PROCEEDINGS BOOK

14TH INTERNATIONAL COCCIDIOSIS CONFERENCE

2-4 JUNE 2026

GHENT, BELGIUM

Dear attendants of the 14th International Coccidiosis Conference (ICC2026)

In animal production, maintaining effective control of parasitic diseases remains essential for improving productivity, reducing reliance on antimicrobials, and safeguarding animal health and welfare. Among these challenges, coccidiosis continues to be one of the most significant intestinal diseases affecting poultry worldwide, driving major scientific and technological efforts to better understand host–parasite interactions, immunity, and gut health resilience.

Over the past decades, substantial progress in coccidia biology, epidemiology, and control strategies has enabled the development of innovative approaches, including improved vaccines, targeted anticoccidial programs, nutritional interventions, and advanced diagnostic tools. These advances are increasingly shaping more sustainable and precision-based poultry production systems.

The 14th International Coccidiosis Conference (ICC2026) continues this global tradition as a leading platform dedicated to the exchange of cutting-edge research and field experience in coccidia and coccidiosis. The conference will bring together researchers, industry experts, academics, and policymakers to foster collaboration and accelerate innovation in animal health and production.

We are pleased to welcome participants to Ghent, Belgium, from June 2–4, 2026, a vibrant European hub for veterinary science and agricultural research.

With this edition, we are pleased to open the conference with a workshop titled “An Overview of Regulatory, Safety, and Efficacy Assessments of Coccidiostats in the European Union”, which will set the stage for discussions by bringing together perspectives from industry representatives and food safety authorities, including the European Commission (DG SANTE) and EFSA.

Building on this opening session, the scientific program will feature internationally recognized keynote speakers who will present the latest advances in coccidiosis research, ranging from parasite biology and host immune responses to applied control strategies and industry-relevant solutions. In addition, short oral communications will highlight emerging findings, while poster sessions accompanied by concise pitch presentations will provide an interactive forum for scientific exchange and discussion.
This conference aims to showcase the latest scientific knowledge, strengthen collaboration across disciplines, and support the development of innovative solutions for sustainable animal production. We look forward to welcoming the global coccidiosis research community for an inspiring and productive meeting in Ghent.

On behalf of the organizers, we sincerely thank all speakers, sponsors, and participants for their contributions and engagement.

14th International Coccidiosis Conference 2026

WORKSHOP SPEAKERS

- Bob Cornez (Huvepharma)
- Fabien Schneegans (European Commission Directorate-General for Health and Food Safety (DG SANTE) G5 – Food Hygiene, Feed and Fraud)
- Vasileios Bampidis - European Food Safety Authority (EFSA) The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)
- Alberto Navarro Villa - European Food Safety Authority (EFSA) The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

KEYNOTE SPEAKERS

- Anja Joachim (Institute of Parasitology, Vetmeduni Vienna, Austria)
- Damer Blake (Royal Veterinary College, United Kingdom)
- Jana Kvičerová (University of South Bohemia in České Budějovice, Czechia)
- Marc Pagès Bosch (Hipra Scientific SLU)
- Rami A. Dalloul (Department of Poultry Science, University of Georgia)
- Woo Kyun Kim (Department of Poultry Science, University of Georgia, U.S.A.)
- Sue Vaughan (Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom)

SCIENTIFIC COMMITTEE

- Damer Blake (Royal Veterinary College, United Kingdom)
- Brecht Maertens (Poulpharm, Belgium)
- Berit Bangoura (University of Wyoming, Laramie, WY, USA)
- Fiona Kenyon (Moredun Research Institute, Penicuik,)
- Fiona Tomley (Royal Veterinary College, London, United Kingdom)
- Gunther Antonissen (Ghent University, Belgium)
- Isa Danladi Jatau (Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria)
- Jana Kvičerová (University of South Bohemia, České Budějovice, Czech Republic)
- Jean-Michel Reperant (French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan, France)
- John Barta (University of Guelph, Guelph, ON, Canada)
- Rodrigo Megía-Palma (Universidad Complutense de Madrid (UCM), Faculty of Biological Sciences, Dep. Biodiversity, Ecology and Evolution, 28040, Madrid, Spain)
- Vladimir Vrba (Bioproperties Pty Ltd, Ringwood, Australia)
- Xun Suo (China Agricultural University, Beijing, China)

ORGANIZING COMMITTEE

- Damer Blake (Royal Veterinary College, United Kingdom)
- Maarten De Gussem (Vetworks, Belgium)
- Giuditta Tilli (Vetworks, Belgium)
- Monita Vereecken (Huvepharma, Belgium)

SUPPORT

- Sirje Soo (Vetworks, Belgium)
- Dóra Máté (Poulpharm, Belgium)

14TH INTERNATIONAL COCCIDIOSIS CONFERENCE 2026

Timetables

General timetable

14th International Coccidiosis Conference Program

START	END	ACTIVITY	PRESENTER	TITLE
TUESDAY – 2026 June 2				
8:00	9:00	**Registration**		
9:00	9:15	**Welcome and opening**	**The ICC organising team**	
Workshop - "An Overview of Regulatory, Safety, and Efficacy Assessments of Coccidiostats in European Union"				
9:15	9:20	**Opening remarks**	Chairs: Manoj Aggarwal (MSD Animal Health) and Maarten De Gussem (Vetworks)	
9:20	9:55	**Lecture 1**	Bob Cornez (Huvepharma)	Coccidiostats in the European Union: Challenges and Future Perspectives
9:55	10:20	**Lecture 2**	Fabien Schneegans (European Commission Directorate-General for Health and Food Safety (DG SANTE) G5 – Food Hygiene, Feed and Fraud)	The legal framework for the authorisation of coccidiostat feed additives in the European Union
10:20	10:50	**Coffee break**		
10:50	11:45	**Lecture 3**	Vasileios Bampidis & Alberto Navarro Villa –European Food Safety Authority (EFSA) The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)	The assessment of coccidiostat feed additives in European Union: General aspects and specific case studies
11:45	12:15	**Round table and discussion**		
12:15	13:45	**Lunch**		
Session 1 – Epidemiology and diagnostic tools – Chair: Monita Vereecken				
13:45	14:30	**Keynote 1**	**Jana Kvicerova (University of South Bohemia in České Budějovice, Czechia)**	**"From samples to letters": methods, conflicts, biases, and pitfalls in coccidian research**
14:30	14:45	**Oral 1**	Patricia Soster de Carvalho (Ghent University and Poulpharm) - Presented by Gunther Antonissen (UGent)	Sound and image analysis to detect coccidiosis-related changes in broiler chickens
14:50	15:05	**Oral 2**	Iram Gladan (Utrecht University)	Evaluating ovotransferrin and calprotectin as biomarkers for Eimeria infections in broilers under field conditions
15:05	15:25	**Coffee break**		

Session 2 – Genomics, genetics, and evolutionary diversity – Chair: Fiona Tomley				
15:25	16:10	**Keynote 2**	**Damer Blake (Royal Veterinary College, UK)**	**Exploring Eimeria genomes to understand Eimeria populations**
16:10	16:25	**Oral 3**	Conor Noonan (Royal Veterinary College)	Comparative Genomic Analysis of Poultry-Infecting Eimeria Genomes
16:25	16:40	**Oral 4**	Brecht Maertens (Poulpharm)	Prevalence of the three cryptic Eimeria species in Europe
16:40	16:43	**Oral (pitch) 5**	Daniel Divín (BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Czech Republic)	Identification of cryptic Eimeria and subsequent evaluation of the situation on Czech production farms
16:43	17:45	**From Posters to Pints: Poster session and beer tasting**		

WEDNESDAY – 2026 June 3				
8:00	9:00	**Registration**		
Session 3 – Immunology and vaccines – Chair: Jana Kvicerova				
9:00	9:45	**Keynote 3**	**Marc Pages Bosch (Hipra Scientific SLU)**	**From First Contact to Protection: The Immunological Journey Against Eimeria**
9:45	10:00	**Oral 6**	Xinming Tang (Institute of Animal Science, Chinese Academy of Agricultural Sciences)	EtAP2_SM Controls Sporogony in Eimeria tenella and Underpins Genetically Engineered Vaccine Design
10:00	10:15	**Oral 7**	Swati Karki (Poulpharm) – presented by Maarten De Gussem (Vetworks)	Impact of Coccidiosis Vaccination on Broiler Performance Under Heat Stress Conditions
10:15	10:18	**Oral (pitch) 8**	Yingying Sun, Xun Suo, Xianyong Liu (State Key Laboratory of Veterinary Public Health and Safety; Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China)	Immunogenicity and protection against infectious bursal disease via a transgenic Eimeria acervulina expressing IBDV VP2-2C3d fusion protein
10:18	10:45	**Coffee break + poster session**		
Session 4 – Cell biology and host-pathogen interaction – Chair: Damer Blake				
10:45	11:30	**Keynote 4**	**Sue Vaughan (Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK)**	**Investigating the 3D ultrastructure of Eimeria tenella asexual and sexual stages**
11:30	11:45	**Oral 9**	Anne Silvestre (INRAE)	Impact of Eimeria tenella rhoptry kinase on host cell signaling: transcriptomic and phospho-proteomic analyses
11:45	12:00	**Oral 10**	Brecht Maertens (Poulpharm)	Evaluation of Eimeria mitis pathogenicity
12:00	12:15	**Oral 11**	Marie Ithurbide (GABI, INRAE, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France)	Understanding the direct and indirect impacts of host response traits on chicken coccidiosis: insights from epidemiological modelling
12:15	12:30	**Oral 12**	Francisca Velkers (Department Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands)	Effect of red-colored feed on Eimeria infection dynamics in Ross 308 broilers
12:30	14:00	**Lunch**		

Session 5 – Experimental and In vitro systems - Chair: Jean-Michel Répérant				
14:00	14:45	**Keynote 5**	**Anja Joachim (Institute of Parasitology, Vetmeduni Vienna, Austria)**	**Research in coccidiosis: experimental and in vitro systems**
14:45	15:00	**Oral 13**	Alireza Khadem (Innovadgroup)	Impact of Natural Coccidiosis on Fat Digestibility and Broiler Performance Under Commercial Conditions
15:00	15:15	**Oral 14**	Oluwayomi Adeyemi (University of Lagos)	Preliminary survey of in-feed mycotoxin contamination and Eimeria co-occurrence in Nigerian chicken farms: implications for disease control and food safety
15:15	15:18	**Oral (pitch) 15**	Matthew Ogwiji (Modibbo Adama University Yola / Ahmadu Bello University Zaria)	Prebiotic, Probiotic and Synbiotic Supplementations Ameliorated E. tenella induced Pathology in Broiler Chickens
15:18	15:45	**Coffee break + poster session**		
Session 6 – Microbiomes and co-infections – Chair: Vladimir Vrba				
15:45	16:30	**Keynote 6**	**Woo K. Kim (Department of Poultry Science, University of Georgia, U.S.A.)**	**Eimeria challenge on gut micro-environment, microbiomes and opportunity for pathogens**
16:30	16:45	**Oral 16**	Jean-Michel Répérant (Anses, Ploufragan-Plouzané-Niort Laboratory, Ploufragan, France)	Effect of some ionophores and a vaccine on the persistence of vancomycin resistant Enterococcus faecium and on efficacy after an Eimeria challenge
16:45	17:00	**Oral 17**	George Tice (Independent Scientific, Policy and Regulatory Consultant)	The use of ionophore coccidiostats in broilers, the narAB gene and its relevance to antibiotic resistance in human clinical isolates of Enterococcus faecium and Enterococcus faecalis
17:00	17:03	**Oral (pitch) 18**	Melanie C. Hay (Royal Veterinary College)	Profiling Eimeria burden, species composition, and genomic heterogeneity in poultry caecal metagenomes using Illumina and Nanopore sequencing
18:30	22:30	**Conference dinner at Yalo Rooftop (only with prior registration)**		

THURSDAY – 2026 June 4

Session 7 – Prophylaxis and treatment, novel approaches for control – Chair: Brecht Maertens				
9:00	9:45	**Keynote 7**	**Rami Dalloul (Department of Poultry Science, University of Georgia)**	**Navigating the complex intervention strategies of coccidiosis control**
9:45	10:00	**Oral 19**	Vereecken Monita (Huvepharma NV)	Efficacy of a trivalent Eimeria vaccine for turkeys when applying spray on birds vaccination
10:00	10:15	**Oral 20**	Jean-Michel Répérant (Anses, Ploufragan-Plouzané-Niort Laboratory, Ploufragan, France)	A model close to actual conditions of use for evaluating disinfectants against coccidia oocysts
10:15	10:30	**Oral 21**	Madalina Diaconu (EW Nutrition, University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca, Romania)	Meta-Analysis of Four Controlled Studies Evaluating the Prophylactic Efficacy of Natural Feed Additives Against Experimental Coccidiosis in Broiler Chickens
10:30	10:45	**Oral 22**	Oluwayomi Adeyemi (University of Lagos)	In silico exploration of the anticoccidial activity of Omi ogi (fermented maize supernatant) against Eimeria tenella
10:45	11:15	**Coffee break + poster session**		
11:15	11:18	**Oral (pitch) 26**	Violette Pousset (NOR-FEED)	Performance and Health Outcomes of a Saponin-Based Solution (Norponin XO2) Compared with Conventional Anticoccidial Programs in Broiler Chickens Reared in Commercial Conditions
11:18	11:21	**Oral (pitch) 27**	Xiaojin Li (State Key Laboratory of Veterinary Public Health and Safety; Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China)	Apicoplast Pyruvate Carrier 1 is a Novel Marker for Diclazuril Resistance in Eimeria
11:21	11:24	**Oral (pitch) 28**	Paulina Abramowicz (Department of Research and Development of AdiFeed LTD)	The effect of phytogenic mixture addition on gut integrity parameters, coccidiosis score in chicken broiler fed two different diets
11:24	11:27	**Oral (pitch) 29**	Rafiullah (The University of Agriculture Peshawar Pakistan)	Phytase Supplementation Enhances Phytate Digestibility and Reduces Phosphorus Run off in Broiler Production
11:27	11:30	**Oral (pitch) 30**	Stéphanie Lecuelle (Novus International Inc.)	Gene Expression Explained the Benefits of Protected Thymol and Carvacrol Under a Chemical-Ionophore Coccidiostat Program on Broiler Performance
11:30	11:33	**Oral (pitch) 31**	Yohannes Tekle Asfaw (Mekelle University)	Clinico-Pathological Study of Avian coccidiosis and its Economical Impact on Small-scale Poultry Farming in Selected Districts of Tigray, Ethiopia
11:33	12:00	**Awards, closing remarks**		
12:00		**End of the conference**		

WORKSHOP LECTURERS

Lecturers' biography

Dr Vasileios Bampidis

Dr Vasileios Bampidis is Professor of Animal and Nutritional Sciences at the Department of Agriculture, School of Geotechnical Sciences, International Hellenic University (IHU, Thessaloniki, Greece). He is Veterinarian (School of Veterinary Medicine, Aristotle University of Thessaloniki – AUTH, Greece) and has a PhD in Animal Nutrition (School of Veterinary Medicine, AUTH, Greece). He has been Post Doctoral Fellow at the Research Institute of Animal Production (Prague, Czech Republic), and at the Department of Animal Science (University of California, Davis, USA). He has been working at the Hellenic Feedstuff Industries SA (Greece), and at the Research Institute of Animal Science (National Agricultural Research Foundation – NAGREF, Greece), and he has been teaching at the Department of Animal Production and the Department of Agricultural Technology (Alexander Technological Educational Institute of Thessaloniki, Greece), at the Department of Veterinary Science (University of Thessaly, Greece), as well as at the Department of Agriculture (AUTH, Greece) and the Department of Agriculture (IHU, Greece). He is scientific expert of the European Food Safety Authority (EFSA) and Member of the Scientific Council of the Hellenic Foundation for Research and Innovation (HFRI), holding the position of member in the scientific area of Agricultural Sciences. He has been the Project Leader in 7 scientific projects financed by European Union and Greek Authorities, as well as participant in 38 other research projects.
He has participated in over 200 international and national conferences and workshops, with scientific papers and/or as Chair/Member in scientific sessions. He has more than 1,300 publications and oral presentations in international and Greek scientific journals and conferences, attracting more than 13,000 citations, with an h-index 48. He has served as Co-Editor-in-Chief (2018–2024) in the international scientific journal Animal Feed Science and Technology (Elsevier, ISSN: 0377-8401), as well as Associate Editor, Member of the Editorial Board and Reviewer in 24 international scientific journals. ORCID (https://orcid.org/0000-0001-8823-6365).

Dr. Bob Cornez

Bob Cornez is Senior Director Regulatory Affairs at Huvepharma and a veterinarian with more than 40 years of international experience in veterinary medicines, pharmaceuticals, and feed additives.
After graduating in Veterinary Medicine from Ghent University in 1982, he held positions in the pharmaceutical and feed additive industries originally involved with commercial activities such as trading, barter trading and area management.
Since 1995 he has held senior regulatory leadership roles in the animal health sector including at Janssen Pharmaceutica and Alpharma, overseeing the development, registration, and lifecycle management of veterinary medicinal products and feed additives across Europe.
Since joining Huvepharma in 2008, he has led the strategic product development programs of the company for veterinary medicines and feed additives in the European Union with an emphasis on live-stock products such as antimicrobials, including coccidiostats. Currently he is also actively engaged in governmental and public affairs initiatives.

KEYNOTE SPEAKERS

Speakers' biography

Anja Joachim

I studied veterinary medicine in Hannover, Germany, and got hooked in parasitology for my PhD and postdoc in Hannover, Sydney (Australia) and Copenhagen (Denmark). Since 2003 I am full professor for veterinary parasitology at the University of Veterinary Medicine, Vienna, Austria. I was President of the European Veterinary Parasitology College (2009-2012) and member of tis educational committee 2016-2021. Currently I am a member of the Executive Committee of the World Association for the Advancement of Veterinary Parasitology and Co-Editor-in chief of Veterinary Parasitology.
My research focus is the biology and control of protozoa. I am interested in improvement of control strategies against protozoal diseases of mammals, especially porcine coccidiosis, and the possibility of vaccination against it, the in vitro cultivation of coccidian parasites and the sexual development of Coccidia in the context of novel control options.
I have been awarded scholarships and research grants (German and Austrian Research Councils, Karl-Enigk-Foundation, Austrian Research Promotion Agency) and have, to date, published, >250 peer-reviewed publications and two European patents through the University of Veterinary Medicine Vienna.

Damer Blake

Following a PhD focused on bacterial genetics from the University of Aberdeen, Damer began working in the area of poultry gut health in 2001 at the Institute for Animal Health (IAH, UK). During his time at IAH he carried out fundamental and applied genetics-led research into coccidiosis, contributing to the Eimeria genome sequencing consortium and working towards new, cost-effective anticoccidial vaccines.
Damer joined the Royal Veterinary College in 2010, becoming Professor of Parasite Genetics in 2016. Current research strands include population genetic analyses of recognised and new Eimeria species, development of novel vaccine delivery strategies, understanding the genetic basis of host resistance to coccidiosis, interactions of Eimeria with bacterial microbiota of poultry, dysbiosis and wider aspects of gut health.
Recent work includes exploring host – parasite – microbiome interactions in Asian and European chicken production systems, understanding the impact of host genotype and antimicrobial exposure. In 2017 Damer became Editor-in-Chief of the journal Avian Pathology.

Jana Kvičerová

Jana Kvičerová obtained her DVM degree in Veterinary Medicine from the University of Veterinary Sciences in Brno, Czech Republic, and her PhD in Parasitology from the University of South Bohemia in České Budějovice, Czech Republic. She is currently employed as an Assistant Professor at the Department of Parasitology, Faculty of Science, University of South Bohemia in České Budějovice, and as a Researcher at the Department of Zoology, Faculty of Science, Charles University in Prague, Czech Republic.
Her research focuses on the morphology, taxonomy, host specificity, and phylogenetic, evolutionary, and population genetic studies of several model systems of apicomplexan parasites, particularly intestinal and blood Coccidia infecting rodent and reptile hosts. In addition, she

is interested in zoonotic pathogens – especially hantaviruses – from a One Health perspective, with a particular focus on their detection, specificity, and phylogenetic relationships in rodent hosts. More recently, her research has expanded to NGS-based analyses of gut, oral, and vaginal microbiota profiles in rodents and their associations with parasitic infections, contributing to a broader understanding of host-microbe-parasite interactions in ecological and evolutionary contexts.
Methodologically, her work is broad-based, encompassing fieldwork, laboratory diagnostic techniques, microscopy, molecular biology, computational methods of molecular phylogenetics, reconstructions of coevolutionary relationships and population structure, and bioinformatic processing of NGS data. She is a mentor of graduate and undergraduate students, a peer reviewer for several international journals in Parasitology, Microbiology, and Molecular Biology, and maintains active collaborations with research laboratories in the Czech Republic and abroad.

Marc Pagès Bosch

Dr. Marc Pagès Bosch is Senior R&D Manager for bacterial and parasitic vaccines in poultry at HIPRA. He obtained his degree in Microbiology and Genetics from the University of Barcelona in 1998 and completed his PhD focusing on the synaptonemal complexes of Eimeria tenella chromosomes during meiosis.
His professional career in poultry coccidiosis began in 2000, when he joined the research group of Dr. Emilio del Cacho at the Department of Parasitology, University of Zaragoza (Spain). Since then, he has played a key role in the development and global registration of several live attenuated anticoccidial vaccines, including HIPRACOX®, EVALON®, EVANT® and EVANOVO®, serving as R&D Project Manager for their worldwide authorization.
In addition to his work on live vaccines, Dr. Pagès spent two years at the laboratory of Dr. Hyun Lillehoj (Animal Parasitic Diseases Laboratory, USDA, Beltsville, USA), where he contributed to the identification and isolation of protective Eimeria antigens for the development of subunit vaccines against poultry coccidiosis.
Beyond coccidiosis, he has also been involved in research projects focused on the development of vaccines or biological products targeting Cystoisospora suis, Dermanyssus gallinae, and Salmonella spp.

Rami A. Dalloul

Dr. Dalloul is the R. Harold Harrison Distinguished Professor of Poultry Science and the Interim Assistant Dean for Research of the College of Agricultural and Environmental Sciences at the University of Georgia (Athens). He grew up in Lebanon and attended the American University of Beirut where he earned dual bachelor's degrees in Agriculture (Animal Sciences) and Agricultural Engineering followed by a Master's degree in Poultry Microbiology. He then joined the University of Maryland for his doctoral studies that focused on poultry immunology and gut health researching coccidiosis, host response, and mitigation tools.
Following a postdoctoral fellowship working on immunity to enteric pathogens, Dr. Dalloul joined the faculty at Virginia Tech University before moving to the University of Georgia Poultry Science in 2020. His research intersects several multidisciplinary focus areas investigating host-pathogen interactions during enteric challenges. Particular emphasis is on coccidiosis and clostridial diseases in chickens and turkeys, very impactful diseases of commercial poultry. In this context, his group explores the molecular mechanisms of mucosal immunomodulation and physio-immunological responses of the host during such challenges. Further, his lab employs unique parasitic and bacterial challenge models to interrogate issues of nutritional immunology, specifically applications for enhancing the early development and competence of the immune system as well as promoting a healthy microbiome in commercial poultry.

Woo Kyun Kim

Woo Kyun Kim is a professor/poultry nutritionist in the Department of Poultry Science, University of Georgia. He received a PhD in nutritional science at Pennsylvania State University.
He was a Postdoc Fellow at Texas A & M University and University of California Los Angeles.
He worked as an assistant professor/monogastric nutritionist at University of Manitoba before his joining as a faculty member at University of Georgia.
His research has focused on roles of feed additives, amino acids, vitamins, and minerals on gut and bone health in poultry under various challenge conditions (coccidiosis, necrotic enteritis, pathogen infection, and heat stress).
He has published over 280 peer-reviewed papers and obtained over $25 million in extramural funding. He was the recipient of the D.W. Brooks Award Excellence in International Agricultural and Environmental Sciences, the PSA American Feed Industry Association Nutrition Research Award, the UGA-CAES Outstanding Mentor Award, and the National Chicken Council Broiler Research Award.
Dr. Kim currently serves as a section editor for Poultry Science Journal.

Sue Vaughan

Sue Vaughan is professor of cell & molecular biology and director of the Oxford Brookes Centre for Bioimaging, UK. Sue studied for a BSc in Microbiology at University of Manchester where her passion for eukaryotic parasites began. Her PhD focused on the flagellum and cell division in the protozoan parasite Trypanosoma brucei where she discovered novel tubulins in eukaryotic parasites. During this time Sue began using high resolution electron microscopy and following her PhD she moved to University of Oxford where she continued to investigation the function of the flagellum using advanced 3D imaging techniques.
In 2010 Sue moved to Oxford Brookes University where she established 3D cellular electron tomography and serial block-face scanning electron microscopy. This broadened her research and collaborations to include other eukaryotic parasites including Eimeria. Her current research interests include gametogenesis in Plasmodium and establishing techniques to study life cycles of eukaryotic parasites in vivo including Eimeria and Toxoplasma. Oxford Brookes centre for bioimaging is part of the UK node of Eurobioimaging, enabling researchers from both the UK and Europe to access cutting-edge microscopes, analysis tools and support. Sue's team supports a wide range of parasite researchers from around the world.

KEYNOTE PRESENTATIONS

Abstracts

Research in coccidiosis: experimental and *in vitro* systems

Anja Joachim
Institute of Parasitology, Vetmeduni Vienna, Austria

As for all relevant infectious diseases, research in coccidiosis spans a wide range of topics, from (comparative) parasite biology (with NGS technologies complementing existing methods) to control measures including chemotherapeutics, vaccines and complementary control. Irrespective of the perspective – host-centered or parasite-centered – the intimate relationship between parasites and their host is one of the most fascinating facets of parasitology. However, when it comes to research on coccidia, it can hamper the use of standard research models, e.g. mice, or of standard monolayer cell cultures which would be suitable for large scale screening. In almost all instances, such models do not support the complicated life cycle of the coccidia in full. The use of the genuine host animal is standard for many applications, e.g. drug screening, or studies on interactions of coccidia with the host microbiome. However, this is only feasible for species that are readily available The use of animals for research in general must also be re-considered under ethical aspects, and replacement research is needed. Lately, advanced animal models (genetically modified rodents) or organ models (including organoids or "organ on a chip" technologies) have been used to advance coccidiosis research, however, these methods are often cost-prohibitive, especially when it comes to veterinary parasitology. As a result of "serendipity", a simple cell-culture-free model for the propagation of *Cystoisospora* suis throughout its life cycle has been developed and applied to study sexual stages of this parasite as a veterinary member of the Sarcocystidae. While other species of coccidia do not seem to surrender to complete *in vitro* cultivation so easily, a combination of *in vitro, in vitro* and *in silico*-derived data and comparative research across different taxa can provide conclusive results in basic research and for applied pre-clinical trials. Further development of well-defined research models with different levels of complexity will support the availability of platforms for the many different research questions in the area of coccidiosis.

Exploring *Eimeria* genomes to understand *Eimeria* populations

Damer P. Blake

Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, AL9 7TA, UK

Eimeria can cause the disease coccidiosis, most notably in chickens where the global cost has been estimated to exceed €12 billion every year. Anticoccidial chemoprophylaxis has become an essential component of modern poultry production, although resistance is widespread. Live parasite vaccines are available, but uptake varies between poultry sectors. In response, interest in the development of cost-effective recombinant vaccines has been rekindled. The successful translation of such vaccines to the field will depend in part on parasite population structure and the extent of pre-existing antigenic diversity, influencing opportunities for recombinant vaccine breakthrough. For *Eimeria* these variables remain almost completely unknown. Seven *Eimeria* species have long been recognised to infect chickens, all with a global enzootic distribution. However, the description of three new species that are not controlled by current vaccines has disrupted long established dogma.

Until very recently genome resources for all *Eimeria* were fragmented and incomplete, precluding analyses beyond 0.01% of each species genome. Now, access to cost-effective long read genome sequencing using technologies such as Oxford Nanopore, requiring relatively modest quantities of temple DNA, has revolutionised *Eimeria* genomics. Improved genome assemblies are represented by 10-100× fewer contigs, or whole chromosomes for some species. Genome-wide single nucleotide polymorphism (SNP)-based genotyping has been used to sample population structures for *Eimeria acervulina* and *E. tenella,* revealing an intriguing dichotomy between species and/or regions. Returning to established loci such as the 18S ribosomal DNA, Illumina deep amplicon sequencing can now be used to track the occurrence and relative abundance of *Eimeria* species and strains (amplified sequence variants; ASVs), characterising the impact of chemoprophylaxis, vaccination, or other interventions on *Eimeria* populations. As sequencing technologies become cheaper and more accessible, metagenomic analyses of *Eimeria* field populations are starting to become feasible, offering opportunities for genomic surveillance using targeted or microbiome datasets.

"From samples to letters": methods, conflicts, biases, and pitfalls in Coccidian research

Jana Kvicerova

University of South Bohemia in České Budějovice, Czechia

Over the years, research on Coccidia has undergone a dramatic shift from descriptive parasitology through Sanger sequencing to NGS and eDNA techniques, colloquially speaking "from faeces to letters". Traditional taxonomy has relied on the morphology of the most accessible life-cycle stage, the sporulated oocyst. However, despite the expertise and experience required for such identification, this approach is limited by polymorphism, overlapping traits, scarcity of diagnostic characters, and observer bias. Therefore, it has become evident that morphology alone is insufficient for reliable species identification and must be interpreted with caution. The introduction of PCR-based tools and Sanger sequencing has improved species discrimination and revealed unexpected phylogenetic relationships, including cryptic species and distinct genetic lineages sharing identical morphology, and led to changes in generic classification. While early studies relied on conserved markers such as 18S rRNA, faster-evolving mitochondrial and apicoplast genes now allow resolution of closely related taxa and intraspecific variation. More recently, modern approaches such as NGS and eDNA have further expanded research possibilities, enabling the detection of coccidia in diverse environmental samples, or assessing their role in the gut microbiota.

Flotation followed by microscopy, the most widely used diagnostic method, has relatively low sensitivity and may underestimate prevalence, whereas PCR can yield false negatives due to incomplete oocyst disruption, PCR inhibition, or primer specificity. Co-infections further complicate detection, as PCR may preferentially amplify certain coccidian species.

These methodological limitations directly affect epidemiological inference. Prevalence, a key epidemiological parameter, reflects the proportion of infected hosts within a population, however, it is affected by sample size, sampling design, host distribution, and whether it targets genus- or species-level. Reported values may therefore differ substantially from real infection levels. Co-infections with multiple coccidian species within a single host further bias prevalence estimates. Such infections are common, for example in rodents, and can often be distinguished microscopically based on morphological differences. However, their detection by PCR remains challenging, as amplification efficiency may vary among species or isolates. This can lead to preferential detection of certain taxa, and potential misidentification or underrepresentation of co-infecting species.

The emergence and dynamics of infection are shaped by interactions among the parasite, the host, and the environment with its characteristics, which together form the so-called "epidemiological triad". Knowledge of the epidemiological background, such as spatial focality or seasonality, also plays an important role. Although sex-related differences in prevalence are generally minimal, ecological drivers such as host density play a key role, with transmission increasing in large, dense populations, which is in line with epidemiological theory. I will attempt to summarize the most common biases and pitfalls of methods used in coccidian research and to highlight the potential consequences they can lead to.

From First Contact to Protection: The Immunological Journey Against *Eimeria*

Marc Pagès Bosch
Senior Manager R&D New Biologicals Poultry, Hipra Scentific SLU

Avian coccidiosis remains one of the most economically significant parasitic diseases in poultry production, driven by infections with *Eimeria* spp. A comprehensive understanding of host–parasite interactions and immune mechanisms is essential for the rational design of effective vaccination strategies. This presentation focuses on the central role of cell-mediated immunity in protection against coccidiosis.

Following primary infection, the host immune response develops in a temporally coordinated manner within the intestinal mucosa. Early innate responses involve heterophils, eosinophils, mast cells, and natural killer (NK) cells, followed by antigen presentation by macrophages and dendritic cells in gut-associated lymphoid tissue. This leads to the activation and expansion of T cell populations, particularly CD8+ cytotoxic T lymphocytes, which play a pivotal role in controlling intracellular stages of *Eimeria* through direct cytotoxicity.

Protective immunity requires repeated exposure, typically achieved after successive infection cycles, resulting in a faster and more efficient secondary response characterized by enhanced cytotoxic activity and improved coordination between immune compartments. While humoral responses may contribute to protection, their role is secondary compared to cellular mechanisms, particularly in the context of live vaccines.

A key determinant of protective efficacy is the polarization and balance of T helper responses. Th1-driven immunity, characterized by increased production of cytokines such as interferon-gamma (IFN-γ) and interleukin-2 (IL-2), is essential for promoting cytotoxic responses and intracellular parasite control. At the same time, a coordinated Th1/Th2 balance ensures effective immune regulation and tissue homeostasis.

Modern vaccination strategies aim to enhance these cellular mechanisms. In particular, the incorporation of immunomodulatory adjuvants into live attenuated vaccines has emerged as a promising approach. These adjuvants stimulate antigen-presenting cells and promote a Th1-biased response, leading to increased IFN-γ and IL-2 production, enhanced formation of organized lymphoid aggregates in the intestinal mucosa, and potentiation of CD8+ and NK cell-mediated cytotoxicity.

In conclusion, advancing vaccine efficacy against avian coccidiosis requires a strong focus on cellular immunity, especially the induction of robust Th1 responses and cytotoxic effector mechanisms. A deeper understanding of these immunological principles provides a solid framework for the development of next-generation vaccines capable of delivering consistent and high levels of protection under field conditions.

Navigating the complex intervention strategies of coccidiosis control

Rami A. Dalloul
Avian Immunobiology Laboratory, Department of Poultry Science, University of Georgia, Athens, GA 30602, USA

Modern control of poultry coccidiosis requires integrated, multi-pronged strategies becauce of continually evolving parasite populations, changing host responses and genetics, and increasing regulatory pressures. Recent advances include new anticoccidial compounds, microbiome-modulation approaches, next-generation vaccines, and biotechnological development of non-drug alternatives. Innovative vaccine technologies and delivery systems encompass live-attenuated and recombinant platforms as well as experimental mRNA-based designs adminstered through spray cabinets, gel appliactions, or in ovo delivery. Although few novel anticoccidial drugs have been introduced in recent years, drug rotation and shuttle programs remain the predominant control strategies in commercial poultry production. Recent efforts have increasingly focused on non-drug strategies, including the use of probiotics, prebiotics, synbiotics, and phytogenic compounds administered through feed or water. In addition, tailored combinations of feed additives – often referred to as precision feeding – can improve early immune development and intestinal health, resulting in measurable economic benefits. Other immunomodulatory approaches, such as cytokine-based products, may further enhance host defenses, although their large-scale production remains costly. Emerging technologies under investigation include CRISPR-mediated gene editing of the parasite, RNA interference, and nanoparticle-based delivery systems, several of which have shown promise for improving the stability and efficacy of drugs and vaccines. Each strategy differs in its mechanism of action, method of application, level of protection, and associated challenges, particularly with respect to production costs and the impact of concurrent infections. Comparative evaluations of these interventions are highly context-dependent and typically consider efficacy, cost, scalability, regulatory approval status, and commercial readiness. Consequently, recommendations are often tailored to specific production systems and may include short-term priorities, such as optimizing vaccine delivery; mid-term goals, including development of next-generation anticoccidials and non-drug alternatives; and long-term strategies that can be focused on host genetics and parasite genomics.

Investigating the 3D ultrastructure of *Eimeria tenella* asexual and sexual stages.

A Karoglu[2]; Alana Burrell[2] V Marugan-Hernandez[1]; DJ Ferguson[2]; F Tomley[1]; S Vaughan[2]

[1] Royal Veterinary College, UK; [2] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK

The Problem

Eimeria species are apicomplexan parasites of major veterinary importance, with complex life cycles involving asexual and sexual stages marked by distinct molecular and morphological changes. Limited genetic tools and in vitro systems restrict understanding of their in vivo development during asexual and sexual development. Using 3D volume electron microscopy methods we have examined organelle organisation and timing of organelle biogenesis in *Eimeria tenella* within infected chicken caeca and explored the organisaton of the apical complex by high resolution cellular electron tomography.

How we investigated or researched the problem

Serial block face scanning electron microscopy (SBF-SEM) was used to create 3D volumetric datasets containing whole individual merizoites and sexual stages. Following invasion of host cells, a single parasite develops into a large multinucleated schizont, which eventually gives rise to numerous motile and invasive merozoites. Qualitative and quantitative approaches were combined to explore the organisation and timing of organelle biogenesis during schizont development within infected chicken tissue following reconstruction of whole individual merozoites. Cellular electron tomography was also used to investigate the detailed ultrastructure of the apical complex.

Results

Our analysis identified four distinct developmental stages of schizonts during organelle development in vivo and provided detailed analysis of the timing of organelle biogenesis as individual merozoites developed. The detailed three-dimensional characterisation of these stages offer novel insights into how *E. tenella* efficiently generates large numbers of invasive merozoites. High resolution cellular electron tomography revealed the distinct organisation of the apical complex offering insights into how the secretory organelles rhoptries and micronemes are organised for secretion.

Implications/ Conclusions

These findings not only deepen our understanding of schizogony, sexual development and the apical complex in this parasite, but also highlight the unique advantages of 3D volume electron microscopy for studying parasite cell biology, revealing perspectives not attainable with conventional two-dimensional imaging techniques.

Eimeria challenge on gut micro-environment, microbiomes and opportunity for pathogens

Woo Kyun Kim

Department of Poultry Science, University of Georgia, U.S.A.

Coccidiosis is a common protozoal enteric disease induced by *Eimeria* spp., causing tremendous economic loss globally. In addition, coccidiosis can affect growth and overall health of the host animals and create gut micro-environment favorable for pathogen colonization and secondary infection. Thus, controlling *Eimeria* infection is not only minizing its direct impact but also reducing secondary infection by other pathogens.

We conducted a *Eimeria* challenge study to evaluate how graded *Eimeria* inoculation (mixed *Eimeria* spp.: *E. acervulina, E. maxima, and E. tenella,*) affects growth performance, gut permeability, nutrient digestion and absorption, intestinal morphology, immunity, and gut microbiome in broilers. There were five treatments: the control group without *Eimeria* inoculation; the Low challenge (31,250 *E. acervulina* oocysts; 6,250 *E. maxima* oocystes; 6,250 *E. tenella* oocysts); the Med-low challenge (62,500 *E. acervulina* oocysts; 12,500 *E. maxima* oocystes; 12,500 *E. tenella* oocysts); the Med-high challenge (125,000 *E. acervulina* oocysts; 25,000 *E. maxima* oocystes; 25,000 *E. tenella* oocysts); and the High challenge (250,000 *E. acervulina* oocysts; 50,000 *E. maxima* oocystes; 50,000 *E. tenella* oocysts). As mixed *Eimeria* challenge dosage increased, body weight gain and feed intake were significantly reduced, and feed conversion ratio was poorer. Further, nutrient digestibility (amino acids, energy, and minerals) were significantly reduced by increasing dosage of *Eimeria* challenge. In addition, gut permeability, intestinal damage, and inflammation and oxidative stress markers were increased as Eimeria challenge dosage increased. Moreover, graded *Eimeria* challenge modulated gut microbiome; the dominat phyrum, Firmicutes, was reduced, whereas Proteobacteria was increased. The family, Enterobacteriacease and Bacillacease, were increased, while Ruminococcaeceae, Christensenellaceae, and Poptostreptococcaecae were linearly decreased. The results suggest that gut damage by *Eimeria* infection reduces available nutrient for host growth and health, while it increases more nutrients and creates favorable gut micro-environment for *Eimeria* and other pathogens.

Mixed *Eimeria spp.* challenge creates more comprehensive influences for *Eimeria* and pathogen activities in the gut because *E. acervuline* and *E. maxima* can affect duodenum and jejunum which are important sites for nutrient digestion and absorption. In order to eliminate undigested feed and nutrients' influence, we conducted another study with single *Eimeria* challenge model (*E. tenella*). Because *E. tenella* damages ceca and thrives their life cycle with a minimum influence by undigested feed and nutrients, we can evaluate more distinct effects of *Eimeria* challenge and gut micro-environment. Graded level of *E. tenella* inoculation linearly increased cecal lesion score, cell debris, and inflammatory cytokines, while it reduced volatile fatty acid production in the ceca, goblet cell count, and antimicrobial peptides. As *E. tenella* challenge dosage increased, the phylum, Firmicutes, decreased, while proteobacteria was increased. Such gut micro-environment changes by *Eimeria* infection may create a favorable environment for pathogens to colonize in the intestine and the internal organs including muscle, liver, lung, kidney, and bones for secondary infection and diseases. Thus, minimizing detrimental effects of *Eimeria* infection, stimulating fast recovery from damage, and restoring gut micro-environment including microbiome are essential for efficient production and animal well-being.

EPIDEMIOLOGY AND DIAGNOSTIC TOOLS

Oral presentations

Sound and image analysis to detect coccidiosis-related changes in broiler chickens

Patricia Soster de Carvalho[1,2], Thorsten Cardoen[3], Tomasz Grzywalski[4], Camila Lopes Carvalho[1], Imad Khan[1], Frank Tuyttens[5,6], Maarten De Gussem[7], and Gunther Antonissen[1]

[1] Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, 9090 Merelbeke-Melle, Belgium.
[2] Poulpharm, Izegem, Belgium.
[3] IDLab, Department of Information and Technology, Ghent University – imec, 9052, Technologiepark-Zwijnaarde 126, Belgium.
[4] Acoustics, Department of Information Technology, Ghent University, Belgium
[5] Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, 9090 Merelbeke-Melle, Belgium.
[6] Flanders Research Institute for Agriculture, Fisheries, and Food (ILVO), 9090 Merelbeke-Melle, Belgium.
[7] Vetworks, Aalter, Belgium

Livestock production is becoming increasingly intensive, driven both by rising global demand for animal protein and by efforts to maintain the affordability and availability of meat. While this improves efficiency, it also challenges health and welfare monitoring in large flocks with limited human surveillance, making disease outbreaks particularly impactful as antibiotic use is reduced or banned. This study aimed to assess how coccidiosis infection affects vocalization patterns and behavioral activity in broiler chickens from 14 to 41 days of age. The experiment included 88 Ross 308 broiler chickens allocated to two treatments (control and coccidiosis), with four replicates of 11 birds per treatment. Birds were housed in 2 m^2 pens with *ad libitum* access to feed and water. Random pen allocation and room rotation were used to minimize environmental bias. At 14 days of age, birds were weight-balanced across treatments and housed in separate compartments to prevent cross-contamination. At 14 days of age, one group was orally challenged with the three major *Eimeria* species in chickens (*E. acervulina, E. maxima, and E. tenella*), while the other served as the control. Continuous audio and video recordings were collected from day 14 to 41 using a centrally mounted microphone and a corner-mounted camera. Vocalizations were analyzed using a custom deep-learning recognizer developed in our group, focusing on four call types: distress calls (stress-related), short peeps (activity-associated), pleasure notes (positive-welfare–associated), and warbles (somnolence-associated). Video data were analyzed using a tracking system combined with an action-recognition model both developed by our research group. Infected birds showed behavioral changes. Drinking, eating, and exploring/foraging were suppressed shortly after infection, while inactivity remained consistently lower in infected birds. Scratching increased after infection and stayed consistently higher than in controls throughout the observation period. Comfort and maintenance behaviors showed mixed responses. After infection, preening increased similarly in both groups and then declined with age, with no clear differences between groups. Dust bathing showed brief, irregular peaks shortly after infection in both groups but was rare thereafter. Gentle feather pecking followed a similar age-related trajectory in both groups, with only minor transient reductions in infected birds. Sleeping and stretching were generally lower in infected birds for much of the period. Panting was absent until 23 days of age but increased more rapidly and to a greater extent in infected birds thereafter. The sound analysis is currently in progress. These findings suggest that behavioral monitoring may serve as an indicator of coccidiosis-related changes and support precision-livestock approaches for early, automated detection of gut-health challenges in broilers.

Evaluating ovotransferrin and calprotectin as biomarkers for Eimeria infections in broilers under field conditions

I. Gladan[1], J.G. Kers[1], F.C.Velkers[1], J.C.M. Vernooij[1], J.M.J.Rebel[2] and J.A. Stegeman[1]

[1] Department Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University;
[2] Wageningen Adaptation Physiology Group, Wageningen University and Research, Wageningen, the Netherlands

The Problem

Eimeria spp. infections in broilers cause intestinal damage and inflammation. Current coccidiosis diagnosis often relies on post-mortem lesion scoring combined with flock performance, clinical signs, and laboratory testing, including fecal oocyst counts. Serum and fecal biomarkers, including ovotransferrin (OVT) and calprotectin (CALP), may offer minimally invasive indicators of intestinal disease compared with post-mortem diagnosis. This study evaluated associations between *Eimeria* lesion scores and serum OVT and CALP levels. We compared individual serum and flock-level fecal biomarker levels to evaluate their suitability as indicators for *Eimeria spp.* infections under commercial conditions.

How we investigated or researched the problem

In two houses (HA and HB) on two Dutch broiler farms, post-mortem was performed and serum samples (n=74) and pooled house-level fecal samples (n=48) were collected at day 13 of age (D13), D21, D28, and D36 in Ross 308 broilers. Macroscopic lesion scores (0-4) were assessed in the duodenum/upper intestine (*E. acervulina,* LS-EA), mid-intestine (*E. maxima*, LS-EM), and ceca (*E. tenella*; LS-ET). Serum and fecal OVT and CALP levels were quantified using commercial ELISA kits. Associations between the currently analyzed biomarker levels and lesion scores were analyzed at bird level, and serum–fecal relationships at house-level, using linear models including lesion scores, day, house and interaction terms.

Results

LS-EA ranged from 0–3; LS-EM from 0–2, and LS-ET from 0–3 for ET. LS-EA and LS-EM were highest at D28–36 in HA and at D21 in HB, while LS-ET was highest at D13 in HA and at D21–28 in HB. Serum OVT was positively associated with increasing LS-ET, with no effect of day and no associations with LS-EA or LS-EM. The serum CALP model (including day, day–house interaction, LS-EM, LS-EA, and day–LS-ET interaction) showed a positive association between mean CALP and LS-ET at D13 only, but no association at later sampling days, or with LS-EA and LS-EM. The serum-fecal analysis showed that mean OVT serum levels were 71% higher than fecal levels, were highest at D28 (60% higher than D13) and were lower in HB (22%) than HA, with similar temporal and house patterns across matrices. In contrast, CALP showed high variability without consistent day or house effects. Mean serum CALP levels were 56% lower than fecal CALP levels.

Implications/Conclusions

Current findings indicate that most associations were found with ET lesion severity, with the most consistent positive association observed for serum and fecal OVT. Controlled infection trials with *Eimeria* are needed to fully evaluate the potential diagnostic value of these biomarkers for *Eimeria* infections, minimizing confounding effects of co-infections and other field-related factors.

Effect of red-colored feed on Eimeria infection dynamics in Ross 308 broilers

F.C.Velkers[1], M.L. Hana[1] and I. C. de Jong[2]

[1] Department Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University;
[2] Wageningen Livestock Research, Wageningen, the Netherlands

The Problem

Coccidiosis in broilers causes considerable economic losses and impairs broiler health and welfare. Although anticoccidial vaccination supports early development of protective immunity, its lower cost-effectiveness compared to antimicrobials has limited its uptake. Previous pilot studies have shown that management factors that stimulate litter pecking behavior, such as using red-colored start feed, may enhance early uptake of *Eimeria* oocysts and promote synchronized infection and earlier development of protective immunity. This study evaluated whether providing red-colored feed during early life influences infection dynamics and immunity against subsequent *Eimeria* spp. challenge in broilers.

How we investigated or researched the problem

A total of 744 Ross 308 male and female broilers, obtained at day of hatch (D0), were housed in 12 pens (3.5 m^2; 6 pens/treatment). Birds received either red-colored feed from day 0–14 (TRT-R) or normal feed (TRT-N). The study included a dynamics phase (D3–D22) and a challenge phase (D22–D38). At the start of the dynamics phase (D3), a group of 70 separately housed broilers (I-birds) were orally inoculated with 50 *E. acervulina,* 50 *E. maxima,* and 200 *E. tenella* sporulated oocysts. On D8, three I-birds were introduced into each pen containing 57 uninfected contact birds (C-birds), to initiate transmission at the start of the challenge phase. On D22, all birds were challenged with 1000 *E. acervulina,* 150 *E. maxima,* and 500 *E. tenella* sporulated oocysts. Oocysts per gram of feces (OPG) were quantified using the McMaster method and body weight and feed intake were recorded for each feed phase (D0, D14, D28) and at the end of the experiment (D38). Post-mortem lesion scoring was performed on D16, D28, and D38. Data were analyzed using linear mixed models including treatment, day, and interaction terms.

Results

During the dynamics phase, no significant differences in OPG or body weight were observed between TRT-R and TRT-N. During the challenge phase, OPG of E. acervulina and E. tenella were lower in TRT-R on D29, D32, and D37, whereas E. maxima showed a different excretion pattern without treatment effects. Absolute OPG levels were low and differences were not clinically relevant. Lesion scores were low throughout the study with on D16, slightly lower lesion scores in TRT-R, particularly for E. maxima. After challenge (D28), lesion scores indicated some development of immunity following initial exposure, but without differences between treatments. Body weight did not differ at any timepoint.

Implications/ Conclusions

Red-colored feed during early life did not significantly affect infection dynamics, lesion, body weight, or protective immunity under the conditions of this study. Exposure to relatively high oocyst numbers at pen level may have limited the ability to detect treatment effects. Under field conditions with lower initial infection pressure, red feed could potentially enhance early oocyst uptake; however, practical benefits are likely limited and may not outweigh additional costs or implementation challenges.

EPIDEMIOLOGY AND DIAGNOSTIC TOOLS

Pitch presentation

Deep Learning-Based Full-Chamber McMaster Analysis for Automated Enumeration and Multi-Species Differentiation of *Eimeria* spp.

D. Mezghiche[1,2,3], M. Verstraete[1], C. Vidal Moreno De Vega[1], G. Antonissen[4], P. Claes[2,3,5]

[1] *Poulpharm bvba, Izegem, Belgium*
[2] *KU Leuven, ESAT/PSI, Leuven, Belgium*
[3] *Medical Imaging Research Center, University Hospital Leuven, Belgium*
[4] *Ghent University, Department of Pathobiology, Pharmacology and Zoological Medicine, Belgium*
[5] *KU Leuven, Department of Human Genetics, Leuven, Belgium*

The Problem

Reliable Eimeria oocyst per gram (OPG) enumeration is essential for coccidiosis monitoring and vaccine trials in poultry production. The McMaster method relies on manual counting of the chamber surface, introducing sampling bias and operator variability. Species differentiation and sporulation assessment are further complicated by overlapping morphology and subjective interpretation. An objective full-chamber approach is required.

How we investigated or researched the problem

We developed a deep learning–based pipeline for automated oocyst detection and enumeration, with exploratory species differentiation.
High-resolution McMaster images were used to train object detection models (YOLOv8n/s, RetinaNet, Faster R-CNN). The entire chamber was digitized to enable full-field analysis, and a patch-based strategy preserved resolution during whole-chamber processing.
The dataset included pure inocula of seven major *Eimeria* species, mixed field samples, and sporulated/non-sporulated oocysts annotated by experts.

Results

Automated analysis was performed on the complete McMaster counting chamber, removing the need for partial sampling bias. High concordance with expert manual counts was achieved, with an overall error rate of 1.10%, and improved reproducibility. YOLOv8n provided the best detection performance.
Preliminary species differentiation displayed promising results; further optimization is ongoing to improve robustness.

Implications/Conclusions

AI-based full-chamber McMaster analysis enhances objectivity and reproducibility of OPG enumeration and reduces operator dependence. This approach represents a significant step toward standardized, scalable coccidiosis diagnostics, with ongoing refinement for reliable species classification and sporulation assessment.

EPIDEMIOLOGY AND DIAGNOSTIC TOOLS

Poster presentation

Preliminary results on Eimeria prevalence in commercial broiler farms in southeastern part of Slovakia

Kožárová Ivona, Šimková Jana, Račka Karol
University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

The Problem

Broiler chickens are frequently affected by a parasitic disease called coccidiosis. Coccidiosis is caused by a single-celled protozoan organisms belonging to the genus *Eimeria*. This infectious disease causes serious economic consequences in the animal production without continuous preventive use of coccidiostats. Monitoring of *Eimeria* spp. infections in conventionally reared broiler chickens can be performed by determining the concentration of oocysts in faeces, expressed as oocysts per gram (OPG). Since OPG count is still the primary diagnostic tool for monitoring coccidiosis in poultry, the aim of our study was to determine OPG count in faecal samples obtained from broiler chickens raised under commercial conditions in the southeastern part of Slovakia.

How we investigated or researched the problem

Pooled faecal samples from two farms (farm 1; farm 2) were initially examined for the presence of *Eimeria* oocysts via qualitative conventional sugar flotation (Sheather's sugar solution, specific gravity ≥ 1.27). All positive samples were subsequently examined for the calculation of the OPG count by a standard McMaster technique using a saturated sucrose solution (Sheather's sugar solution, specific gravity ≥ 1.27). Count of *Eimeria* oocysts in the positive samples was performed using microscopic examination.

Results

All samples were positive to *Eimeria* oocysts in farm 1 and two samples were positive from farm 2. OPG values ranged between 1650 – 42 900 in farm 1 and between 3000 – 4200 in farm 2. According to the microscopy, there was a mixed infection of several *Eimeria* species, but exact determination will need further microscopic micrometry and molecular analysis.

Implications/Conclusions

Coccidiosis is a constant problem in intensive poultry farming conditions, and that is why prevention of the disease using in-feed coccidiostats or live vaccination is very important. Except that, long term monitoring of *Eimeria* in commercial poultry farms, species determination and intensity of infection is also required to set relevant conditions for the protection of animal health, including the consumer.

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GENOMICS, GENETICS, AND EVOLUTIONARY DIVERSITY

Oral presentations

Comparative Genomic Analysis of Poultry-Infecting *Eimeria* Genomes

Conor Noonan[1], Melanie Hay[1], Sarah Hill[1], Damer P. Blake[1], Dong Xia[1]

[1] Department of Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms AL9 7TA, UK

The Problem

Eimeria are a genus of apicomplexan protists that can infect all livestock, the ingestion of which can lead to coccidiosis. Species which infect chickens are of particular economic importance, incurring costs upwards of £10.4 billion annually to global production. Despite their impact, little is known about the genetic diversity of these parasites and how this variation contributes to the rising level of resistance to current control strategies. These knowledge gaps exist primarily due to the limited number of loci used in population studies, as well as the fragmented and incomplete reference genomes available due to the technical limitations of short read sequencing and the inherent repeat-rich content of *Eimeria* genomes.

How we investigated or researched the problem

We developed an experimental workflow and a hybrid Illumina and Nanopore sequencing pipeline to improve upon the quality of *Eimeria* reference genomes by generating long reads capable of spanning repeat-rich genomic regions. Acting as scaffolds, these large sequences improve the *de novo* assembly of *Eimeria* genomes by properly orienting shorter sequences and bridging gaps between them.

Results

We improved the contiguity and compositional quality of six existing reference genomes and generated novel high-quality reference sequences for the newly characterised species *E. zaria* and *E. lata.* Comparative genomic analysis revealed evidence of extensive genomic rearrangements within the genus, including chromosomal fission and fusion events. Assessment of orthogroups across the genus highlighted the lineage-specific expansion of gene families involved in the invasion process, as well as novel families of uncharacterised function. Furthermore, lineage-specific expansions of transposon families were identified, reflecting patterns of genome expansion in *E. mitis*, the largest known *Eimeria* genome to date.

Implications/Conclusions

By analysing whole genome sequencing data against the improved reference sequences, we can gain a more complete understanding of the genetic diversity of these organisms, their local and regional population structures, as well as the genetic determinants of clinically relevant traits such as drug resistance and precocious development.

Prevalence of the three cryptic Eimeria species in Europe

Brecht Maertens (B.M.)[1], Sander Verbeke (S.V.)[1], Monita Vereecken (M.V.)[2], Ben Dehaeck (B.D.H.)[2]

[1] Poulpharm BV, Prins Albertlaan 112, 8870 Izegem, Belgium
[2] Huvepharma NV, Uitbreidingstraat 80, 2600 Antwerp, Belgium

The Problem

Coccidiosis is one of the most economically devastating diseases of poultry industry. The infestation can be caused by several Eimeria species, each with its own characteristics. For chickens, seven species: *E. acervulina, E. maxima, E. tenella, E. brunetti, E. necatrix, E. praecox* and *E. mitis,* are acknowledged and have been extensively described in the past. In 2021 however, three new cryptic operational taxonomic units (OTU's) were described based on genetical and biological characterization: *E. lata* (OTUx), *E. nagambie* (OTUy) and *E. zaria* (OTUz). To this day, standard coccidiosis field diagnostics is done by lesion scoring and determination of morphology by microscopy or (q)PCR, neglecting these OTU's. Subsequently, little is currently known regarding the prevalence of each of these cryptic OTU's in Europe.

How we investigated or researched the problem

Faecal samples from poultry farms in geographically different areas within the European continent were collected over a period of six months. In addition, differences in prevalence across the various production types (broilers, layers, layer breeders and broiler breeders) were included. Samples were analyzed by both microscopy and specific polymerase chain reaction (PCR) for the seven recognized *Eimeria* species and three cryptic OTU species.

Results

One hundred forty samples originating from 14 European countries were analyzed. As expected, the most prevalent species was *E. acervulina* (93%), followed by *E. tenella, E. maxima, E. mitis, E. praecox, E. necatrix* and *E. brunetti* with respectively 68%, 67%, 65%, 56%, 22% and 18%. For the cryptic OTU's, 11 samples (8%) tested positive for *E. zaria* (OTUz), 7 samples (5%) for *E. nagambie* (OTUy) and 1 sample (1%) for *E. lata* (OTUx). Three samples were positive for more than one of the three cryptic species. The species were detected across the different chicken production types.

Implications/Conclusions

This prevalence study shows that all three cryptic species: *E. lata, E. nagambie* and *E. zaria* occur in European poultry farms, yet their presence is low compared with the seven original *Eimeria* species described in chickens.

GENOMICS, GENETICS, AND EVOLUTIONARY DIVERSITY

Pitch presentation

Identification of cryptic *Eimeria* and subsequent evaluation of the situation on Czech production farms

Daniel Divín[1, 2], Lucie Minaříková[1], Jiří Kalina[1], Damer Blake[3] and Jitka Mucksová[1]

[1] *BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Czech Republic*
[2] *Charles University, Faculty of Science, Department of Zoology, Czech Republic*
[3] *Royal Veterinary College, United Kingdom*

The Problem

Seven *Eimeria* species have long been recognized to infect chickens. Recent studies focused on genome sequencing identified three new cryptic *Eimeria* genotypes in particular chicken populations. They are considered as novel operational taxonomic units (OTUs) divided into three clusters, OTUx, y, and z. According to Blake et al. 2021 (doi: 10.1016/j.ijpara.2020.12.004) these new cryptic *Eimeria* genotypes are likely to be distinct species, named *Eimeria lata* n. sp. (OTUx), *Eimeria nagambie* n. sp. (OTUy) and *Eimeria zaria* n. sp. (OTUz), all of which are capable of escape from immunity induced by current commercially available anticoccidial vaccines, suggesting that they may pose a notable threat to chicken health. Therefore, it is useful to have an overview of the possible presence of these species in chicken populations.

How we investigated or researched the problem

DNA isolated from fecal field samples from Czech and Slovak production farms were tested for the presence of OTUx, OTUy, and OTUz by PCR amplification with published genotype-specific primers (Blake et al., 2021). PCR products were sequenced and compared to available sequences of cryptic *Eimeria* in online databases.

Results

Sequences homologous to OTUy were recorded in two out of 33 farm samples. These sequences showed greatest similarity to sequences available online from *Eimeria nagambie* and OTUy genes.

Implications/Conclusions

We were able to detect signals of at least one of the new cryptic *Eimeria* species in samples from two Czech production farms, suggesting the presence of *Eimeria nagambie* circulating in Czech poultry.

IMMUNOLOGY AND VACCINES

Oral presentations

Establishment of a Gene Editing Platform in *Eimeria tenella* and Its Application in Vaccine Development

Dandan Hu[1], Peiyao Li[1], Rina Su[1], Ying Pang[1], Xinming Tang[2], Xianyong Liu[3], Xun Suo[3]

[1] College of Animal Science and Technology, Guangxi University, Nanning, 530004, China;
[2] Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China;
[3] College of Veterinary Medicine, China Agricultural University, Beijing, China

To enable precise genome editing in *Eimeria tenella,* we introduced the CRISPR/Cas9 system and successfully generated a transgenic parasite line stably expressing the Cas9 protein. This Cas9-expressing strain exhibited no significant differences in major biological characteristics compared with the wild-type parasite. Using this platform, we achieved targeted random mutagenesis and homologous recombination in *E. tenella,* with a homologous recombination efficiency of approximately 29%. Based on this genome-editing system, endogenous tagging of the EtGRA9 gene was performed, allowing dynamic visualization of its subcellular localization. The results demonstrated that EtGRA9 is a secreted protein that is released into the parasitophorous vacuole in a development-dependent manner. In addition, systematic disruption of the ApiAP2 transcription factor gene family using this approach revealed that 23 of the 33 ApiAP2 factors expressed by *E. tenella* are essential for parasite development and survival within the host.

Given the pronounced antigenic polymorphism among different *Eimeria* field strains, there is a clear need to develop customized gene-deleted attenuated vaccines targeting locally prevalent strains. Accordingly, we employed CRISPR/Cas9-mediated gene knockout to disrupt the sporozoite-specific ApiAP2 transcription factor EtAP2-S1 and the invasion-associated gene EtAMA3. Integrated RNA-seq and CUT&Tag analyses revealed that EtAP2-S1 directly binds to the promoter regions of multiple genes, including members of the SAG gene family, and that deletion of EtAP2-S1 resulted in significant upregulation of up to 59 SAG genes. Moreover, the EtAMA3-deficient strain exhibited downregulation of several moving-junction associated genes, accompanied by extensive remodeling of the expression profiles of multiple transmembrane proteins. Notably, both gene-deleted strains displayed significantly reduced invasion efficiency, oocyst output, and virulence, indicating marked attenuation. Despite this attenuation, the mutant parasites retained the ability to elicit robust protective immunity, highlighting their potential as candidate live attenuated vaccine strains. Collectively, this study establishes a robust CRISPR/Cas9-based genome editing platform for *E. tenella* and provides important theoretical and experimental support for the development of gene-deleted attenuated vaccines against coccidiosis.

EtAP2_SM Controls Sporogony in *Eimeria tenella* and Underpins Genetically Engineered Vaccine Design

Yaru Li, Runqi Luo, Lin Liang, Yanzhen Liao, Ruiying Liang, Dandan Hu, Jiabo Ding, Xinming Tang

Key Laboratory of Animal Biosafety Risk Prevention and Control (North) & Key Laboratory of Veterinary Biological Products and Chemical Drugs of MARA, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China

The Problem

Coccidiosis control would greatly benefit from blocking sporogony – the environmental maturation step that makes *Eimeria tenella* oocysts infective – yet the transcriptional regulators of this stage in *Eimeria* are largely uncharacterized. The study asks whether an ApiAP2 transcription factor, EtAP2_SM, controls sporogony and could thereby inform safer, genetically attenuated vaccine design.

How we investigated or researched the problem

(1) Candidate discovery & expression profiling: Cross-species transcriptomic screening identified EtAP2_SM (ETH2_0700600) as a sporogony-stage candidate; a dual-fluorescent reporter line tracked its expression during 0–48 h of sporulation.
(2) Gene perturbation: Constructed a CRISPR-Cas9 knockout (EtAP2_SM-KO) and a conditional mAID line to acutely degrade EtAP2_SM with auxin; validation used PCR, Western blot, fluorescence microscopy.
(3) Phenotyping in vivo: Infected chickens (dose-titration), quantified oocyst output, lesion scores, weight gain; measured sporulation rates; evaluated immunoprotection in a floor-rearing (field-like) mode.

Results

(1) Attenuated pathogenic readouts with KO: Relative weight gain increased in KO birds (≈ +22% at 2,000 oocysts; +11% at 20,000), whereas lesion scores were not significantly different; total oocyst output fell by ~25–28%.
(2) Conditional depletion works as designed: mAID tagging enabled auxin-dependent loss of EtAP2_SM signal and protein within 48 h.
(3) Reduced transmission potential in "field" model: Birds immunized with EtAP2_SM-deficient parasites showed markedly lower oocyst shedding upon reinfection, improving biosafety.

Implications/Conclusions

EtAP2_SM acts as a key transcriptional regulator orchestrating gene essential for *E. tenella* sporogony. Its deletion reduces oocyst shedding and transmission without abolishing viability, suggesting a promising strategy for developing safer, attenuated live vaccines against avian coccidiosis. Future structural and genomic mapping of EtAP2_SM targets could refine rational vaccine design.
This study was supported by National Natural Science Foundation of China (32273035) and the Youth Innovation Program of CAAS (Y2023QC10).

Impact of Coccidiosis Vaccination on Broiler Performance Under Heat Stress Conditions

Swati Karki[1] and M. De Gussem[1,2]

[1] *Vetworks BVBA, Knokstraat 36, 9880 Poeke, Belgium*
[2] *Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
Corresponding author: swati.karki@vetworks.eu

In broiler production systems exposed to heat stress and or high-altitude conditions, coccidiosis control strategies should be assessed not only for their effectiveness against Eimeria spp., but also for their potential impact on bird physiology and metabolic demand.

Materials and Methods

A commercial field study was conducted in broilers raised under conditions of heat stress and high altitude. Four key zootechnical indicators—mortality, live body weight, daily weight gain, and feed conversion ratio (FCR)—were used to compare performance across different coccidiosis control programs.

The evaluated treatments were:

T1 (control): Chemical A + Ionophore A | T2: Chemical B + Ionophore B | T3: Chemical B | T4: Ionophore B | T5: Commercial coccidiosis vaccine

Results

There were numerical differences and more consistent results in two of the performance parameters (mortality at 40 days of age and FCR corrected at 2.3 kg) with an advantage for the houses that were vaccinated (T5):

Mortality: T1(control)=9.0+/-1.6(17.5%), T2=9.8+/-2.4(24.4%), T3=6.2+/-4.3(70.0%), T4= 6.4+/-3.2(50.6%) and T5= 6.5+/0.8(11.6%)
FCR: T1(control)=1.41+/0.06(4.5), T2=1.47+/0.08(5.4), T3=1.44+/0.02(1.4%), T4=1.52+/0.07(4.8) and T5=1.43+/0.05(3.7)

Conclusions

Broilers exposed to heat stress tend to reduce feed intake as a mechanism to limit heat production, while birds raised at high altitude are challenged by lower oxygen availability and increased cardiopulmonary workload. Under such environmental stressors, maintaining a lower metabolic burden becomes essential for sustaining performance.
Certain anticoccidial programs, although effective against coccidiosis, may contribute to increased metabolic demand, potentially affecting bird performance under these conditions. In contrast, coccidiosis vaccination promotes the development of immunity without imposing additional metabolic stress, which may help support performance, as suggested by the results of this field study.
Further investigations are needed to confirm these observations and establish statistical significance.

IMMUNOLOGY AND VACCINES

Pitch presentation

Immunogenicity and protection against infectious bursal disease via a transgenic Eimeria acervulina expressing IBDV VP2-2C3d fusion protein

Yingying Sun , Xun Suo , Xianyong Liu

State Key Laboratory of Veterinary Public Health and Safety; Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China

The Problem

Infectious bursal disease (IBD), caused by the infectious bursal disease virus (IBDV), significantly threatens global poultry health by inducing immunosuppression and causing economic losses. To enhance vaccination efficacy, we engineered a transgenic strain of *Eimeria acervulina* (Ea-2C3d) expressing a fusion protein composed of IBDV VP2 and three tandem C3d segments (3C3d), utilizing C3d's adjuvant properties to boost immune responses.

How we investigated or researched the problem

The transgene was generated by integrating codon-optimized VP2 and 3C3d sequences into the *E. acervulina* genome using restriction enzyme-mediated transfection. PCR, protein, and genome sequencing confirmed the successful integration and expression of VP2 fusion C3d, but only two copies of C3d were successfully expressed, due to a partial deletion of one C3d copy during the transfection process.

Results

In vivo studies demonstrated that Ea-2C3d elicited significantly higher anti-VP2 antibody titers than the parental Ea-VP2 strain ($P < 0.05$), especially following second immunization. Upon challenge with virulent IBDV, chickens immunized with Ea-2C3d displayed reduced bursal lesions (histopathological score ≤ 1) and maintained bursal integrity (bursal index >0.7), comparable to those receiving a commercial subunit vaccine. Despite reduced reproductive capacity in the transgenic parasites, Ea-2C3d maintained its immunogenicity and safety.

Implications/Conclusions

These findings highlight that C3d adjuvant enhances VP2-mediated protection in a coccidial vector, presenting a novel dual-protection strategy against IBD and coccidiosis.

Acknowledgements

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"Sun, Y., Liu, Y., Wang, H., Guo, Q., Suo, J., Zhang, S., Tang, X., Yin, G., Suo, X., & Liu, X. (2025). Immunogenicity and protection against infectious bursal disease via a transgenic *Eimeria acervulina* expressing IBDV VP2-2C3d fusion protein.Vaccine, 64, 127715."

IMMUNOLOGY AND VACCINES

Poster presentation

Toward a Safer Live Vaccine: Targeting the Key Gene EtMob1 to Block Sporogony in *Eimeria tenella*

Yaru Li, Lin Liang, Ruiying Liang, Jiabo Ding, Xinming Tang

Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, China

The Problem

Avian coccidiosis is one of the most critical diseases jeopardizing the development of the poultry industry and impacting farming profitability. According to recent estimates, the global annual economic loss to the poultry industry attributable to coccidiosis exceeds $14.5 billion. The control of chicken coccidiosis primarily relies on two strategies: chemoprophylaxis and vaccination. The major limitations of chemoprophylaxis are drug resistance and residue concerns. Live vaccines can provide flocks with robust immune protection; however, vaccination often affects the production performance of young broilers intended for early market. Unsporulated oocysts are non-infective. Under suitable conditions, unsporulated oocysts undergo sporogony to form sporulated oocysts, which are infective. Following ingestion, sporulated oocysts release sporozoites under the physical and chemical actions of the chicken's digestive system. These sporozoites then invade intestinal epithelial cells to develop and multiply. Consequently, constructing parasite strains with developmental defects or reduced efficiency in sporogony represents a promising breakthrough for addressing the current application bottlenecks of existing vaccines.

How we investigated or researched the problem

We used EtMob1—a gene specifically highly expressed in unsporulated oocysts—as the starting point. Immunofluorescence assay (IFA) was employed to investigate the expression pattern of EtMob1 during the initiation of sporogony. A conditional knockout strain of EtMob1 was constructed to elucidate its biological function and mechanism in the DNA replication stage of sporogony. Finally, the safety profile of the EtMob1 conditional knockout strain was validated.

Results

A monoclonal antibody against EtMob1 was successfully generated. Using this antibody for IFA, we determined that EtMob1 expression peaked at the 8-hour time point during sporogony and ceased upon its completion. We successfully constructed a conditional knockout strain of EtMob1 and conducted a simulated field trial. Compared to the wild-type strain (ETH), the EtMob1-deficient strain caused significantly less damage to the chicken ceca. Furthermore, oocyst shedding was markedly reduced during both the primary and secondary peak shedding periods, leading to a significant decrease in total oocyst output.

Implications/Conclusions

EtMob1 is specifically expressed during the initiation of sporogony. Its deficiency inhibits the sporogonic process, thereby significantly reducing the biosafety risks associated with post-vaccination infection in both non-immunized and young chickens, ultimately enhancing vaccine safety. This study offers novel insights into the regulation of Eimeria sporogony and paves the way for safer genetically engineered vaccines. *This study was supported by National Natural Science Foundation of China (32273035) and the Youth Innovation Program of CAAS (Y2023QC10).*

CELL BIOLOGY AND HOST-PATHOGEN INTERACTION

Oral presentations

Impact of Eimeria tenella rhoptry kinase on host cell signaling: transcriptomic and phospho-proteomic analyses

Sharvani S. Shintre[1,2], Laura Sedano[1], Sonia Lacroix-Lamande[1], Najma Rachidi[2], Anne Silvestre[1]

[1] ISP, INRAE, Université de Tours, 37380 Nouzilly, France
[2] Institut Pasteur, Université de Paris and INSERM U1201, Unité de Parasitologie Moléculaire et Signalisation, Paris, France

The Problem

In the face of the concerning rise in resistance to anticoccidials, the identification of new therapeutic targets has become a strategic priority in veterinary parasitology. Upon infection, Apicomplexan parasites secrete effector molecules, among which rhoptry kinase proteins (ROPK) that modify their host cells to create a permissive environment. Conversely to *Toxoplasma gondii*, their study in *Eimeria* is made difficult by the lack of efficient tools to invalidate parasite genes.

How we investigated or researched the problem

To decipher ROPK functions in avian coccidiosis and possibly develop new treatments, we produced a knock-in strain that overexpresses EtROP1 (ETH2_0808400) fused to YFP, under the control of the actin promoter. In parallel, we produced a wild-type strain (Et-INRAE) that constitutively expresses m-Cherry fluorescent marker. We performed RNAseq analyses of infected and uninfected chicken epithelial cells, to establish the host cell signature for *E. tenella* infection and to determine the contribution of EtROP1 in host cell-signature. The phospho-proteomic analysis was also conducted in order to identify the underlying molecular mechanisms.

Results

The wild-type strain infection modified numerous KEGG pathways, such as metabolic processes, apoptosis, lysosomal function, focal adhesion and oxidative phosphorylation. Infection by the strain that overexpresses EtROP1 resulted in the modulation of the ribosome pathway, the polycomb repressive complex, DNA replication, apoptosis, and proteolysis via ubiquitination, for the main modified KEGG pathways. The phospho-proteomic analysis confirmed the EtROP1-overexpressing parasites led to a substantially higher number of host modulated phosphosites, and also an increase of phosphorylation level of proteins. The KEGG pathways were related to RNA metabolism and RNA splicing. These results suggest that EtROP1 modulates RNA splicing through targeted phosphorylation of host substrates.

Implications/Conclusions

As parasite ROPKs are highly divergent from eukaryotic protein kinases from hosts, ROPK may be relevant drug target candidates to control parasite infections. Our preliminary data support the essential role of EtROP1 N-terminal domain in substrate binding and catalytic activity. Although, it is unlikely that existing kinase inhibitors maybe be repurposed easily, crystallography, high-throughput inhibitor screening and *in vitro* phenotypic screening should facilitate the research for new anticoccidial molecules.

Evaluation of *Eimeria* mitis pathogenicity

Brecht Maertens (B.M.)[1], Janne Snoeck (S.N)[1], Maarten De Gussem (M.D.G)[1]

[1] *Poulpharm BV, Prins Albertlaan 112, 8870 Izegem, Belgium*

The Problem

Coccidiosis is one of the most economically devastating diseases of poultry industry. The infestation can be caused by several Eimeria species, each with its own characteristics. Johnson and Reid have published in 1970 a scoring system for *Eimeria* species specific intestinal lesions induced by *E. acervulina, E. maxima, E. tenella, E. necatrix* and *E. brunetti.* Yet, for *E. mitis* and *E. praecox,* no pure clones were available at that time. These two species are commonly considered as less pathogenic compared to the other species but the actual pathogenicity and the potential lesions they induce have not been described so far.

How we investigated or researched the problem

A dose response study was performed with pure *E. mitis* clones. Per infection dose (500, 5,000, 25,000, 50,000, 100,000 and 500,000 sporulated oocysts) twenty five birds were infected by oral gavage. Body weight and intestinal lesions were monitored on day 4, 5, 6 and 7 post challenge. In order to determine the pathogenicity of *E. mitis* in relation to the other species, birds infected with *E. acervulina* and *E. maxima* at similar doses were included in the study. Furthermore, also daily feed intake, feed conversion ratio and daily oocyst excretion were monitored.

Results

For *E. acervulina* a clear dose response was observed on daily weight gain reduction in relation to the infection dose, while both *E. maxima* and *E. mitis* displayed a saturation level from 25,000 sporulated oocysts onwards. At this specific infection dose it was observed that *E. maxima* had the highest impact of the three *Eimeria* species on daily weight gain, followed by *E. mitis*. Yet at higher doses, from 100,000 sporulated oocysts onwards, daily weight gain was more reduced by *E. acervulina* compared to *E. mitis.* Observations of the intestinal lesions indicated increased induction of petechiae for all three species compared to the uninfected control birds. More specifically, the highest number of petechiae were observed for *E. maxima,* followed by *E. mitis.* Besides the abundancy, also the location of the petechiae variated between the different species. While for *E. maxima* and *E. acervulina,* the petechiae were primarily observed in duodenum and the cranial part of the mid intestine, *E. mitis* infected birds displayed a higher abundancy of petechiae in the cranial part of the mid intestine.

Implications/Conclusions

Our findings show that *E. mitis* is able to induce intestinal lesions by the presence of petechiae in the cranial part of the mid intestine. Yet, setting a scoring system would need further investigation with more pure *E. mitis* clones due to the absence of other intestinal parameters like mucosal spots, ballooning and blood. Furthermore, our results indicate that the pathogenicity of *E. mitis*, especially at lower doses, is higher than expected. Therefore its potential role in sub-clinical coccidiosis is likely underestimated.

Understanding the direct and indirect impacts of host response traits on chicken coccidiosis: insights from epidemiological modelling

Ithurbide[1], M., A. Doeschl-Wilson[2], Y. Gao[3,4], A.D. Hulst[4], M.C.M. De Jong[4], M.H. Pinard van der Laan[1]

[1] *GABI, INRAE, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France*
[2] *The Roslin Institute, University of Edinburgh, Easter Bush Estate, UK*
[3] *Animal Breeding and Genomics Group, Wageningen University & Research, Wageningen, The Netherlands*
[4] *Infectious Disease Epidemiology, Wageningen University & Research, Wageningen, The Netherlands*

The Problem

Coccidiosis, caused by *Eimeria* parasites, remains a major economic challenge for the poultry industry. As resistance to anti-parasitic drugs increases and vaccines prove costly, genetic selection for disease resilience offers a promising alternative. Understanding how individual host traits affect both the infected animal and its flock-mates is crucial for developing effective disease control strategies. Traditional approaches focus only on direct effects on individual performance, missing the indirect effects on parasite transmission that determine population-level outcomes.

How we investigated or researched the problem

We developed an individual-based epidemiological model of coccidiosis in chickens to evaluate the relative impact of five key host response traits: susceptibility, infectivity, recoverability, tolerance, and compensatory growth. Model parameters were estimated using maximum likelihood methods from published experimental transmission data involving *Eimeria acervulina.* The model captures both epidemiological dynamics through environmental pathogen transmission and growth impacts of infection, including the development of acquired immunity after successive infections.

Results

Our simulations revealed distinct patterns for each trait. Reduced infectivity primarily operated through indirect effects, substantially decreasing environmental contamination and benefiting flock-mates without direct benefits to carriers. Reduced susceptibility showed mainly direct protective effects on individual animals. Enhanced recoverability emerged as particularly valuable, demonstrating both strong direct effects (reduced infection duration, improved final weight) and substantial indirect benefits (reduced infection frequency in flock-mates). Increased tolerance dramatically improved weight parameters but led to increased pathogen circulation.

Implications/Conclusions

Our findings suggest that recoverability may be a particularly valuable target for genetic improvement, offering population-level benefits through both direct and indirect protective effects. This modelling framework can guide breeding strategies and disease control interventions by identifying traits that most effectively improve flock-level resilience to coccidiosis, representing an important step toward reducing reliance on antimicrobials in commercial poultry production.

CELL BIOLOGY AND HOST-PATHOGEN INTERACTION

Pitch presentation

Haemogregarina stepanowi: lower host specificity, broad geographical range

Turkan Gurbanova[1], Jana Kvičerová[2]

[1] *Department of Protozoology, Institute of Zoology, Ministry of Science and Education Republic of Azerbaijan, AZ 1004, Str. A.Abbaszadeh, 1128th side street, 504th block, Baku, Azerbaijan Republic*
[2] *Department of Parasitology, Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic*

The Problem

The genus *Haemogregarina* (Coccidia: Adeleorina) is among the most common blood protists in turtles. It is heteroxenous, circulating in the erythrocytes of turtles as intermediate hosts, and in the intestinal epithelial cells of leeches as definitive hosts. Once transmitted, it develops into pre-erythrocytic meronts in various organs, such as the lungs, liver, and/or spleen, releasing secondary merozoites during asexual reproduction. Due to the high morphological similarity of erythrocytic stages in vertebrate hosts, species description, identification, and assessment of the real diversity of these parasites have long been challenging. Therefore, taxonomic studies are now preferably based on a complex approach that combines morphological and molecular data.

How we investigated or researched the problem

Altogether, only three species of turtles occur in Azerbaijan: *Emys orbicularis, Mauremys caspica,* and *Testudo graeca. E. orbicularis* and *M. caspica* are widely distributed across all geographical regions of Azerbaijan, and these species play a major role in maintaining the stability of biodiversity in freshwater and marsh ecosystems. Two of the three turtle species occurring in Azerbaijan (the freshwater turtles *E. orbicularis* and *M. caspica*) have been screened for blood protists. The study was performed in Agzibircala Lake, Shabran District. Blood smears were collected from each examined turtle, and partial 18S rRNA was sequenced from the recorded blood protists.

Results

Microscopic examination of blood smears revealed that both examined *E. orbicularis* individuals (100%) and two of six *M. caspica* individuals (33.6%) were infected with *Haemogregarina.* Parasitaemia in infected individuals ranged from 0.05 to 1.18%. Various developmental stages of *Haemogregarina* were recorded in infected erythrocytes. Distinct premeronts and gamonts were frequently observed, whereas trophozoites were not detected. The size and other morphological traits of the developmental stages of *Haemogregarina* observed in the peripheral blood of Azerbaijani turtles corresponded to the morphology of the respective stages of *Haemogregarina stepanowi,* described by Danilewsky (1885) and Reichenow (1910). PCR analysis demonstrated the same sensitivity as microscopy; all microscopically *Haemogregarina*-positive samples yielded the corresponding PCR products. All 18S rDNA sequences generated in the present study were identical in nucleotide composition and, based on BLAST analysis, were identified as *H. stepanowi* from *E. orbicularis* and *M. caspica.* Therefore, our isolates were considered to represent this species. All collected leeches from turtles were identified as *Placobdella costata.*

Implications/Conclusions

Considering other published studies on this topic, it is evident that *H. stepanowi* is a species with lower host specificity and a broad geographical range. Since it has only been reported in freshwater turtles and is transmit-

ted by leeches, its distribution appears to be closely linked to its definitive host – leeches – which are not host-specific and may spread *H. stepanowi* among turtle populations. The present study on *H. stepanowi* in *E. orbicularis* and *M. caspica* in Azerbaijan represents a new record, further expanding the known geographic range of this haemogregarine.

INTESTINAL MICROBIOTA

Poster presentation

Identifying Novel Vaccine Candidates and Drug Targets Against *Eimeria* in Chickens

Lois Cowie[1], Laura Toni[2], Tatiana Küster[3], Damer Blake[1], Dong Xia[1]

[1] *Royal Veterinary College*
[2] *University College London*
[3] *Boehringer Ingelheim*

The Problem

Control of *Eimeria* in chickens remains challenging. Resistance to anticoccidial drugs is widespread and public opinion/legislative changes are encouraging more precise drug use. Immunisation using live vaccines is effective, but relatively costly and limited by production capacity. Therefore, new drug and vaccine options to control *Eimeria* in chickens are required.

How we investigated or researched the problem

An *in-silico* approach to identify new drug targets and vaccine candidates in *Eimeria* is being applied, using *Eimeria tenella* as a model species. To identify vaccine candidates, the *Vacceed* pipeline (S. Goodswen *et al,* Bioinformatics 2014, 30 (16), 2381–2383) has been adapted and implemented. Potential drug targets are being identified using subtractive genomics methods, predicting protein properties relating to homology, function, and structure of the parasite's proteome. These methods are being supplemented by generation of host-pathogen and intra-parasite protein-protein interaction (PPI) networks. By integrating muti-lifecycle stage transcriptomics data, these networks can identify master regulators that drive pathogenic transitions via "silent" topological rewiring. Such regulators are often invisible to traditional differential expression analysis, as their own abundance may remain unchanged. However, they can be detected as high-traffic, topologically essential hubs by a graph neural network (GNN). Disruption of these proteins could dysregulate entire downstream cascades, making them ideal targets for pathogen control.

Results

Initial results from the Vacceed pipeline identified experimentally tested immunoprotective proteins as potential vaccine candidates with a success rate of ~77%. Work to further refine the pipeline is ongoing. The subtractive genomics pipeline has excluded ~3000 (~41%) proteins from consideration as drug targets in the initial homology stages. Further proteins will be excluded during function and structural stages, providing a shortlist for future screening. An intra-parasite PPI reference network has been generated using the STRING database (D. Szklarczyk et al, Nucleic Acids Res 2025, 53 (D1), D730–D737). The *de novo* prediction by STRING suggested a network with 3,272 nodes interacting via 99,340 edges. An orthology-based parasite network and a host-parasite interactome are currently being constructed. These will be modelled with temporal expression profiles from transcriptomics data to identify key proteins at different parasite lifecycle stages.

Implications/Conclusions

In-silico approaches provide promising insight to novel drug targets and vaccine candidates, particularly for complex pathogens like *Eimeria*. By leveraging these computational methods, recombinant vaccines can be developed, with the potential to provide more affordable and scalable options for vaccination against *Eimeria* in chickens and other species. Furthermore, target-based drug design aids the development of novel drug mechanisms to overcome drug-resistant strains, and contributes to tackling wider antimicrobial resistance.

EXPERIMENTAL AND IN VITRO SYSTEMS

Oral presentations

Impact of Natural Coccidiosis on Fat Digestibility and Broiler Performance Under Commercial Conditions

Alireza Khadem[1,2] and Christos Gougoulias[1]

[1] *Innovad, NV/SA, Cogels Osylei 33, 2600 Berchem, Belgium*
[2] *Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

The Problem

Coccidiosis caused by Eimeria spp. is a major subclinical challenge in commercial broiler production.. Coccidial infection damages the intestinal epithelium, impairing the digestion and absorption of key nutrients, particularly fats, thereby reducing energy efficiency and compromising growth performance in broilers.

How we investigated or researched the problem

Thirty-six pens of 36 broilers each were allocated to six dietary treatments: three with coccidiostat and three without. Diets were supplemented with graded levels of the emulsifier Maxilys® (Innovad) at 0, 250, or 500 ppm. Birds were raised under standard commercial conditions with natural *Eimeria* exposure. Growth performance was evaluated at Days 14 and 28, and apparent ileal fat digestibility was determined at Day 28. Dose-response analysis was performed separately for birds with and without coccidiosis control to evaluate how natural coccidial exposure affected nutrient digestibility and growth performance, and to assess the response to graded levels of the emulsifier

Results

At Day 14, emulsifier supplementation significantly influenced performance in a dose-dependent manner in both groups (P = 0.043 with coccidiostat; P = 0.032 without coccidiosis control). The effect was more pronounced in birds without coccidiosis control, reflecting greater intestinal compromise under natural coccidial exposure. At Day 28, fat digestibility showed a significant dose-response to emulsifier in both groups (P = 0.039 with coccidiostat; P = 0.037 without). Although digestibility remained responsive, the greater performance impairment observed at Day 14 in birds without coccidiosis control suggests that early intestinal damage temporarily compromised nutrient utilization, even when fat digestion later showed a measurable response.

Implications/Conclusions

Natural coccidiosis compromises intestinal integrity and negatively affects broiler performance under commercial conditions. While fat digestibility remained responsive to dietary emulsifier supplementation, early reductions in growth performance in birds without coccidiosis control indicate that intestinal damage can transiently impair nutrient utilization. These findings highlight the critical importance of effective coccidiosis management to preserve intestinal health, optimize nutrient absorption, and sustain broiler productivity.

Preliminary survey of in-feed mycotoxin contamination and Eimeria co-occurrence in Nigerian chicken farms: implications for disease control and food safety

Oluwayomi O. Adeyemi[1]*, Idowu A. Aneyo[2], Doyinsola M. Akingbehin[1], Serah D. Adeniyi[2], Onyinye B. Nwosa[2], Olufemi P. Sotayo[3], Patrick A. Ebomese[4], Abiodun K. Olakiigbe[5], Mayowa Oyewunmi[1], Barakat A. Amusat[1], Ayomide F. Aboyade[1], Ayobola M. Taiwo[1], Zainab, H. Jubril[2], Tolulope O. Oke[1], Praise C. Chukwuma[1]

[1] Parasitology unit, Department of Zoology, University of Lagos, Akoka-Yaba, Lagos, Nigeria
[2] Ecotoxicology unit, Department of Zoology, University of Lagos, Akoka-Yaba, Lagos, Nigeria
[3] Department of Microbiology, University of Lagos, Akoka-Yaba, Lagos, Nigeria
[4] Department of Statistics, University of Lagos, Akoka-Yaba, Lagos, Nigeria
[5] Nigerian Institute of Medical Research (NIMR), Yaba, Lagos
** Corresponding author: yadeyemi@unilag.edu.ng; +2348033954967*

The Problem

Mycotoxins in poultry feed may act synergistically with *Eimeria* infections, leading to increased severity of coccidiosis and mycotoxicosis, thereby compromising effective disease control and food safety. Field-based data on the dynamics of their co-occurrence however remain limited. This study provides baseline data on the prevalence and associated risk factors of *Eimeria* parasites and in-feed mycotoxigenic fungi co-occurrence on commercial poultry farms in Lagos, Nigeria.

How we investigated or researched the problem

Pooled faeces/litter and composite feed samples were collected from 126 chicken farms located in four poultry settlements. *Eimeria* oocysts were detected using the salt floatation method and classified into three size categories corresponding to the ten recognized *Eimeria* species of chickens. Feed samples were cultured on Potato Dextrose Agar, and pure isolates were obtained and characterized based on macroscopic and microscopic features. Farm management, biosecurity, and feeding practices were assessed using semi-structured questionnaires.

Results

Eimeria parasites and mycotoxigenic fungi occurred concurrently in 23/126 (18.3%) farms and did not vary across settlements ($P > 0.05$). Farms positive for small-sized oocysts showed a strong association with mycotoxigenic fungi contamination ($P < 0.001$). Most farms with *Fusarium*-contaminated feed were also positive for *Eimeria* oocysts ($P < 0.01$). High-Performance Liquid Chromatography revealed four Aflatoxin classes (AFB1, AFB2, AFG1, AFG2) in at least 60% of the *Aspergillus*-contaminated feed samples. Co-occurrence was significantly higher in broiler farms (5/10, 50%) compared with layer farms (18/116, 15.5%) ($P < 0.01$) but did not differ between farms using manufactured versus self-compounded feed ($P > 0.05$). Wet feeding troughs (OR: 80.1, $P < 0.05$) and the presence of flies or insects (OR: 9.1, $P < 0.05$) were significant predictors of co-occurrence.

Implications/Conclusions

Further research is needed to characterize the risks of coccidiosis and mycotoxicosis co-occurrence to poultry, public, and environmental health from a One Health perspective.

Prebiotic, Probiotic and Synbiotic Supplementations Ameliorated *E. tenella* induced Pathology in Broiler Chickens

Ogwiji M*[1, 2], Jatau ID[2], Natala AJ[2], Gasaliyu AK[2]

[1] *Modibbo Adama University Yola, P.M.B 2076, Yola 640101, Faculty of Agriculture, Department of Animal Science and Range Management*

[2] *Ahmadu Bello University Zaria, P.M.B 1045, Zaria 810107, Faculty of Veterinary Medicine, Department of Veterinary Parasitology and Entomology*

The Problem

Eimeria tenella, the widespread and deadly coccidian parasite causing caecal coccidiosis, impairs bird performance and haematological parameters by distorting nutrient intake and metabolism, with resultant high mortality in poultry. Coccidiosis generates substantial financial losses, estimated at 14.4 billion US dollars per year worldwide. Hence, for the poultry sector to realize its full potential, birds must be protected against coccidiosis. In addition, following increased concerns about *Eimeria* drug resistance and restrictions on the frequent use of synthetic anticoccidials, the search for sustainable strategies that can promote disease tolerance and increase productivity has intensified. Prebiotics, probiotics, and synbiotics have been recognized for their potential to promote gut health, modulate immunity, and mitigate the negative consequences of enteric infections.

How we investigated or researched the problem

This research studied the impact of dietary prebiotics (sugarcane molasses), probiotics (*Saccharomyces cerevisiae*), and synbiotics (combination of some probiotics and prebiotics) on hematological parameters, performance, and the severity of caecal coccidiosis in broiler chickens. 90-day-old Cobb 500 chicks were randomly allocated into six groups: negative (uninfected, unsupplemented), positive (*E. tenella*-infected, unsupplemented), standard drug (*E. tenella*-infected, Amprolium treated) control groups, and three supplemented groups receiving prebiotics, probiotics, and synbiotics products from day-old. Birds were challenged with *E. tenella* sporulated oocysts (2.0×10^4) at 21 days.

Results

The experimental infection elicited classical clinical manifestations of caecal coccidiosis, including as depression, ruffled feathers, diarrhoea, and bloody droppings, with 100% morbidity in infected groups. The severity of the infection scored base on clinical signs and mortality suggested the infected control group had severe caecal coccidiosis. There was reduced clinical severity and absence of mortality in the supplemented groups. Supplemented groups showed significantly higher packed cell volume (28 – 32%) and haemoglobin (Hb) concentration (9.5 – 10.8 g/dL) compared to the positive control (PCV: 18%; Hb: 7.2 g/dL). Synbiotic supplementation led to the most significant ($p<0.05$) weight gain and feed conversion ratio, compared to the positive control. Caecal lesion scores and oocyst shedding were significantly reduced in supplemented groups, with synbiotics achieving the most reduction.

Implications/Conclusions

These findings highlight the potential of the evaluated dietary supplements, particularly synbiotics to enhance poultry productivity, improve haematological indices, and mitigate *E. tenella*-induced pathologies. Thus showing that functional feed additives can act as effective nutritional strategies for boosting resilience against caecal coccidiosis and minimizing dependency on conventional anticoccidial drugs in poultry production systems.

MICROBIOMES AND CO-INFECTIONS

Oral presentations

Effect of some ionophores and a vaccine on the persistence of vancomycin resistant *Enterococcus faecium* and on efficacy after an *Eimeria* challenge

Jean-Michel RÉPÉRANT[1], Martine THOMAS-HÉNAFF[1], Chantal BENOIT[1], Pierre LE BIHANNIC[1], Gwenaelle MOURAND[2], Léa DUVAL[2], Eunice BRUYANT[2], Isabelle KEMPF[2], Florence TARDY[2]

[1] Anses, Ploufragan-Plouzané-Niort Laboratory, VIPAC Research Unit, 22440 Ploufragan, France
[2] Anses, Ploufragan-Plouzané-Niort Laboratory, MBA Research Unit, 22440 Ploufragan, France

The Problem

Ionophores are widely used to prevent coccidiosis in poultry. In some poultry-derived Enterococcus strains, resistance genes to narasin have been identified on the same plasmids as resistance genes to clinically important antibiotics, such as vancomycin. This has raised concerns about the potential co-selection of antibiotic resistance in bacteria of relevance to human health. The aim of this study was to determine whether chicks inoculated with a strain of *E. faecium* resistant to narasin and vancomycin, and exposed to narasin or monensin in their feed, were more likely to harbour these bacteria for a prolonged period. The study also evaluated whether ionophores in feed had a positive effect on coccidia control after an experimental challenge, in comparison with an anticoccidial vaccine.

How we investigated or researched the problem

A total of 210 chicks were allocated to five groups and housed in three separate rooms to prevent cross-contamination. In room 1, one group received no *Enterococcus* and served as a negative control. The remaining four groups, housed in room 2 and 3, were inoculated on day 1 and 3. In room 2, two groups received either narasin or monensin in the feed throughout the entire trial. In room 3, one group was vaccinated with an anticoccidial vaccine, while the other group received no treatment. At 16 days of age, each group was divided into two subgroups: one that received a coccidia challenge dose and one that received a placebo. Samples of cecal and intestinal contents were collected regularly to test for resistant bacteria. Body weight, weight gain, feed intake and feed conversion ratio were recorded, along with droppings appearance, oocysts per gram and coccidia lesion scores.

Results

The inoculation of *Enterococcus* had no significant effect on body weight. Birds receiving monensin or narasin had significantly lower weights on day 15 compared with the other groups, among which no significant differences were observed. The *Eimeria* challenge on day 16 induced clinical coccidiosis, which was less severe in the groups receiving monensin or narasin, and to a lesser extent, in the vaccinated group. The greatest weight gain was observed in the narasin-treated group. The populations of resistant *Enterococcus* declined at similar rates across all the groups, and neither narasin nor monensin increased their persistence in the chicken gastrointestinal tract.

Implications/Conclusions

Narasin was the most effective option for controlling the coccidia challenge; however, neither ionophore promoted prolonged persistence of the *Enterococcus* strain carrying resistance genes to narasin and vancomycin.

The use of ionophore coccidiostats in broilers, the narAB gene and its relevance to antibiotic resistance in human clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis*

George Tice[1], Shabbir Simjee[2], Jayanth Balakuntla[3]

[1] Independent Science, Policy and Regulatory Consultant, Wicklow, Co. Wicklow, Republic of Ireland
[2] Elanco Animal Health, Form 2, Bartley Way, Bartley Wood Business Park, Hook, UK;
[3] Elanco Innovation and Alliance Centre India LLP, Bengaluru, India.

The Problem

It has been proposed by researchers in Canada, Norway and Holland that the use of ionophore coccidiostats in broilers may select for the narAB gene in *Enterococcus faecium* and *Enterococcus faecalis* and that AMR genes of clinical significance may be associated with this, which then may colonise humans and pose antimicrobial resistance risk in hospital infections. The researchers hypothesise that this is because there is evidence of the presence of narAB on the same genetic elements as AMR genes of clinical relevance. The assumption his that there is therefore gene linkage and co-selection between narAB and antibiotic resistance genes, particularly vancomycin.

How we investigated or researched the problem

Firstly, a review of Antimicrobial National Resistance Programs between 2011 and 2020 in Norway, Sweden, Denmark and Netherlands, looking at 5181 *E. faecium* / *E. faecalis* poultry isolates, indicated that the prevalence of resistance to clinically relevant antibiotics for the treatment of human enterococcal infections was either absent or extremely low.
Secondly a genomic survey of over 25,000 *E. faecium* / *E. faecalis* genomes using internationally available genetic databases found that: **A.** The narAB is significantly more prevalent in broilers (26% *E. faecalis*, 39.2% *E. faecium*) and is extremely rare in isolates from human clinical infections (2.8% *E. faecalis,* 2.0% *E. faecium*). **B.** Genes associated with resistance to medically important antibiotics were not found close to the narAB gene. **C.** The sequence types found in broilers were not present in human clinical isolates and are distinct from them. In addition, the pattern of genes near narAB differed between them.

Results and implications

The above indicates that genetic linkage between clinically relevant AMR genes and narAB is highly unlikely and that salinomycin and narasin use and the associated higher incidence of narAB in enterococci from broilers will not lead to co-selection of antimicrobial resistance genes that may inpact clinical infections in humans. In addition clones, sequence types and plasmidomes are not shared between broilers and humans.

Implications and Conclusions

The probability that narasin and salinomycin use in broilers contributes to antimicrobial resistance in human clinical isolates through co-selection and gene transfer mechanisms is extremely low. Concurrently the withdrawal of access to ionophore coccidiostats for broiler producers and veterinarians would lead to significant and predictable risks to animal welfare, animal health, food safety and environmental and economic sustainability of broiler production.

MICROBIOMES AND CO-INFECTIONS

Pitch presentation

Profiling Eimeria burden, species composition, and genomic heterogeneity in poultry caecal metagenomes using Illumina and Nanopore sequencing

Melanie C. Hay[1], Conor J. Noonan[1], Patricia Conway[2], Fiona Tomley[1], Dong Xia[1], Damer P. Blake[1]

[1] Pathobiology and Population Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, AL9 7TA, UK,
[2] Nanyang Technological University, Singapore, Singapore

The Problem

Eimeria species are ubiquitous enteric parasites of poultry and a major cause of coccidiosis, with substantial impacts on animal health and productivity. Despite their importance, most large-scale poultry microbiome studies do not explicitly quantify *Eimeria* burden or explore species-level and genomic variation across production systems.

How we investigated or researched the problem

Here, we analysed deep shotgun metagenomic data from 120 poultry caecal samples collected across diverse production contexts in India, Bangladesh and Vietnam. Using curated reference genomes for multiple *Eimeria* species, we mapped both Illumina short-read and Oxford Nanopore long-read sequencing data to quantify overall *Eimeria* burden, species composition, and within-genome coverage patterns. *Eimeria* burden estimates were further used to stratify samples for exploratory integration with bacterial microbiome community profiles.

Results

Initial results reveal substantial variation in *Eimeria* load across samples, spanning orders of magnitude. Across both sequencing platforms, *Eimeria tenella* consistently emerges as the dominant species, in line with its known caecal tropism, while mixed-species profiles are common. Comparison of Illumina and Nanopore data from matched samples shows concordant detection of *Eimeria* presence and dominant species, supporting the robustness of the approach across technologies.

Implications/Conclusions

By enabling identification of samples with elevated *Eimeria* burden, this framework provides a basis for targeted integration with bacterial microbiome analyses to explore potential dysbiosis-associated patterns. Ongoing analyses focus on genome-wide coverage patterns to identify conserved and variable regions within the different *Eimeria* genomes, providing insight into population structure, potential strain diversity, and region-specific signatures. Together, this work demonstrates the utility of metagenomic sequencing for high-resolution profiling of *Eimeria* ecology and genomic variation in poultry systems, with implications for surveillance and control strategies.

PROPHYLAXIS AND TREATMENT, NOVEL APPROACHES FOR CONTROL

Oral presentations

Efficacy of a trivalent Eimeria vaccine for turkeys when applying spray on birds vaccination

Monita Vereecken*[1], Brecht Maertens[2], Davy Van Gaver and Koen De Gussem[1]

[1] *Huvepharma NV, Antwerp, Belgium*
[2] *Poulpharm, Izegem, Belgium*

The Problem

In intensively reared turkey production systems, feed-administered coccidiostats remain the primary method of managing coccidiosis. This study evaluates the efficacy of a trivalent *Eimeria* vaccine administered to turkeys via spray application against a challenge with *Eimeria meleagrimitis,* the most prevalent *Eimeria* species in Europe, as an additional tool to control coccidiosis in turkeys.

How we investigated or researched the problem

One group of turkeys was vaccinated by spray on birds at day old with a trivalent turkey coccidiosis vaccine containing strains of *Eimeria meleagrimitis, Eimeria gallopavonis* and *Eimeria adenoeides.* A second group remained unvaccinated. Birds were reared in floor pens till the age of 20 days after they were allocated to cages. One day later, a subset of unvaccinated turkeys and the vaccinated group were challenged with a recent European isolated *Eimeria meleagrimitis* strain (respectively IUC and IV). An additional subset of unvaccinated turkeys were not challenged and served as the negative control group (UUC). Protection against the challenge was evaluated by means of lesion scoring (5 days after challenge), oocyst excretion, clinical scores and body weights till 35 days of age.

Results

No significant differences in body weights and clinical scores were observed between vaccinated and unvaccinated turkeys in the first 3 weeks of life. Following the challenge at 21 days of age, the IUC group developed a pronounced infection, including one challenge related mortality. Compared with the UUC, IUC turkeys exhibited significantly lower body weights and daily weight gain in the two weeks after challenge, higher intestinal lesion scores for *Eimeria meleagrimitis* (2.0 vs 0.0; $p<0.001$), higher oocyst shedding and elevated clinical coccidiosis scores. Vaccinated turkeys (IV) demonstrated significant improvements compared with the IUC group. Post challenge, IV birds body weight and daily weight gain were significantly higher in comparison to the IUC. Lesion scores were markedly reduced (0.0 versus 2.0 for IUC; $p<0.001$), and oocyst shedding was significantly lower from D24 to D35 (414,900 vs 1,338,733; $p=0.0001$). Clinical coccidiosis scores were also significantly reduced, especially during peak infection periods.

Implications/Conclusions

Spray application of the trivalent coccidiosis vaccine to day old turkeys did not negatively affect health or body weight. A strong protective effect was seen against a challenge with *Eimeria meleagrimitis* as both zootechnical and parasitological parameters were significantly improved in comparison to the challenged control group. Given the limited number of registered coccidiostats for turkeys, a trivalent coccidiosis vaccine could become a valuable additional tool for coccidiosis control.

A model close to actual conditions of use for evaluating disinfectants against coccidia oocysts

Jean-Michel RÉPÉRANT[1], Catherine CAZEAUX[2], Martine THOMAS-HÉNAFF[1], Chantal BENOIT[1], Pierre LE BIHANNIC[1], Stéphanie LA CARBONA[2]

[1] Anses, Ploufragan-Plouzané-Niort Laboratory, Ploufragan site, VIPAC Research Unit, 22440 Ploufragan, France
[2] Actalia, 310 rue Popielujko, 50000 Saint Lo, France

The Problem

According to European recommendations, the evaluation of disinfectant efficacy against coccidia oocysts is extrapolated from in vitro tests using *Cryptosporidium parvum* oocysts. This parasite has oocysts with a size, structure, and cell wall composition very different from those of coccidia of the genus *Eimeria*. The conditions under which these in vitro tests are carried out are very different from those found in poultry houses: temperature, tests in suspension or on non-porous physical supports (stainless steel), and absence of biofilm on surfaces.
Our objective was to develop a model for exposing chicken coccidia oocysts to disinfectants in order to evaluate their effectiveness under conditions close to those encountered in poultry houses.

How we investigated or researched the problem

Two solid surfaces, one porous and one non-porous, were defined. Oocysts were mixed with a defined amount of biofilm representative of what might remain in a building after washing: dust from livestock, traces of animal feed, and manure. The mixture was then applied to the substrates, and the disinfectant was used according to the manufacturer's instructions. The percentage of oocysts recovered after contact was used for the evaluation of the direct destruction effect of the oocysts. The recovered oocysts were then administered to chickens to assess their viability and infectivity, by comparing them to parasite development in birds receiving increasing doses of control infective oocysts.

Results

Four disinfectants recognized as oocysticides according to European guidelines for validation by in vitro tests were assessed in our model. The achieved results suggest a lack of efficacy against *Eimeria* oocysts.

Implications/Conclusions

The developed model attempts to mimic the conditions of use of disinfectant products in poultry houses (i.e. biofilms and porous surfaces). This model is complex and requires the inoculation of oocysts into chickens to assess the viability of the oocyst and their ability to cause typical lesions. Nevertheless, it allows us to obtain results directly applicable to chicken coccidia, which may differ from those obtained via the in vitro test recommended at the European level. This work suggests that the current European requirements for the evaluation of these products against coccidia of the genus *Eimeria* should be reconsidered.

Meta-Analysis of Four Controlled Studies Evaluating the Prophylactic Efficacy of Natural Feed Additives Against Experimental Coccidiosis in Broiler Chickens

Madalina Diaconu

The Problem

Coccidiosis, caused by Eimeria spp., remains a major enteric disease in poultry. With restrictions on chemotherapeutic coccidiostats, there is a need for sustainable and effective natural alternatives.

How we investigated or researched the problem

A meta-analysis of four controlled studies (total n=720 broilers per product group) was conducted. Ross 308 broilers received prophylactic in-feed supplementation of one of three products (P1, P2, or P3) from day 1 to 35. Birds were challenged at day 17 with a mixed Eimeria spp. inoculum (75,000 oocysts/bird). Outcomes included Anticoccidial Index (ACI), oocyst shedding (OPG), lesion scores, body weight gain (BWG), feed conversion ratio (FCR), and mortality. A chemical coccidiostat control was included and is referred to as CC.

Results

P3 achieved medium anticoccidial efficacy with ACI=146.8, comparable to CC (155.0). It reduced oocyst shedding by 46.7% and lowered lesion scores by 52.9%. Growth performance improved (BWG +2.95%) with FCR similar to CC. P1 and P2 showed partial efficacy with reductions in OPG (44.1% and 41.9%) and lesion scores (40.0% and 43.9%). Mortality remained <2.8% across treatments.

Implications/Conclusions

P3 demonstrates medium-level anticoccidial efficacy and performance comparable to CC, supporting its use as a natural alternative in integrated coccidiosis control. P1 and P2 provide partial protection and may be suited to lower-challenge conditions.

In silico exploration of the anticoccidial activity of *Omi ogi* (fermented maize supernatant) against *Eimeria tenella*

Gbolahan Oladipupo Oduselu[1,4]*, Oluwayomi Oluwatoyin Adeyemi[2]*, Olufemi Isaac Sotayo[3], Olamide Daniel Shoyinka[4]

[1] West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana, P.O. Box LG 54, Legon, Accra, Ghana
[2] Department of Zoology, University of Lagos, Lagos, Nigeria
[3] Department of Microbiology, University of Lagos, Lagos, Nigeria
[4] Drug Discovery Innovation Hub, Ota, Ogun State, Nigeria
Corresponding author: Oluwayomi Oluwatoyin Adeyemi (yadeyemi@unilag.edu.ng; +2348033954967)

The Problem

Control options for chicken coccidiosis have become increasingly limited due to the emergence of drug resistance and the high cost of vaccines. This challenge has created a pressing need for sustainable alternatives, with growing attention directed toward natural products and traditional remedies. *Omi ogi,* the fermented water obtained from maize slurry, is rich in lactic acid bacteria metabolites and organic acids and is indigenously used in West Africa as a remedy against gastroenteritis. However, its anticoccidial potential in poultry remains largely unexplored. This study aims to bridge this gap by employing *in silico* approaches to evaluate the activity of bioactive compounds derived from *omi ogi* against key molecular targets associated with *Eimeria tenella* pathogenicity.

How we investigated or researched the problem

Eight (8) protein targets relevant to *E. tenella* development were retrieved from the Protein Data Bank based on literature evidence. These targets included dihydroorotate dehydrogenase (DHODH), apical membrane antigen 1 (AMA1), calmodulin-like domain protein kinase, phosphotransferase, microneme protein 3, microneme protein 5 precursor, SAG family member (SAG19), and enoyl-acyl carrier protein reductase. Thirteen (13) bioactive compounds previously reported in *omi ogi* were docked against these targets using Maestro Schrödinger. Binding affinities and binding free energies were subsequently analyzed to identify promising compound–target interactions.

Results

The results varied across the targets, with most interactions exhibiting moderate binding affinities. Notably, 2-methoxy-4-vinylphenol demonstrated the most favorable overall binding profile across multiple targets and exhibited a stronger binding affinity than the co-crystallized ligand of apical membrane antigen 1 (2-acetamido-2-deoxy-α-D-galactopyranose, A2G). Three other phenolic compounds showed particularly strong binding against the calmodulin-like domain protein kinase (PDB ID: 4SYJ), including phenol-2,6-dimethoxy (−6.78 kcal/mol; −34.42 kcal/mol), 2',4'-dimethoxyacetophenone (−6.03 kcal/mol; −39.33 kcal/mol), and 2,4-di-tert-butylphenol (−5.87 kcal/mol; −27.80 kcal/mol). Furthermore, squalene, a non-phenolic compound, showed considerable binding affinity toward the 6AJE target (−5.63 kcal/mol; −46.61 kcal/mol). Overall, these compounds exhibited relatively stronger and more stable interaction profiles compared to other evaluated candidates.

Implications/ Conclusions

Collectively, the findings provide preliminary computational evidence that *omi ogi* constituents interact with *E. tenella* proteins, suggesting potential anticoccidial activity. However, further in vitro and in vivo investigations are required to validate these computational predictions.

PROPHYLAXIS AND TREATMENT, NOVEL APPROACHES FOR CONTROL

Pitch presentations

Performance and Health Outcomes of a Saponin-Based Solution (Norponin XO2®) Compared with Conventional Anticoccidial Programs in Broiler Chickens Reared in Commercial Conditions

Violette Pousset, Sekhou Cissé and Mohammed el Amine Benarbia

The Problem

Today, poultry producers are confronted with the need to make choices in coccidiosis management to address different challenges. Many solutions are proposed; however, most alternatives to conventional solutions are supported only by evidence from in vitro and/or in vivo models. While data from these models are useful, there is a clear need to generate data for these alternative solutions under commercial farming conditions. The objective of this study was to generate data for a natural saponin-based solution, Norponin XO2®.

How we investigated or researched the problem

The study was conducted over four production cycles (35 days each) using four buildings with a capacity of 15,000 birds each (A, B, C, and D). Birds in buildings A and B were supplemented with a saponin-based solution (Norponin XO2®; 250 ppm) throughout the four cycles, from day 1 to day 35. Birds in buildings C and D were supplemented with either clopidol (125 ppm; cycle 1), lasalocid (75 ppm; cycles 2 and 3), or salinomycin (60 ppm; cycle 4). Zootechnical performance parameters were monitored, and the European Poultry Efficiency Factor was calculated for each building and cycle. Data were recorded and statistical analyses were performed. Statistical analysis was performed by Fischer t-test using GraphPad® prism 7 software. Statistical significance was considered at $P < 0.05$.

Results

During the four study cycles, no cases of coccidiosis were reported in any of the buildings. This indicates that the disease was effectively managed with both the conventional and the saponin-based solutions (Norponin XO2®). No differences were observed in growth performance parameters between chickens supplemented with the saponin-based solution and those receiving conventional solutions (ADG, FCR). However, a significant reduction in mortality was observed in birds supplemented with the saponin-based solution compared with the conventional groups. This was reflected in a higher European Poultry Efficiency Factor in birds supplemented with the saponin-based solution (Norponin XO2®), although the difference was not statistically significant.

Implications/ Conclusions

Based on growth performance and health parameters, this study showed that, under commercial conditions, supplementation with a saponin-based solution (Norponin XO2®) was as effective as conventional solutions for the management of coccidiosis in broiler chickens. In addition, a reduced mortality rate was observed in chickens supplemented with the saponin-based solution (Norponin XO2®). This effect has already been reported for saponin-based solutions, including Norponin XO2®, using other experimental models. Overall, these results indicate that the tested saponin-based solution (Norponin XO2®) represents a suitable option for professionals seeking greater flexibility in coccidiosis management.

Apicoplast Pyruvate Carrier 1 is a Novel Marker for Diclazuril Resistance in *Eimeria*

Xiaojin Li[1], Yuanyuan Zhang[1], Qi An[1], Xiaomin Ge[1], Zhili Hao[1], Yingying Sun[1], Xun Suo[1]*, and Xianyong Liu[1]*

[1] State Key Laboratory of Veterinary Public Health and Safety; Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China

The Problem

Anticoccidial drug resistance represents a major challenge for the control of coccidiosis and the maintenance of poultry health and productivity. Diclazuril is one of the most important broad spectrum anticoccidial drugs, exhibiting high efficacy at low doses against all pathogenic *Eimeria* species and multiple developmental stages in chickens. Although several genes have been proposed to be associated with diclazuril activity, the definitive resistance genes and causative mutations have remained unclear.

How we investigated or researched the problem

We applied an advanced backcross mapping strategy to generate introgressed resistant lines with highly similar genetic backgrounds but segregating diclazuril resistance phenotypes. By comparing the genetic frequency spectrum between resistant and sensitive progeny, candidate genes and loci responsible for the phenotype were precisely identified. Functional validation was performed by overexpressing candidate genes carrying resistance-associated mutations in diclazuril-sensitive *Eimeria* strains. In addition, diclazuril-resistant field isolates were analyzed using whole-genome resequencing and alignment analysis. Cross-species validation was conducted using homologous mutations from *Toxoplasma gondii.* Finally, a PCR-based sequencing assay was developed to detect resistance-associated alleles in field samples.

Results

Genetic mapping localized diclazuril resistance to a region on chromosome 9 containing six genes with missense mutations. Functional assays demonstrated that a single mutation in the pyruvate transporter gene *EtAPC1* conferred diclazuril resistance in *Eimeria*. Analysis of field-derived resistant isolates identified two additional resistance-associated mutations in *APC1.* In addition, a homologous resistance-associated mutation identified in *T. gondii APC1* also conferred diclazuril resistance when introduced into sensitive *Eimeria*, indicating functional conservation of *APC1*-mediated resistance across apicomplexan parasites. PCR amplification and sequencing confirmed the presence of *APC1* resistance alleles in field samples.

Implications/Conclusions

This study identifies *APC1* as a key determinant of diclazuril resistance in *Eimeria* and demonstrates that *APC1*-mediated resistance is evolutionarily conserved across apicomplexan parasites. *APC1* mutations therefore represent sensitive and practical molecular markers for field surveillance of diclazuril resistance and evaluation of anticoccidial drug efficacy. We thank Dr. Hongyu Han for providing a diclazuril-resistant strain! This study was supported by National Natural Science Foundation of China (31873007, 32373031) and China Agriculture Research System of MOF and MARA (CARS-43).

The effect of phytogenic mixture addition on gut integrity parameters, coccidiosis score in chicken broiler fed two different diets

Paulina Abramowicz[1], MonikaMichalczuk[2], Małgorzata Majewska[3], Misza Kinsner[3], Dominika Szkopek[3], Arkadiusz Matuszewski[2], Damian Bień[2], Jakub Urban[2], Patrycja Ciborowska[2], Pawel Konieczka[3,4]

[1] R&D, AdiFeed LTD, Grodzisk Mazowiecki, Poland;
[2] Institute of Animal Sciences, Warsaw University of Life Sciences, Warsaw, Poland;
[3] University of Warmia and Mazury, Olsztyn, Poland;
[4] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland.

The Problem

Phytobiotic mixtures (PM) are increasingly considered potential alternatives to coccidiostats and antibiotic growth promoters due to their growth-promoting, immunomodulatory, anti-inflammatory, and antimicrobial properties. However, their mechanisms of action remain incompletely understood. This study evaluated the effects of a phytogenic mixture on gut integrity parameters and coccidiosis lesion scores in broiler chickens fed either a soybean meal-based diet (SB) or a diet containing sunflower and rapeseed post-extraction meal (SR).

How we investigated or researched the problem

A total of 500 one-day-old Ross 308 chicks were randomly allocated to four treatments, each comprising 125 birds distributed into five replicates (25 birds per pen). The first group (K1) served as a negative control and received the SB diet without supplementation, while the second group (K2) served as a negative control fed the SR diet without supplementation. The remaining groups received supplementation of 0.3 kg of phytogenic mixture per ton of complete feed based on the SB diet (A1) or the SR diet (A2), respectively. The phytogenic product used in the experiment was a commercial mixture containing hot pepper fruit, white mustard seed, soapwort root, turmeric rhizome, and thymol (AdiCox® AP, AdiFeed® LTD). The experiment was conducted for 42 days under floor-pen conditions. On day 28, six birds from each treatment were randomly selected and euthanized for blood and intestinal sample collection. Gut health was assessed using the Johnson and Reid lesion scoring method. The following markers of intestinal integrity were analyzed: diamine oxidase in blood (gut permeability), secretory IgA in the small intestinal content, and ovotransferrin in feces followed by CLDN-3, OCLN, ZO-1, ZO-2.

Results

Supplementation of the phytogenic mixture in the SB diet resulted in lower concentrations of all three markers: diamine oxidase in blood (gut permeability), secretory IgA in the small intestinal content, and ovotransferrin. The results showed that the addition of PM significantly ($p \leq 0.001$) impacted the expression of claudin-3, occludin, zonula-1 and zoonula-2 in comparison with control group subjects. The composition of feed did not affect the expression of selected gut integrity genes, nevertheless, the rape and sunflowersuggesting improved intestinal barrier function. In birds fed the SR diet, a similar effect was observed for secretory IgA. Moreover the rapeseed and sunflower-based diet increased the *E. maxima* lesion score in the K2 group, whereas phytogenic supplementation reduced the *E. tenella* lesion score in this group.

Implications/ Conclusions

These findings indicate beneficial effects of phytogenic mixture supplementation on the functional status of the gastrointestinal tract. The observed effects were associated with improved regulation of intestinal permeability and maintenance of gut health in broiler chickens.

Phytase Supplementation Enhances Phytate Digestibility and Reduces Phosphorus Run off in Broiler Production

Rafiullah, Asad Sultan, and Sarzamin Khan

Department of Poultry Science, The University of Agriculture, Peshawar, Pakistan

Abstract

High density poultry production can result in the leaching of significant amounts of nutrients into the environment, particularly phosphorus, due to incomplete digestion of phytate and nitrogen present in cereal grains, which constitute up to 70% of poultry feed. This study investigated the effects of phytase supplementation on nutrient digestibility and phosphorus run-off in broilers. Phytase enzyme (10,000 FTU/g) was added at two inclusion levels 0.01% (F1) and 0.015% (F2) to sorghum-based diets (918 g/kg), while a non-supplemented control (F0) was maintained. Celite served as an indigestible marker. At day 42, nitrogen, phosphorus, and phytate contents in feed, digesta, and feces were analyzed. Ileal nitrogen digestibility increased by 2.6% and 5% in F1 and F2, respectively, while fecal nitrogen losses decreased by 45% compared with the control. Excreta phosphoru0s digestibility improved (0.31 and 0.32 in F1 and F2) compared to the control (0.17). Phytate digestibility also increased (0.751 and 0.754), and litter pH was numerically reduced (6.7 vs. 7.6 in control). Soluble reactive phosphorus (SRP) levels were significantly ($p < 0.05$) lower in treated groups. These findings indicate that microbial phytase supplementation improves nutrient digestibility and reduces phosphorus losses, offering a sustainable strategy to mitigate environmental pollution. Further studies are warranted to assess phosphorus and nitrogen leaching and their effects on aquatic ecosystems.

Keywords

Phytase, Broilers, Phosphorus run-off, Nutrient digestibility, Environmental sustainability

Gene Expression Explained the Benefits of Protected Thymol and Carvacrol Under a Chemical-Iionophore Coccidiostat Program on Broiler Performance

***Hugo Romero[1], Mireille Huard[1], Silvia Peris[1], Stéphanie Lecuelle[1], Laura M. Payling[2], and Luis F. Romero[2]**

[1] Novus International, Inc., Chesterfield, MO, USA;
[2] Biofractal Lda., Portugal

The Problem

Coccidiosis remains a major challenge in broiler production, and coccidiostat-based programs are widely used for control. However, these programs may affect intestinal function and nutrient utilization. This study investigated whether changes in gene expression could explain improvements in growth performance and intestinal integrity in broilers fed a blend of essential oils under a chemical-ionophore coccidiostat program.

How we investigated the problem

A total of 560 one-day-old Ross 308 male broilers were allocated to two dietary treatments in a completely randomized design with 14 replicate pens per treatment and 20 birds per pen. Birds were reared under reused litter contaminated with *E. coli* (8.5×10^3 CFU/g measured through *E.coli* B-glucoronidase), *Clostridium perfringens* (<10 CFU/g), and coccidia oocysts (744 oocysts/g). All birds received a standard shuttle coccidiostat program consisting of the chemical nicarbazin in combination with the ionophore narasin (40 mg/kg of each) in the starter phase (0–14 d), followed by the ionophore salinomycin (60 mg/kg) in the grower (14–28 d) and finisher (29–42 d) phases. Treatments included a control diet and the same diet supplemented with protected thymol and carvacrol (NEXT ENHANCE® 150) at 30 g/ton during the starter and grower phases and 15 g/ton during the finisher phase. Growth performance was recorded throughout the study. On day 28, jejunum and liver samples were collected for RNA sequencing to characterize changes in gene expression. Differential expressions were analyzed using DESeq2, and pathway activation was assessed using Quantitative Pathway Activation Analysis (adjP<0.05). Additionally, calprotectin, IL-10, IgA, and IFN-γ were measured in blood on day 28. Pairwise comparisons using LSMEANS were used to evaluate performance data.

Results

Supplementing the coccidiostat diet with protected thymol and carvacrol tended to improve body weight and BW gain during the starter (P=0.07) and significantly during grower phases (P<0.05). The trend to significance is also observed for the adjusted feed conversion ratio in the grower phase (P=0.08) and the overall feed intake (P=0.1). Blood analysis did not detect biomarkers, except for a trend to lower IgA in the supplemented diet (P<0.1). However, the jejunum transcriptomic analysis identified 918 differentially expressed genes in response to supplementation, whereas no differences were observed in the liver. Supplemented with protected thymol and carvacrol, activated pathways related to tight junction assembly, epithelial maintenance, and intestinal motility, along with suppression of apoptosis and cell-cycle pathways, suggesting greater tissue stability and reduced stress.

Oxidative stress-induced senescence was downregulated, indicating a lower inflammatory burden. Jejunum tissue showed down-regulation of IL-10 (adjP<0.05), supporting these results. This also explained the lower trend for mortality in the treated group during the grower phase (P<0.1). In addition, the transcriptomic response suggested activation of gut motility and lipid absorption pathways (transport of bile salts, portomicron assembly; adjP<0.01).

Implications

The benefits of protected thymol and carvacrol in a chemical-ionophore shuttle coccidiostat program were associated with changes in jejunal gene expression. These transcriptomic shifts help explain the improved growth performance, intestinal integrity, and metabolic function observed in broilers reared on reused contaminated litter. Supplementation with protected thymol and carvacrol may complement conventional coccidiosis control programs by supporting intestinal function at the molecular level.

Clinico-Pathological Study of Avian coccidiosis and its Economical Impact on Small-scale Poultry Farming in Selected Districts of Tigray, Ethiopia

A.T. Yohannes[1]*, M. T. Habtamu[1], M. T. Abreha[1] and R. T. Yisehak[1]

[1] Mekelle University, College of Veterinary adn Animal Sciences, P. O. Box: 1436, Mekelle, Tigray, Ethiopia.
** yohannes.tekle@mu.edu.et, https://orcid.org/0000-0001-9707-3887*

The Problem

Despite the economic significance of coccidiosis in Tigray region, Ethiopia, no substantial research have been done and there was scanty information. Hence, the present work was designed to provide information on the clinico-pathology and socioeconomic impacts of avian coccidiosis on chickens farmed under small-scale system.

How we investigated or researched the problem

Clinico-pathomorphological study, morphometric oocyte per gram (OPG) determination and questionnaire survey were done.

Results

Out of 2,000 chicken populations, 350 (17.5%) showed clinico-pathomorphological manifestations of coccidiosis, and 5% mortality due to clinically-suspected coccidioisis was reported. The major clinical findings were depression, bloody diarrhea, mucoid droppings and loss of production. Eleven percent of birds examined through necropsy showed mild to moderate thickened, wrinkled and oedematous intestinal wall with multiple focal, ecchymotic haemorrahge and congestion and with mucoid content. Seven percent of samples reported to have greatly enlarged and distended caecal pouch with clotted blood and diffused haemorrhagic enteritis. Histopathological examinations of 8.5% of the representative tissue sections showed nnumerous oocytes invading the mucosal and submucosa layers, loss of enterocells, hemorrhage, necrosis of mucosal layer, infiltration of heterophils and lyphocytes in the submucosal, desquamation and blunting of villi. On morphometric examination, out of 96, 31 and 51 droppings, litter and intestinal content samples, 66 (68.75%), 8 (25.81%) and 39 (76.47%) were found positive for coccidian oocyts respectively. The questionnaire based survey indicated that coccidiosis attributed to an estimated ~USD 137.5 loss per farm per month. Risk factors such as poultry farmers/employees with little background on poultry production and poor litter management were found to favour the occurrence of avian coccidiosis.

Implications/ Conclusions

The study indicated that avian coccidiosis is very important disease of chickens reared under small-scale farming in Tigray (Ethiopia), with significat socioeconomics impacts. Low level of education and technical knowledge of owners and employees, poor veterinary access and inputs, and cocciodiosis management practices, and weak integrated and systematic coccidiosis surveillance and control activities are key factors contributing for the high prevalence of coccidiosis.

PROPHYLAXIS AND TREATMENT, NOVEL APPROACHES FOR CONTROL

Poster presentations

Effects of Quillaja Saponins on Enveloped Viruses and Bacteria in Broiler Chickens and Their Contributions to Performance Parameters

Murat Bilgiç

DVM,Poultry Disease(+90)05379566594 vet.hekimmuratbilgic@gmail.com Orcid ID:0009-0009-6612-8334

The global ban on antibiotic growth promoters (AGPs) has prompted the poultry industry to seek safe and sustainable alternatives. Saponins derived from the Quillaja saponaria tree are at the forefront of this search thanks to their biological properties. This article examines the antimicrobial mechanisms of quillaja saponins against enveloped viruses, particularly Newcastle disease virus and infectious bronchitis virus, and against the pathogenic bacteria Clostridium perfringens and Salmonella. Furthermore, this article evaluates the effects of these phytobiotics on live weight gain, feed conversion ratio (FCR), immune response, mortality, intestinal health and meat quality in broilers in light of current literature data. The conclusion section discusses the advantages of using Quillaja saponins in broiler farming in terms of improving performance and controlling disease.

Effects of dietary supplementation with essential oil-derived compounds on growth performance in naked neck chickens naturally infected with coccidia (*Eimeria spp.*)

Marta Raffaelli[1], Alessia Zoroaster[2], Riccardo Dadà[1], Margerita Marzoni Fecia di Cossato[1], Davide Buzzanca[3], Ilario Ferrocino[3], Roberto Marani[4], Antonio Frangipane di Regalbono[2], Stefania Perrucci[1]

[1] Department of Veterinary Sciences, University of Pisa, Italy
[2] Department of Animal Medicine, Production and Health, University of Padova, Italy
[3] DISAFA- University of Torino Italy
[4] La Veterinaria s.r.l., Perugia, Italy

The Problem

Coccidiosis is considered the cause of major bird health and economic problems in the poultry industry worldwide [1]. Due to drug-resistance and drug residues in the environment and food [2], and the high cost of vaccines which do not include three newly identified *Eimeria* species [3], the control of chicken coccidiosis is challenging. This study aimed to evaluate the effects of thymol and a commercial blend (EP) containing cinnamaldehyde, carvacrol, and eugenol, on chickens naturally infected by coccidia (*Eimeria* spp.).

How we investigated or researched the problem

Naked-neck chickens (n. 99) were divided into three groups, each consisting of three replicates of 11 animals, based on the diet offered: control feed group (C), 5 mg thymol/kg feed group (T), and 50 mg EP/kg feed group (EP). Pooled faecal samples were collected and analysed by a modified McMaster method. Sampling and analysis were performed from 15 days of age until the end of the study. *Eimeria* species identification was carried out by endpoint PCR [4]. Average body weight (BW), average daily weight gain (ADG) and feed conversion ratio (FCR) were recorded. Carcass yield and caecal microbiota composition were also assessed. All data were statistical analysed.

Results

Eimeria praecox and the highly pathogenic species *E. necatrix* and *E. tenella* were identified. EP significantly reduced coccidian peak oocyst shedding ($P < 0.001$) and improved BW ($P < 0.001$) and ADG ($P < 0.001$) when compared to C and T groups. T improved the FCR ($P < 0.01$) but did not show a significant anticoccidial activity. Among the 25 most abundant taxa identified with microbiota analysis, beta-diversity was significantly higher in C and EP compared to T. The greatest intergroup distance was observed between C and EP ($P < 0.05$). Among the most represented bacteria, a significant higher abundance of *Phocaeicola* in EP and T groups compared to the C group ($P < 0.05$) resulted.

Implications/ Conclusions

This study provides new evidence on the effectiveness of EP and thymol in chickens naturally challenged with highly pathogenic *Eimeria* spp. EP improved growth performance, reduced oocyst excretion, and maintained good carcass yield. In addition, EP and thymol modulated caecal microbiota, with a greater abundance of bacteria considered beneficial for intestinal functionality and immune response [6], and this is consistent with performance results observed.

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Comparative Anticoccidial Efficacy and Growth Performance of Narasin/Nicarbazin and Phytogenic Feed Additives in Broiler Chickens Under a Mixed Eimeria spp. Challenge

Felipe Penagos-Tabares[1], Greg Mathis[2], Brett Lumpkins[2], Marko Vasiljevic[1]

[1] Agromed Austria GmbH, 4550 Kremsmünster, Austria;
[2] Southern Poultry Feed & Research Inc, Georgia, USA;
[3] PatentCo, 24211 Misicevo, Serbia

The Problem

Coccidiosis remains a major constraint to broiler performance and intestinal health. Besides conventional anticoccidial programs, phytogenic feed additives containing plant bioactives may support gut integrity and immunity for mitigating *Eimeria*-associated losses.

How we investigated or researched the problem

This study evaluated anticoccidial efficacy and growth performance of a conventional anticoccidial program (based on narasin/nicarbazin) compared with two phytogenic feed additives in broilers exposed to a mixed *Eimeria* challenge. A 42-day floor-pen trial was conducted using male Cobb 500 broilers assigned to 50 pens (50 birds/pen) in a randomized complete block design (5 treatments; 10 replicate blocks). Treatments included: (1) unchallenged control (no additive), (T2) challenged control (no additive), (3) challenged + narasin/nicarbazin (NN) (included at 1 lb/US ton feed [=551.2 g/MT feed (ppm)]), (4) challenged + *Quillaja saponaria* extract (QE; 30 g/MT), and (5) challenged + a phytogenic blend (PB; 500 g/MT) containing extracts of *Q. saponaria, Castanea sativa, Thymus vulgaris* and *Origanum vulgare.* On day 21, all challenged groups received a mixed *Eimeria inoculum* (*E. acervulina* 100,000; *E. maxima* 50,000; *E. tenella* 75,000 oocysts/bird). Growth performance was assessed based on feed intake, body weight gain (BWG), and adjusted feed conversion ratio (FCR). Lesion scoring was performed on day 27 (Johnson and Reid method) and oocyst shedding (OPG) was determined on day 28. Data were analyzed by ANOVA and Tukey's HSD test ($p \leq 0.05$).

Results

The mixed Eimeria challenge reduced performance by day 42. Challenged, untreated birds exhibited lower BWG and poorer FCR compared with unchallenged controls (2.135 kg and 1.81 vs. 2.426 kg and 1.64, respectively). Narasin/nicarbazin improved BWG and FCR relative to challenged control (2,318 kg; 1.72), while the PB achieved comparable improvements (2.299 kg; 1.71). QE provided intermediate performance responses under challenge (2.210 kg; 1.75). Total oocyst shedding was was markedly higher in challenged control (17,696 OPG) than unchallenged birds (227 OPG), whereas all treated challenged groups reduced total OPG (7,557–12,200). Average lesion scores were highest in challenged controls (2.65) and decreased with narasin/nicarbazin (1.99), QE (2.10) and the PB (1.62).

Implications/Conclusions

In a mixed *Eimeria* spp. challenge, NN and the evaluated phytogenic feed additives (QE and PB) reduced lesion severity and oocyst shedding while improving broiler performance compared to the challenged, untreated birds. The tested PB, showed the strongest lesion-mitigation response and competitive performance, supporting its potential as a nutritional tool for coccidiosis control programs.

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