiment for testing. Birds were challenged at 10 weeks of age with 100 EID50 per bird delivered via intranasal route. Results demonstrate that three of the four H7 vaccine isolates tested provided 100 % protection, whereas the fourth isolate provided 90 % protection. In the second experiment, a Mexican-lineage LPAI H7N3 isolate from wild birds, with 98 % sequence similarity to the HPAI virus, was formulated into an inactivated vaccine and applied to 2 week old birds. Birds were challenged as previously described and demonstrated 100 % protection from challenge. All vaccines tested reduced shedding of virus compared to sham vaccinated birds. Taken together, these results indicate that both U.S. and Mexican vaccine isolates can provide protection to poultry against this recent HPAI H7N3 virus.

Key Words: highly pathogenic avian influenza, vaccine, poultry, H7N3, protection

T92 Comparison of serological methods for the detection of antibodies to Chicken Anemia Virus in chicken sera. Chintia Lamichhane*, Haichen Song, Dan Domingo  Pfizer Animal Health, College Park, MD

Chicken Anemia virus (CAV) is the etiological agent of infectious anemia of chicken. The clinical symptoms are severe anemia with low hemocrit, retardation of growth, atrophy of the thymus, bone-marrow and bursa of fabricius, hemorrhages in skeletal muscles, and destruction of lymphoid tissues. Chicks are immunosuppressed and high morbidity and mortality are attributed to secondary infection.

Virus neutralization and indirect immunofluorescent antibody tests are considered as gold standard methods for detecting antibodies to CAV. The enzyme-linked immunosorbent assay (ELISA) was developed for the rapid and efficient large scale screening of antibodies to Chicken Anemia Virus (CAV). This study examines the sensitivity and specificity of a new commercial CAV ELISA assay (ProFLOK ® CAV PLUS Antibody Test Kit, Pfizer Animal Health), Indirect Immunofluorescence Antibody (IFA), and Virus neutralization (VN) tests on sera from controlled and field exposure conditions.

The efficacy data for the new CAV PLUS ELISA Kit demonstrate that the kit can accurately detect CAV antibody. Masked suitability panels show that the analytic specificity was 100% compared to IFA, detecting antibodies to CAV and not antibodies to other tested pathogens. The new CAV ELISA is highly correlated to currently available tests with an overall agreement of greater than 95% between the ELISA, IFA and VN tests. The kit demonstrated excellent well-to-well (%CV less than 5.7%) and plate-to-plate (%CV equal to 4.5%) reproducibility. The kit also demonstrated the ability to detect antibody to CAV one week post-infection (after maternal antibody is depleted, at which point, any antibody level is attributed to response to field exposure).

The new ProFLOK CAV PLUS ELISA can be used for quantitative and qualitative analysis of antibodies to Chicken Anemia Virus (CAV) in chicken sera.

Key Words: Chicken Anemia Virus ELISA, ProFLOK CAV PLUS ELISA, Chicken Anemia Virus, ELISA, Chicken Infectious Anemia Virus