



---

**XI Avian Immunology Research Group Meeting**

# **Scientific program & Proceedings**

---

**Budapest, Hungary, 7-10 October 2010**

## OUR SPONSORS



Contact: [diana-kruse@uiowa.edu](mailto:diana-kruse@uiowa.edu)



Contact: [zsbenyeda@prophyl.hu](mailto:zsbenyeda@prophyl.hu)



Contact: [peter.erdei@sp.intervet.com](mailto:peter.erdei@sp.intervet.com)



Contact: [vilmos.palya@ceva.com](mailto:vilmos.palya@ceva.com)



Contact: [plszha@freemail.hu](mailto:plszha@freemail.hu)



Contact:  
[nemeskeri.agnes@med.semmelweis-univ.hu](mailto:nemeskeri.agnes@med.semmelweis-univ.hu)

## PUBLISHED BY:

Diamond Congress Ltd., the secretariat of the Conference  
H-1012 Budapest, Vérmező út 8.  
Editor and responsible publisher: Robert Hohol  
[www.diamond-congress.hu](http://www.diamond-congress.hu)

ISBN 978-963-88019-2-0



## WELCOME

Time is flying, thus it is the time to think and speak about our beloved animal at the XI. Avian Immunology Research Group (AIRG) Meeting and the place, Budapest, Hungary.

From time to time we have to stop the lab and field works, and summarize what we did in the past year or two and to travel to another part of the world to share our knowledge with other people, to meet with old friends or making new ones. The world rapidly changes, new challenges emerge, lots of information come through the net, so the personal contact now is more important than ever before. More than fifty years ago the discovery of the function of the bursa of Fabricius initiated the explosive development of classical immunology and few decades later the chicken became a fundamental living model for the vertebrate developmental biology. We do believe, that the future AIRG meetings in addition to the traditional immunology, immunopathology, and immunosuppression should address novel research areas related with immunology - like developmental immunology. I encourage everyone, who "tasted" the amazing process of development of chicken, to send an abstract.

All of us are local patriots. In the opening address we used to praise the place of meeting to be attractive for the expecting participants. I do not praise the pearl of the Danube, Budapest. Please, come see the city of Budapest with your own eyes. We will try to do our best that you feel yourself both professionally and socially well.

Welcome to XI. AIRG 2010

Welcome to Budapest

Imre Oláh  
president of the XI. AIRG Meeting

Nándor Nagy  
secretary of the XI. AIRG Meeting



**Budapest, Hungary, 7-10 October 2010**

---



## GENERAL INFORMATION

### Venue

#### **Danubius Thermal and Conference Hotel Helia**

*H-1133 Budapest, Kárpát u. 62-64., Hungary*

The four-star Danubius Thermal and Conference Hotel Helia spa, wellness and conference hotel, built in Scandinavian style, was opened in 1990 and partially renovated in 2003. Natural thermal water is pumped to the hotel spa from springs on the nearby Margaret Island, which is famous in Budapest for its high quality medical and relaxation services.

### Registration fees

#### **Registration fees include:**

Participation in scientific sessions, Book of abstracts, congress bag, lunches on October 8, 9, 10, coffee breaks, welcome reception on October 7, city tour in Budapest by bus on October 8, farewell party on a river boat on October 9.

#### **Registration fees for accompanying persons include:**

Welcome reception on October 7, city tour in Budapest by bus on October 8, farewell party on a river boat on October 9, lunches are not included, daily lunch ticket for accompanying persons is available for 20 EUR.

### Optional programs

#### **19.00-20.30, 7 October 2010, Thursday – Welcome reception**

**Location:** Danubius Thermal and Conference Hotel Helia - Conference venue, Restaurant  
H-1133 Budapest, Kárpát u. 62-64., Hungary

#### **13.30-16.00, 8 October 2010, Friday – City Tour by bus; meeting at the main entrance of the Hotel Helia at 13.15.**

#### **19.00-22.00, 9 October 2010, Saturday – Farewell Party on the Danube; meeting at the lobby of the Hotel Helia at 18.30**

### Foreign exchange, banking facilities

The Hungarian currency is the Hungarian Forint (HUF). Currency exchange booths are available at the airport terminals, railway stations, travel agencies, banks and various places in the city. Traveller's cheques and convertible currency may be exchanged at these facilities. Major credit cards are usually accepted in most hotels, restaurants and certain shops in the city. Obtaining cash against ATM or credit cards is very easy from the ATM cash machines that can be found at almost each bank office, hotel or on the street.

### Climate and weather

The climate of Budapest is continental. At the beginning of October a cool, usually an autumn weather is expected with rains, the maximum temperature is about 10-15°C.



Budapest, Hungary, 7-10 October 2010

---

### **Clothing**

---

Informal for all occasions.

### **Shopping**

---

Shops in Budapest usually open at 10:00 and close at 18:00 (Monday-Friday), on Saturday they open at 9:00 and close at 13:00. A lot of hypermarkets open at 9:00 and close at 20:00.

**THE NUMBERS AFTER O (ORAL) OR P (POSTER) INDICATE  
THE PAGE NUMBER OF THE ABSTRACT.**



## SCIENTIFIC PROGRAM – ORAL PRESENTATIONS

### Session 1- Innate immunity

8 October, Friday – 8:30 - 10:00

Chairs: Jansen, Ch. –Parmentier, H.

- 8:30 O-39: **Enhanced NK-cell function after infection with avian influenza virus: studies in a natural host**  
*Christine Jansen, Peter van de Haar, Daphne van Haarlem, Willem van Eden, Thomas Göbel, Lonneke Vervelde*
- 9:00 O-82: **Across-line SNP association study for (innate) immune traits in laying hens**  
*Jan van der Poel, Filippo Biscarini, Johan van Arendonk, Henk Parmentier, Annemieke Jungerius, Henk Bovenhuis*
- 9:15 O-66: **Innate immune responses to infection with H5N1 highly pathogenic avian influenza virus in different duck species**  
*Mary Pantin-Jackwood, Caran Cagle, Jamie Wasilenko*
- 9:30 O-67: **Post translational polymorphism of Natural Antibodies: rapid protection to infection?**  
*Henk Parmentier, M.G.B. Nieuwland, T. Bergstra, A. Lammers*
- 9:45 O-13: **Resistance of B2 lines to avian coronavirus compared with B19 lines correlates with monocyte function**  
*Ellen Collisson, Ghida R. Banat, Lisa Griggs, Maisie Dawes, Suzana Tkalcic, Miguel Saggese, Worthie Briles, H.L. Shivaprasad, Mark W. Jackwood, Yvonne Drelchsler*

### Session 2- APC, B cells

8 October, Friday – 10:30 - 12:15

Chairs: Göbel, Th. – Heller, D.

- 10:30 O-29: **The chicken Ig-like receptor family: complexity, structure and function**  
*Thomas Göbel*
- 11:00 O-34: **Identification of regulators in early chicken B cell development by combined differential transcriptome and proteome analysis of the Bursa of Fabricius**  
*Sonja Haertle, Julia Korte, Stefan Schmieder, Georg J. Arnold, Bernd Kaspers*
- 11:15 O-35: **Characterization of the germinal centre reaction in chicken**  
*Susanne Hainke, Bernd Kaspers, Sonja Haertle*
- 11:30 O-33: **Effects of CD40L on chicken B-cell development and differentiation**  
*Sonja Haertle, John Young, Bernd Kaspers, Magdalena Schumacher, Katharina Schenk-Weibhauser, John Young, Bernd Kaspers*
- 11:45 O-90: **Further characterisation of chicken bone marrow-derived dendritic cells**  
*Zhiguang Wu, Lisa Rothwell, Colin Butter, Pete Kaiser*
- 12:00 O-19: **Intratracheally administered antigens are taken up by chicken respiratory tract phagocytes that acquire a mature DC-like phenotype**  
*Eveline De Geus, J.M. Rebel, L. Vervelde*



Budapest, Hungary, 7-10 October 2010

---

### Session 3- Interferons and receptors

9 October, Saturday – 8:30 - 10:00

Chairs: Dalgaard, T. – Kaiser, P.

---

- 8:30 O-54: **Chicken interferons**  
*John.W.Lowenthal, Adam Karpala, Kate Goossens, Audrey Baule, Kristie Jenkins, Mary Broadway, Andrew Bean*
- 9:00 O-22: **Characterization of novel type I chicken interferons**  
*Ibrahim Eldaghayes, Jean-Remy Sadeyen, Lisa Rothwell, Zhiguang Wu, Zhengxing Lian, Changlin Chen, Peter Russell, Pete Kaiser*
- 9:15 O-31: **The chicken IFN-lambda receptor complex**  
*Kate Goossens, Alister C. Ward, Andrew G. D. Bean*
- 9:30 O-78: **Lack of antiviral activity of IFN and the IFN effector protein Mx during HPAIV infection in the chicken**  
*Benjamin Schusser, Carsten Krohmann, Nicola Penski, Thomas Vahlenkamp, Peter Staeheli, Bernd Kaspers, Sonja Haertle*
- 9:45 O-56: **The antiviral role of chicken type I interferons (INF) against in vitro fowlpox virus (FPV) infection**  
*Heba Mahgoub, Mark Fife, Colin Butter, Mick Bailey, Pete Kaiser*

### Session 4- Influenza virus and cytokine

9 October, Saturday – 10:30 - 12:00

Chairs: Lowenthal, J. – Vervelde, L.

---

- 10:30 O-64: **IFN- $\gamma$  does not appear to contribute to the immunity conferred by DNA vaccination against avian influenza**  
*Dele Ogunremi, Hilary Kelly, Qigao Fu*
- 10:45 O-04: **Reduced levels of cytokine expression and mortality in ducks compared to chickens during H5N1 avian influenza infection**  
*Andrew Bean, Simon Burggraaf, Adam J. Karpala, Sue Lowther, John W. Lowenthal, John Bingham*
- 11:00 O-12: **Avian influenza virus hemagglutinin and nucleocapsid proteins induce chicken effector, memory and effector memory CD8<sup>+</sup> T lymphocytes**  
*Ellen Collisson, Haroldo Toro, De-Chu Tang, Worthie E. Briles, Linda Yates, Renee Kopulos, Shaibala Singh*
- 11:15 O-45: **Intranasal application of alpha interferon reduces morbidity associated with low pathogenic avian influenza infection**  
*Darrell R. Kapczynski, Hai Jun Jiang*
- 11:30 O-38: **Enhanced activation of chicken dendritic cells upon infection with high pathogenic avian influenza virus**  
*Christine Jansen, Lonneke Vervelde, Sylvia Reemers, Jacob Post, Annemarie Rebel*
- 11:45 O-81: **Chicken dendritic cells are infected and activated by avian influenza virus**  
*Karen Staines, R. Ruiz-Hernandez, Colin Butter*





### Session 5- Influenza-vaccination

9 October, Saturday – 13:30 - 15:00

Chairs: Ogunremi, D. – Juul-Madsen, H.

- 13:30 O-63: **Vaccination with a DNA construct encoding hemagglutinin gene resulted in protection against the avian influenza virus in chickens**  
*Dele Ogunremi, John Pasick, Yohannes Berhane, Gary Kobinger, Drew Hannaman, Alfonso Clavijo*
- 13:45 O-40: **CD8+ T-cell responses against avian influenza virus: the identification of influenza specific CD8+ T-cell epitopes in chickens**  
*Christine Jansen, Sylvia Reemers, Alice Sijts, Lonneke Vervelde*
- 14:00 O-79: **Development of a virosome-based vaccine against avian influenza virus**  
*Shayan Sharif, Amirul Mallick, Payvand Parvizi, Leah Read, Eva Nagy, Shahriar Behboudi*
- 14:15 O-52: **Vaccinal potency of a recombinant NDV-H5 vaccine against an Asian Highly Pathogenic clade 1 H5N1 virus in day-old-chickens with or without maternal-derived-antibodies**  
*Amélyne Lardinois, Mieke Steensels, Bénédicte Lambrecht, Dierk Rebeski, Thierry van den Berg*
- 14:30 O-14: **Evaluation of intracellular cytokine staining by flow cytometry for assessment of vaccine outcomes in chickens**  
*Tina S. Dalgaard, L. R. Norup, E. Watrang, H. R. Juul-Madsen*
- 14:45 O-74: **Differences in host response and virus replication in chickens infected with low pathogenic or high pathogenic avian influenza viruses**  
*Johanna Rebel, J. Post, J. B. Cornelissen, D. Burt, B. Peeters*

### Session 6- Host response to infections

9 October, Saturday – 15:30 - 17:00

Chairs: Schat, K. – Rautenschlein, S.

- 15:30 O-76: **Humoral immune responses to Mycoplasma gallisepticum infection in house finches (Carpodacus mexicanus)**  
*Karel Schat, Jessica Grodio, Dana M. Hawley, Keila V. Dhondt, Erik E. Osnas*
- 15:45 O-77: **Salmonella has impact on spatial-temporal immunological processes during chicken jejunal development**  
*J.M.J. Rebel, Dirkjan Schokker, M.A. Smits*
- 16:00 O-37: **Salmonella enterica serovar enteridis (SE) infection in 5 commercial white egg and 3 commercial brown egg strains of laying hens: comparison of SE colonization with immune responsiveness**  
*Peter Holt, Lara E. Vaughn, Richard K. Gast, Kenneth E. Anderson*
- 16:15 O-71: **Gut-associated immunological responses after nematode infection of chickens**  
*Silke Rautenschlein, A. Scharz, H. Abel, M. Gauly, G. Breves*
- 16:30 O-27: **Immune responses in chicks following vaccination with live infectious bronchitis vaccines**  
*Kannan Ganapathy, Lisa Rothwell, Pete Kaiser, Stephane Lemiere, Richard Jones*
- 16:45 O-16: **The biological effects of the avian macrophage migration inhibitory factor (MIF) on immune cell function**  
*Rami Dalloul, Sungwon Kim, Kate B. Miska, Mark C. Jenkins, Ray H. Fetterer, Chasity M. Cox, Lindsay H. Summers*



Budapest, Hungary, 7-10 October 2010

## Session 7- Immunogenetics

10 October, Sunday – 8:30 - 10:00

Chairs: Magor, K. – Ragland, W.

- 8:30 O-83: **Upregulation of immune genes in duck lung and intestine during high and low pathogenic avian influenza infection**  
*Hillary Vandervon, Robert Webster, Kathy Magor*
- 8:45 O-88: **B cell chemoattractants and the Marek's disease viral chemokine**  
*Victoria Waters, Pete Kaiser*
- 9:00 O-28: **A genetic and functional analysis of the chicken interleukin-1 gene family**  
*Mark Gibson, Pete Kaiser, Mark Fife, Steve Bird, Pat Woo*
- 9:15 O-03: **Association of RIG-I with innate immunity of ducks to influenza**  
*Megan Barber, J.R. Aldridge, R.G. Webster, Kathy Magor*
- 9:30 O-55: **Allelic diversity of MHC class I in wild Mallard ducks**  
*Kathy Magor, Shawna Jensen, Christine Mesa, Julie Parks-Dely, Deb Moon*
- 9:45 O-30: **From genes to markers: characterization of putative chicken NK cell markers**  
*Marie-Luise Neulen, Birgit C. Viertlboeck, Thomas W. Göbel*

## Session 8- GALT and immunomodulation

10 October, Sunday – 10:30 - 12:00

Chairs: Kaspers, B. – Erf, G.

- 10:30 O-23: **Immunopathology in autoimmune vitiligo in Smyth line chickens: a central role of IL-21?**  
*Gisela Erf, Fengying Shi*
- 10:45 O-32: **Automated differential blood count in the chicken**  
*Bernd Kaspers, Sonja Haertle, Christian Seeliger*
- 11:00 O-46: **The use of high throughput sequencing for avian transcriptome analysis**  
*Calvin Keeler, Cynthia Boettger, Michele N. Maughan, John K. Rosenberger, Carl Schmidt*
- 11:15 O-95: **Multi-platform next generation sequencing of the domestic turkey (*Meleagris gallopavo*): Genome assembly and analysis**  
*Rami A. Dalloul, Julie A. Long, Aleksey V. Zimin, Luqman Aslam, Kathryn Beal, Le Ann Blomberg, David W. Burt, Oswald Crasta, Richard P. M. A. Crooijmans, Kristal Cooper, Roger A. Coulombe, Supriyo De, Mary E. Delany, Jerry B. Dodgson, Jennifer J. Dong, Clive Evans, Paul Flicek, Liliana Florea, Otto Folkerts, Martien A. M. Groenen, Tim T. Harkins, Javier Herrero, Steve Hoffmann, Hendrik-Jan Megens, Andrew Jiang, Pieter de Jong, Pete Kaiser, Hee-bal Kim, Kyu-Won Kim, Sungwon Kim, David Langenberger, Mi-Kyung Lee, Taeheon Lee, Shrinivasrao Mane, Guillaume Marcais, Manja Marz, Audrey P. McElroy, Thero Modise, Mikhail Nefedov, Cédric Notredame, Ian R. Paton, William S. Payne, Geo Pertea, Dennis Prickett, Daniela Puiu, Dan Qioa, Emanuele Raineri, Steven L. Salzberg, Michael C. Schatz, Chantel Scheuring, Carl J. Schmidt, Steven Schroeder, Edward J. Smith, Jacqueline Smith, Tad S. Sonstegard, Peter F. Stadler, Hakim Tafer, Zhijian (Jake) Tu, Curtis P. Van Tassell, Albert J. Vilella, Kelly Williams, James A. Yorke, Liqing Zhang, Hong-Bin Zhang, Xiaojun Zhang, Yang Zhang, Kent M. Reed, Jim Biedler*
- 11:30 O-21: **Synovial fluid of *Mycoplasma synoviae* infected chickens contains proinflammatory cytokines and autoantibodies**  
*Daliborka Dusanic, Sasa Kastelic, Dusan Bencina, Mojca Narat*
- 11:45 **Closing remark**



## SCIENTIFIC PROGRAM – POSTERS

- P-01:** **Evolution of Toll-like receptor 4 in passerine birds**  
*Hana Bainová, Marta Promerová, Anna Bryjová, Josef Bryja, Tomas Albrecht, Michal Vinkler*
- P-02:** **Differential susceptibility of inbred chicken lines to influenza-A infection in vitro and in vivo: is it still Mx?**  
*Devanand Balkissoon, Karen Staines, Raul Ruiz-Hernandez, John Young, Adrian Smith, Jim Kaufman, Colin Butter*
- P-05:** **Increased inducible nitric oxide synthase expression in organs is associated with a higher severity of H5N1 influenza virus infection**  
*Simon Burggraaf, John Bingham, Jean Payne, Wayne G. Kimpton, John W. Lowenthal, Andrew G. D. Bean*
- P-06:** **Common immunocytochemical feature of the FDC and type II pneumocyte**  
*Zsófia Benyeda, Katalin Kocsis, Nándor Nagy, Ildikó Bódi, Vilmos Palya, Imre Oláh*
- P-07:** **Origin of the splenic reticular cells influences the effect of the infectious bursa disease virus (IBDV) on the extracellular matrix**  
*Éva Bíró, Nándor Nagy, Katalin Kocsis, Susanne Kabell, Vilmos Palya, Imre Oláh*
- P-08:** **Follicular dendritic cell marker; 74.3 mAb identifies cells clusters in the thymic medulla**  
*Ildikó Bódi, Nándor Nagy, Imre Oláh*
- P-09:** **Development of novel T cell vaccines against avian influenza in domestic chickens**  
*Connor Carson, Raul Ruiz-Hernandez, Devanand Balkissoon, Karen Staines, Sarah Gilbert, Colin Butter - WITHDRAWN*
- P-10:** **Changes in chicken innate immunity induced by in vivo administration of baculovirus**  
*Silvina Chimeno Zoth, Juan Manuel Carballeda, María José Gravisaco, Evangelina Gómez, Elisa Carrillo, Analía Berinstein*
- P-11:** **Studying avian immune response to an intermediate strain of IBDV**  
*Juan Manuel Carballeda, Silvina Chimeno Zoth, Evangelina Gómez, María José Gravisaco, Analía Berinstein*
- P-15:** **Five-color flow cytometry used for immunophenotyping of peripheral chicken T cells in an attempt to define correlates of vaccine-induced protection**  
*Tina S. Dalgaard, L. R. Norup, A. R. Pedersen, K. J. Handberg, P. H. Jørgensen, H. R. Juul-Madsen*
- P-17:** **Dietary beta-glucan effects on the T helper cytokine balance in the intestinal tract of broiler chicks**  
*Chasity M. Cox, Lindsay H. Sumners, Sungwon Kim, Audrey P. McElroy, Mike R. Bedford, Rami A. Dalloul*
- P-18:** **In vitro uptake of antigen by chicken BM-DC and effect on phenotype**  
*Eveline D. de Geus, B. Tefsen, I. van Die, L. Vervelde*
- P-20:** **CD45 hemopoietic cells in the early chicken embryo**  
*Dávid Dóra, Dávid Molnár, Imre Oláh, Nándor Nagy*
- P-24:** **Avian dendritic cell express gamma-actin**  
*Balázs Felföldi, Nándor Nagy, G. T. Pharr, Imre Oláh*
- P-25:** **Cloning of the chicken integrin alpha4 gene**  
*Dorte R. Fink, G. Jungersen*
- P-26:** **Expression of duck chemokine receptor CCR7 and ligands in lymphoid tissues and influenza infection**

- P-36:** *Ximena Fleming, Craig Brusnyk, Katie Ross, Jianguo Xia, Deb Moon, Katharine Magor*  
**Thoracic aorta region colonizes lympho-myeloid organs**  
*Krisztina Minkó, Arianna Caprioli, Karine Bollérot, Francoise Dieterlen-Lievre, Thierry Jaffredo, Nándor Nagy*
- P-41:** **Genetic diversity of chicken interleukin-17 cDNA from Chinese native and western breeds**  
*Wen Zhao, Zhiming Pan, Shizhong Geng, Qiang Fang, Xinan Jiao*
- P-42:** **Development and application of an MAb-based sandwich ELISA in the assessment of chicken interferon- $\gamma$  response to the lasota vaccine of Newcastle disease virus**  
*Hua Dai, Junhua Chen, Zhiming Pan, Xinan Jiao*
- P-43:** **Mannose-binding lectin affects phenotype and functions of HD11 chicken macrophages**  
*Hong Shen, Liselotte R. Norup, Tina S. Dalgaard, Helle R. Juul-Madsen*
- P-44:** **The effect of *Ascaridia galli* oral and intra-muscular immunisation on chicken immune response, as well as on parasite egg excretion and worm load following infection**  
*Janne Pleidrup, Liselotte R. Norup, Tina S. Dalgaard, Rikke M. Kjærup, Helle R. Juul-Madsen*
- P-47:** **Structural gene variants of chicken Mannose-binding lectin and association with serum concentration**  
*Rikke M. Kjærup, Tina S. Dalgaard, Liselotte R. Norup, Janne Pleidrup, Helle R. Juul-Madsen*
- P-48:** **Preventive administration of *Enterococcus faecium* 55 to *Salmonella* Enteritidis infected chicks and immune response**  
*Martina Kolesárová, V. Revajová, Z. Ševčíková, A. Lauková, V. Strompfová, V. Spišáková, M. Leokut*
- P-49:** **Immunomodulatory effect of mycotoxins studied by flow cytometry in chickens**  
*Z. Slaminková, L. Grešáková, Martina Kolesárová, V. Revajová, M. Leokut*
- P-50:** **Dust and its components can modulate broiler's immune system**  
*Huong Thi Lan Lai, André J. A. Aarnink, Henk K. Parmentier*
- P-51:** **Sustained effects of early-life oral colistin treatment on immune reactivity to intratracheally administered LPS and HuSA in chicken**  
*Aart Lammers, Linda J. W. van Zutphen, Ger de Vries Reilingh, Henk K Parmentier*
- P-53:** **Immunogenicity of transgenic *Eimeria* and *Toxoplasma* vaccine vectors in the chicken mode**  
*Xianyong Liu, Xiaoxi Huang, Jun Zou, Guangwen Yin, Huali Su, Jianan Li, Xun Suo*
- P-57:** **Microglial cell population expresses B cell specific antigen**  
*Dávid Molnár, Dávid Dóra, Imre Oláh, Nándor Nagy*
- P-58:** **A novel cell type of hemopoietic origin in the avian enteric nervous system**  
*Nándor Nagy, G. Csikós, Dávid Molnár, A. M. Goldstein, Imre Oláh*
- P-59:** ***Mycoplasma synoviae* induced modifications in respiratory activity of chicken chondrocytes analyzed by phenotype microarrays**  
*Mojca Narat, Daliborka Dusanic, Dusan Benina, Irena Oven*
- P-60:** **Assessment of local adaptive immunity after infection with *Ascaridia galli* in chickens**  
*Liselotte R. Norup, Tina S. Dalgaard, Janne Pleidrup, Rikke M. Kjærup, Gregers Jungersen, Helle R. Juul-Madsen*
- P-61:** **Immunoglobulin levels in chicken serum and bile during an *Ascaridia galli* infection**  
*Liselotte R. Norup, Tina S. Dalgaard, Janne Pleidrup, Rikke M. Kjærup, Torben W. Schou, Anders Permin, Helle R. Juul-Madsen*



- P-62: Electroporation-mediated delivery of a DNA vaccine against H5N1 avian influenza led to an enhanced memory antibody response**  
*Dele Ogunremi, Drew Hannaman, Sylvia van Drunen Little-van den Hurk, Gary P. Kobinger, John Pasick, Yohannes Berhane, Alfonso Clavijo*
- P-65: In vitro assessment of potency of a DNA vaccine by measuring gene expression**  
*Dele Ogunremi, Hyun Lillehoj, Hilary Kelly, Qigao Fu*
- P-68: Avian beta-defensin 2 is weakly active against enteric bacterial pathogens but is chemotactic towards leukocytes.**  
*Anne-Christine Lalmanach, Hélène Marty, Pascale Quéré*
- P-69: Antibody response to H5N9 inactivated vaccine and MHC diversity in the chicken**  
*Pascale Quéré, Pierre Sibille, Magali Bergès, Nicolas Bruneau, Tran Xuan Toàn, Jean-Charles Maillard, Bertrand Bed'Hom*
- P-70: Macrophage activation-induced thymosin beta 4 production: a tissue repair mechanism**  
*Narayan C. Rath, Lakshmi Kannan, Rohana Liyanage, Jackson O. Lay*
- P-72: Assessment of the influenza specific cell-mediated immune response in chickens by detection of chicken interferon-gamma after ex vivo antigenic stimulation on lymphocytes**  
*Fabienne Rauw, Sofia Anbari, Thierry van den Berg, Bénédicte Lambrecht*
- P-73: Immune reaction in chickens and ducks in response to low pathogenic avian influenza**  
*J. B. W. J. Cornelissen, J. Post, B. Peeters, H. Fijten, L. Vervelde, Johanna M. J. Rebel*
- P-75: Effect of microbial-nutrition interaction on chicken immune system after the early administration of probiotic with organic acids in young chicks**  
*J. C. Rodríguez-Lecompte, J. Brady, G. Camelo-Jaimes, S. Sharif, G. Crow, G. Ramirez-Yanez, W. Guenter, J. House*
- P-80: Characterization of immune response to a T cell epitope of the haemagglutinin (HA) antigen of avian influenza virus H5 subtype in chickens**  
*Hamid R. Haghighi, Leah R. Read, S.M. Mansour Haeryfar, Shahriar Behboudi, Shayan Sharif*
- P-84: Adaptive immunity in conjunctiva associated lymphoid tissue after ocular immunization**  
*Frederik W. van Ginkel, Stephen L. Gulley, Aart Lammers, Haroldo Toro*
- P-85: Identification of core elements involved in antibacterial and immunomodulatory activities of chicken cathelicidin 2**  
*Albert van Dijk, E. Margo Molhoek, Floris Bikker, Edwin J. A. Veldhuizen, Henk P. Haagsman*
- P-86: Involvement of avian collectins in host defence**  
*Edwin J. A. Veldhuizen, Astrid Hogenkamp, Lonneke Vervelde, Sylvia S. Reemers, Martin van Eijk, Henk P. Haagsman*
- P-87: Toll-Like receptor repertoire in grey partridge**  
*M. Vinkler, O. Tomasek, H. Bainova, P. Opatova, T. Albrecht, A. Bryjova, J. Bryja*
- P-89: Local perforin mRNA expression at experimental Eimeria tenella infection of naive and immune chicken**  
*Sofia E. Magnusson, Per Thebo, Adrian L. Smith, Anna Lundén, Eva Wattring*
- P-91: Phylogeny and genetic variability of red junglefowls and native chickens assessed by means of blood group system in Southeast Asia**  
*Yoshio Yamamoto, Takao Namikawa*
- P-92: Experimental evidence for the ectodermal origin of the epithelial anlage of the bursa of Fabricius**  
*Nándor Nagy, Imre Oláh*



**Budapest, Hungary, 7-10 October 2010**

---

- P-93:**            **Elucidating specific responses against influenza in vaccinated and/or infected chickens using elispot assays**  
*Raul Ruiz-Hernandez, Connor Carson, Dave Balkissoon, Karen Staines, Debra Clayton, Sarah Gilbert, Colin Butter*
- P-94:**            **Pathogen-specific responses are generated in the avian lymphoid lung**  
*Colin Butter, Laura Fyfe, Karen Staines*





---

# Proceedings





**P-01****EVOLUTION OF TOLL-LIKE RECEPTOR 4 IN PASSERINE BIRDS**

Hana Bainová<sup>1</sup>, Marta Promerová<sup>2</sup>, Anna Bryjová<sup>2</sup>, Josef Bryja<sup>2</sup>, Tomas Albrecht<sup>1,2</sup>, Michal Vinkler<sup>1,2</sup>

<sup>1</sup> Faculty of Science, Charles University in Prague, Viničná 7, Prague, 128 44, Czech Republic,  
e-mail: hanulay@gmail.com

<sup>2</sup> Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8,  
603 65 Brno, Czech Republic

Toll-like receptors (TLRs) are the first receptors of the vertebrate immune system to sense the pathogen incursion into the host organism. It has been well established that their optimal function is of a crucial importance for successful defence against various infection agents. However, as different hosts may harbour distinct types of parasites, comparative research in TLRs may shed some light on the molecular basis of the evolution of host-parasites interactions. Despite increasing number of fish and mammalian species with completely described sets of TLRs the structural variability of these genes in birds is only very imperfectly explored. This is especially true for passerine birds (taxon comprising more than half of the total avian species richness) where only Zebra finch TLRs are known. In this contribution we therefore describe TLR4 variability among selected passerine species (Barn swallow *Hirundo rustica*, Bluethroat *Luscinia svecica*, Great tit *Parus major*, Great reed warbler *Acrocephalus arundinaceus* and Scarlet rosfinch *Carpodacus erythrinus*) representing several distinct passerine evolutionary lineages. Moreover, in the Great tit we also provide preliminary results of the intrapopulation polymorphism analysis of the TLR4 gene.

**Keywords:** alleles variability, avian immunology, evolution, Pattern recognition receptors, PRRs, Toll-like receptor 4, TLR4, passerine, polymorphism

## P-02

### DIFFERENTIAL SUSCEPTIBILITY OF INBRED CHICKEN LINES TO INFLUENZA-A INFECTION *IN VITRO* AND *IN VIVO*: IS IT STILL MX?

Devanand Balkissoon<sup>1,4</sup>, Karen Staines<sup>1</sup>, Raul Ruiz-Hernandez<sup>1</sup>, John Young<sup>1</sup>, Adrian Smith<sup>2</sup>, Jim Kaufman<sup>3</sup>, Colin Butter<sup>1</sup>

<sup>1</sup> *The Institute for Animal Health, Compton, Berkshire, RG20 7NN, United Kingdom*

<sup>2</sup> *Department of Zoology, The Tinbergen Building, South Parks Road, Oxford, OX1 3PS, United Kingdom*

<sup>3</sup> *Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom*

<sup>4</sup> *The Cambridge Infectious Diseases Consortium, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, United Kingdom*

Inbred chicken lines have been associated with differing levels of resistance to bacteria (e.g. *Salmonella*), viruses (e.g. Marek's disease) and parasites (e.g. *Eimeria*). Permissibility of the replication of low pathogenicity avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) of chicken embryo fibroblasts (CEFs) was determined, alongside host cytokine responses. Significant differences in were observed between cells of two chicken lines (61 & C b12) possessing different MHC and Mx haplotypes, when infected with LPAI H5N3. An F1 population from these lines and a subsequent back-cross population were produced and tested, demonstrating an intermediate phenotypic response in the F1 and a tendency towards one of the parental phenotypes (C b12) used to generate the back-cross. siRNA-mediated knockdown of the Mx gene yielded a small increase in influenza matrix gene mRNA levels; whilst pIRES2-EGFP plasmid-mediated over-expression of line C Mx showed an increased susceptibility to influenza virus infection. The work indicates that Mx has a function in the response to influenza; though it is not as key as in the human and mouse, and that other factors may contribute to differences observed between the inbred lines.

**O-03****ASSOCIATION OF RIG-I WITH INNATE IMMUNITY OF DUCKS TO INFLUENZA**

Megan R.W. Barber<sup>1</sup>, Jerry R. Aldridge Jr.<sup>2</sup>, Robert G. Webster<sup>2</sup>, Katharine E. Magor<sup>1</sup>

<sup>1</sup> *Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada*

<sup>2</sup> *Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, United States*

Ducks and wild waterfowl perpetuate all strains of influenza viruses in nature. In their natural host, influenza viruses typically cause asymptomatic infection and little pathology. Ducks are often resistant to influenza viruses capable of killing chickens. Here, we show that the influenza virus sensor, RIG-I, is present in ducks and plays a role in clearing an influenza infection. We show evidence suggesting that RIG-I may be absent in chickens, providing a plausible explanation for their increased susceptibility to influenza viruses compared with ducks. RIG-I detects RNA ligands derived from uncapped viral transcripts and initiates the IFN response. In this study, we show that the chicken embryonic fibroblast cell line, DF-1, cannot respond to a RIG-I ligand. However, transfection of duck RIG-I into DF-1 cells rescues the detection of ligand and induces IFN- $\beta$  promoter activity. DF-1 cells expressing duck RIG-I have decreased low and highly pathogenic avian influenza virus replication. Additionally, RIG-I transfected DF-1 cells have an augmented innate immune response to influenza, as revealed by a 44K Agilent microarray. Differences in the gene expression profile were noted, depending on the influenza strain. Implicating RIG-I in the antiviral response to an infection in vivo, we found that RIG-I expression is induced 200 fold, early in an innate immune response in ducks challenged with the H5N1 virus A/Vietnam/1203/04. Finding this natural disease resistance gene in ducks opens the possibility of increasing influenza resistance through creation of a transgenic chicken.

**O-04****REDUCED LEVELS OF CYTOKINE EXPRESSION AND MORTALITY IN DUCKS COMPARED TO CHICKENS DURING H5N1 AVIAN INFLUENZA INFECTION**

Simon Burggraaf<sup>1,2</sup>, Adam J. Karpala<sup>1</sup>, Sue Lowther<sup>1</sup>, John W. Lowenthal<sup>1</sup>, John Bingham<sup>1</sup>, Andrew G. D. Bean<sup>1</sup>

<sup>1</sup> CSIRO AAHL, Private Bag 24, Geelong, Victoria 3220, Australia

<sup>2</sup> School of Veterinary Science, The University of Melbourne, Victoria, Australia  
E-mail: andrew.bean@csiro.au

---

There are dramatic differences in the severity of disease between chickens and ducks following H5N1 influenza infection. Chickens show a high level of mortality and associated pathology, whilst ducks show relatively minor symptoms. It is not clear how this varying pathogenicity comes about, although it has been suggested that an overactive inflammatory immune responses to infection in the chicken, compared to the duck response, may be to blame for the disparity in observed pathology. Nevertheless, the mechanisms of disease severity caused by H5N1 influenza virus infection remain somewhat unclear. Studies have indicated that a high viral load and an associated hyper inflammatory immune response are influential during the onset of infection. Therefore, we investigated the expression of several key proinflammatory cytokines during the infection of chickens and ducks with H5N1 influenza. Two H5N1 strains, A/Muscovy duck/Vietnam/453/2004 (Vt453) and A/Duck/Indramayu/BBVW/109/2006 (Ind109) were compared. Intriguingly, in the chicken these viruses caused severe infection, with a high viral load and increased production of proinflammatory molecules, such as Interleukin 6 (IL6) and the acute phase protein Serum Amyloid A (SAA). Contrastingly, ducks displayed only small changes in these cytokines and at later time-points. These observations support that hypercytokinemia may contribute to disease in chickens, whilst the lower cytokine response may explain the decrease in mortality observed in ducks.

**Keywords:** H5N1 Avian Influenza, Cytokine Storm, Interleukin, Interferon, Inflammation

**P-05****INCREASED INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION IN ORGANS IS ASSOCIATED WITH A HIGHER SEVERITY OF H5N1 INFLUENZA VIRUS INFECTION**

Simon Burggraaf<sup>1,2</sup>, John Bingham<sup>1</sup>, Jean Payne<sup>1</sup>, Wayne G. Kimpton<sup>2</sup>,  
John W. Lowenthal<sup>1</sup>, Andrew G. D. Bean<sup>1</sup>

<sup>1</sup> CSIRO AAHL, Private Bag 24, Geelong, Victoria 3220, Australia

<sup>2</sup> School of Veterinary Science, The University of Melbourne, Victoria, Australia  
E-mail: andrew.bean@csiro.au

Nitric oxide (NO) is an anti-viral effector of the innate immune system that acts to inhibit the replication of a variety of viruses. Moreover, enzymes of the nitric oxide synthase (NOS) family, such as the inducible form of NOS (iNOS), generate NO to combat infection in combination with acute phase proteins and cytokines. However, excessive production of iNOS and subsequent high levels of NO infection may have negative effects, acting with other damaging oxidants to promote excessive inflammation or induce apoptosis. Therefore, a controlled and balanced iNOS-mediated response is required for beneficial outcomes to viral infection. In this study we identify and investigate iNOS gene expression in ducks and chickens during H5N1 influenza infection. At 24 h post H5N1 influenza infection chickens show a 6-fold increase in serum NO levels, while ducks show very little increase. Nevertheless, by 72 h post infection, duck serum NO levels appear to be a little over 2-fold higher. However, analysis of iNOS expression in infected chickens showed a marked increase in iNOS expression in a wide range of organs, including the lung, spleen, caecal tonsil and liver. Contrastingly, infected duck tissues appear to have lower levels of tissue related iNOS expression. The differences in iNOS expression levels observed between chickens and ducks during H5N1 avian influenza infection may be important in the inflammatory response that contributes to the pathology. Understanding the regulation of iNOS expression and its role during H5N1 influenza infection may provide insights for the development of new therapeutic strategies in the treatment of avian influenza infection.

**Keywords:** H5N1 Avian Influenza, iNOS, Nitric oxide, Inflammation

**P-06****COMMON IMMUNOCYTOCHEMICAL FEATURE OF THE FDC AND TYPE II PNEUMOCYTE**

Zsófia Benyeda<sup>1</sup>, Katalin Kocsis<sup>2</sup>, Nándor Nagy<sup>2</sup>, Ildikó Bódi<sup>2</sup>, Vilmos Palya<sup>1</sup>, Imre Oláh<sup>2</sup>

<sup>1</sup> Ceva-Phylaxia Zrt., 1107 Budapest, Szállás u. 7, Hungary, vilmos.palya@ceva.com

<sup>2</sup> Semmelweis University, Department of Human Morphology and Developmental Biology  
1094 Budapest, Tűzoltó u.58, Hungary, kocsis.katalin@med.semmelweis-univ.hu

Jeurissen et al (1992) described a novel monoclonal antibody (mAb), designated CVI-ChNL-74.3, which identified follicular dendritic cells (FDC) in the chicken peripheral lymphoid organs and bursal secretory dendritic cells (BSDC) in the bursa of Fabricius. The 74.3 mAb recognized few ellipsoid associated cells (EAC) in the spleen, which are the precursors of the FDC. The 74.3 mAb showed two kinds of staining clusters at the corticomedullary border of the thymus. In one of them, the antigen is intracellularly located while in the other clusters the 74.3 positivity appeared to be extracellularly. Over the thymic medulla, scattered, individual, 74.3 positive cells also occur. In a quail-chick chimeric experiment, an accidental finding revealed, that the 74.3 mAb specifically stained epithelial cells in the parabronchi and atrium of the chicken lung. Analysis of this finding elucidated, that these epithelial cells are type II pneumocytes, which produce biologically active, secreted substance; surfactant. Surfactant is a phylogenetically highly conserved, phospholipid, which emerged about 300 million years ago in the air-breathing animals. The platelet activating factor (PAF) is also a phospholipid, which may be present in the granules of the BSDC in the medullary region of the bursal follicles and the FDC. The PAF or PAF-like substance in the dendritic cells can activate inflammatory leukocytes during infection of bursal disease virus. Clinical and immunocytochemical studies are necessary to determine the effect of pathogens for the production and secretion of the surfactant in the lung and the 74.3 antigen in the bursa and FDC. The 74.3 mAb does not cross-react with other species studied, like turkey, guinea fowl and quail, and according to our knowledge the 74.3 antibody is the only monoclonal one, which recognizes type II pneumocytes. The occurrence of the 74.3 positive antigen in different types of cells and tissue, makes the molecular characterization of the antigen is highly necessary.

*The first two authors contributed equally.*

*Grant sponsor: OTKA K 69061.*

**P-07****ORIGIN OF THE SPLENIC RETICULAR CELLS INFLUENCES THE EFFECT OF THE INFECTIOUS BURSA DISEASE VIRUS (IBDV) ON THE EXTRACELLULAR MATRIX**

Éva Bíró<sup>1</sup>, Nándor Nagy<sup>1</sup>, Katalin Kocsis<sup>1</sup>, Susanne Kabell<sup>2</sup>, Vilmos Palya<sup>3</sup>, Imre Oláh<sup>1</sup>

<sup>1</sup> *Semmelweis University, Department of Human Morphology and Developmental Biology  
1094 Budapest, Tűzoltó u.58, Hungary, olah.imre@med.semmelweis-univ.hu*

<sup>2</sup> *Department of Poultry, Fish and Fur Animals, Danish Institute for Food and Veterinary Research,  
Høngvej 2, 8200 Aarhus N, Denmark*

<sup>3</sup> *Ceva-Phylaxia Zrt., 1107 Budapest, Szállás u. 7., Hungary*

After infectious bursal disease virus (IBDV) infection the splenic extracellular matrix (ECM) was studied by immunohistochemical method. After inoculation of classical IBDV strain (52/70) the major fibrillar components, type I and type III collagens and the main ECM organizing glycoproteins (laminin, tenascin and fibronectin) were used to follow the changes in the spleen up to eleven days postinfection (p.i). The ellipsoid and periellipsoidal white pulp (PWP) are the antigen trapping and B dependent compartments of the spleen, respectively. Reticular cells of the ellipsoid and PWP express CD45 hemopoietic marker, indicating their blood - borne origin. In these compartments the ECM produced by the reticular cells is very susceptible for the IBDV infection, because by day 3 p.i. ECM is drastically deteriorated. The splenic tissue emerges from mesenchyme of the dorsal mesogastrium from which the reticular cells of the periarteriolar lymphatic sheath (PALS), germinal center (GC) and the red pulp develop. The ECM produced by these reticular cells is much less or not susceptible for IBDV infection. This portion of the splenic ECM does not change significantly after infection. The laminin in the basement membrane of the penicillar capillaries is possibly produced exclusively by the reticular cells of the ellipsoid, because by day three p.i. there is no laminin staining. The tenascin in the capsule of Schweigger-Seidel sheath (CSS of the ellipsoid) disappears by day three p.i.. Tenascin is capable for both promotion and inhibition of the cell migration, and together with the fibronectin regulates the position and migration of the ellipsoid associated cells (EAC). This physiological function of the CSS is completely damaged by the IBDV. Disturbance of the position of EAC may be subsequently resulted in heavily impairment in the adaptive, humoral immune response, at least for blood-borne antigen. It may contribute to the permanent immunosuppression.

*First two authors contributed equally.*

*Grant sponsor OTKA; K 69061.*



**P-08****FOLLICULAR DENDRITIC CELL MARKER; 74.3 MAB IDENTIFIES CELLS CLUSTERS IN THE THYMIC MEDULLA**

Ildikó Bódi, Nándor Nagy, Imre Oláh

*Semmelweis University, Department of Human Morphology and Developmental Biology**1094 Budapest, Tűzoltó u.58., Hungary**E-mail: bodi.ildiko@med.semmelweis-univ.hu*

---

The 74.3 monoclonal antibody (mAb) recognizes follicular and bursal dendritic cells as well as few cells in the spleen around the ellipsoids (Jeurissen and Janse 1994) Guillemot et al (1984) isolated a cell type of dendritic morphology, which expressed MHC class II antigen. However, the thymic dendritic cell (TDC) has not been identified in vivo. The aim of this study was to determine the TDC using 74.3 mAb. The 74.3 positive cells show cytoplasmic staining and appear as individual cells over the medulla or they are clustered at the cortico-medullary border. In the clusters the individual cells are either well-identified and loosely packed or the whole cluster is "homogenously-stained". In the latter clusters the cells may be degranulated. The clustered 74.3 positive cells raised the possibility, that these cells may be identical with the earlier observed myeloperoxidase positive cells (Olah et al (1991). However, the double staining (74.3 mAb immunocytochemistry and endogenous peroxidase histochemistry) did not show colocalization, proving that the 74.3 and endogenous peroxidase positive cells represent two different cell populations. The 74.3 positive cells emerged in the thymus of the 13 day old embryos, two days later than in the bursa of Fabricius. Before development of the thymic cortex and medulla the 74.3 positive cells covers the whole thymus and as the medulla has been formed the cells were restricted to the medulla. The cluster formation of the cells starts after 1 week of age. The chick quail chimeras were made to determine the origin of 74.3 positive cells. The quail branchial region (branchial arches, pouches and grooves) was implanted into chicken coelomic cavity and after 17 day incubation the graft was studied for thymus formation and hemopoietic cell colonization. These chimeric experiments indicated that the 74.3 positive cells are of blood-borne origin. The histological identification of the 74.3 positive thymic cells and the biochemical determination of the 74.3 positive materials are in progress.

*This work was supported by OTKA grant number: K-69061.*





**P-09**

## **DEVELOPMENT OF NOVEL T CELL VACCINES AGAINST AVIAN INFLUENZA IN DOMESTIC CHICKENS**

Connor Carson<sup>2</sup>, Raul Ruiz-Hernandez<sup>1</sup>, Devanand Balkissoon<sup>1</sup>, Karen Staines<sup>1</sup>, Sarah Gilbert<sup>2</sup>, Colin Butter<sup>1</sup>

<sup>1</sup> *Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berks, RG20 7NN, United Kingdom*

<sup>2</sup> *The Jenner Institute, Old Road Campus Research Building, Oxford University - Nuffield Department of Clinical Medicine, Roosevelt Drive, Oxford OX3 7DQ, United Kingdom*

**This abstract has been withdrawn.**

## P-10

### CHANGES IN CHICKEN INNATE IMMUNITY INDUCED BY *IN VIVO* ADMINISTRATION OF BACULOVIRUS

Silvina Chimeno Zoth, Juan Manuel Carballeda, María José Gravisaco, Evangelina Gómez, Elisa Carrillo, Analía Berinstein

*2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

Baculoviruses (BVs) are dsDNA viruses which infect insects. They have been used as selected bioinsecticides and for the expression of recombinant proteins in insect cells. Recent studies showed that BVs have strong adjuvant effects on the mammalian immune system. The purpose of this work was to study the effect of *in vivo* administration of baculovirus in chickens.

SPF chickens of 20 days were intravenously inoculated with  $10^8$  pfu of *Autographa californica nuclear* polyhedrosis virus. Three hours later, chickens were bled, euthanized and their spleen, bursa, duodenum and cecal tonsils were excised in order to take samples for RNA extraction and Real Time PCR, and to isolate the lymphocytes, which were stained and analyzed by flow cytometry. Furthermore, splenocytes were cultured at 41°C and nitric oxide (NO) production was measured in the culture supernatant by the Griess reaction. gIFN production was also evaluated in the culture medium of purified splenocytes.

RNA was extracted from spleen tissue and the expression of gIFN, aIFN, IL-8, LITAF, IL-15, IL-6 and TGFb was measured by real time RT-PCR. The results obtained showed that baculovirus inoculation up-regulates the expression of gIFN, IL-6 and LITAF in spleen cells when compared with samples from chickens inoculated with supernatant of mock-infected cells. This result was in agreement with those obtained by ELISA which showed a very strong increase of gIFN in splenocytes supernatants and in chicken plasma.

Flow cytometry analysis revealed that BV inoculation induced a decrease in the percentage of T-lymphocytes, mainly of CD3<sup>+</sup>CD4<sup>+</sup>, accompanied by an increase in the frequency of NK cells in cecal tonsils. On the other hand, in spleen, an increment in the percentage of monocyte/macrophage population was detected, together with an increase in T lymphocytes CD3<sup>+</sup>CD4<sup>+</sup>. In bursa, a drop in the frequency of monocyte/macrophage population due to BV inoculation was observed. Finally, intraepithelial lymphocytes of the gut did not show differences between BV and control treated animals.

Although, further studies in order to understand the mechanisms, by which baculoviruses affect the avian immune response, are needed, results obtained in the present work demonstrate the ability of baculoviruses to stimulate the innate immunity in chickens, modifying the expression pattern of related genes and the profile of immunocompromised cells.



## P-11

# STUDYING AVIAN IMMUNE RESPONSE TO AN INTERMEDIATE STRAIN OF IBDV

Juan Manuel Carballeda, Silvina Chimeno Zoth, Evangelina Gómez, María José Gravisaco, Analía Berinstein

*2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

Infectious Bursal Disease (IBD) is a highly contagious, wide spread immunosuppressive chicken disease caused by the Infectious Bursal Disease Virus (IBDV). IBDV is a two segmented double-strand RNA virus, member of the Birnaviridae family.

In order to study the interaction between IBDV and the immune system, chickens were exposed to an intermediate IBDV strain by both, intramuscular and oral routes, and sacrificed at 1, 3, 5 and 28 days post inoculation (DPI). Using Real Time PCR we analyzed the expression of a panel of avian cytokines and chemokines in duodenum, spleen and Bursa of Fabricius. Also, splenic nitric oxide (NO) production and immunocompromised cellular populations were analyzed by Griess reaction and flow cytometry, respectively.

Intramuscular IBDV inoculation promoted an over expression of proinflammatory cytokines as IL-6, IL-15 and gINF in spleen, which correlated with an increase of gINF plasma concentration measured by ELISA. At 3DPI, gINF expression was still up-regulated in spleen. At 5DPI, an over expression of IL-15 was observed in the bursa. Splenic NO production was measured in supernatants of splenocytes stimulated (or not) with Concanavalin A (ConA). Intramuscular inoculation of IBDV caused an increment of NO concentration in supernatants at 1DPI. At 3 and 5DPI, no increase of NO was detected in splenocytes from IBDV when compared with PBS treated animals. In addition, at 3 and 5 DPI splenocytes of chickens inoculated with IBDV appeared to be insensitive to ConA stimulation.

When IBDV was orally administered, plasma concentration of gINF was strongly increased at 3DPI compared with control animals. Flow cytometry analysis of the bursa showed a strong T-lymphocyte infiltration and a decrease in B- lymphocyte populations in animals inoculated with IBDV at 5DPI. The Adherent/no Adherent splenic cells ratio displayed an increment of adherent (macrophages) cells in the spleen at 1DPI but no differences were detected at 3DPI in IBDV inoculated animals. At 5DPI, splenocytes of IBDV treated chickens showed a lower Ad. / Non Ad. ratio, suggesting a drop in macrophage population of the spleen.

Splenic NO production was higher in IBDV inoculated chickens only at 1DPI.

In summary, results obtained in the present work showed that IBDV of intermediate virulence induced similar effects as previously described for highly virulent IBDV, in terms of up regulation of inflammatory cytokines expression in spleen and bursa, early increase of spleen macrophages with augmented NO production followed by a significant decrease in the number of these cells, and infiltration of T lymphocytes in the bursa, among others. These results allow us to be confident in the study of the immune response against IBDV using non virulent virus as a model.

**O-12****AVIAN INFLUENZA VIRUS HEMAGGLUTININ AND NUCLEOCAPSID PROTEINS INDUCE CHICKEN EFFECTOR, MEMORY AND EFFECTOR MEMORY CD8<sup>+</sup> T LYMPHOCYTES**

Ellen W. Collisson<sup>1,2</sup>, Haroldo Toro<sup>3</sup>, De-Chu Tang<sup>4</sup>, Worthie E. Briles<sup>4</sup>, Linda Yates<sup>4</sup>,  
Renee Kopulos<sup>4</sup>, Shailbala Singh<sup>1</sup>

<sup>1</sup> *Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843, United States*

<sup>2</sup> *College of Veterinary Medicine, Auburn University, Auburn, AL 36849, United States*

<sup>3</sup> *Vaxin, 1500 First Ave N., Birmingham, AL 35203, United States*

<sup>4</sup> *Northern Illinois University, DeKalb, IL 60115, United States*

<sup>5</sup> *College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA 91786, United States*

---

A non-replicating adenovirus vector encoding hemagglutinin derived from an H5N9 avian influenza virus (AIV) had been shown to protect chickens against challenge with homologous virus and heterologous H5 strains. The T lymphocyte responses mediated by vectors expressing either the HA or the NP were evaluated in chickens following intramuscular (i.m.) inoculation. Specific T cell responses were determined by quantifying expression of IFN $\gamma$  following *ex vivo* stimulation of infected, non-professional antigen presenting cells. AIV specific, MHC-I restricted, CD8<sup>+</sup> effector responses, were identified at 10 days post-inoculation (p.i.), but were undetectable by 2 weeks p.i. The viral specific memory responses, detected by 3 weeks significantly declined by 8 weeks p.i. Effector memory responses, detected by 1 week following a booster inoculation, were significantly greater than the primary responses, but also declined within a few days. Following i.m. inoculation of non-replicating vectors expressing influenza nucleocapsid protein, CD8<sup>+</sup> responses to the avian virus proved to be even greater than CD8<sup>+</sup> responses to the HA. Their capacity to cross-react with the heterologous H7N2 AIV strain, indicates the potential to protect against a broad spectrum of variant AIV strains. It is of future interest to determine the mechanisms for the predictable control or decline observed following all anti-AIV T cell responses.

**O-13****RESISTANCE OF B2 LINES TO AVIAN CORONAVIRUS COMPARED WITH B19 LINES CORRELATES WITH MONOCYTE FUNCTION**

Ellen W. Collisson<sup>1</sup>, Ghida R. Banat<sup>1</sup>, Lisa Griggs<sup>1</sup>, Maisie Dawes<sup>1</sup>, Suzana Tkalcic<sup>1</sup>, Miguel Saggese<sup>1</sup>, Worthie Briles<sup>2</sup>, H.L. Shivaprasad<sup>3</sup>, Mark W. Jackwood<sup>4</sup>, Yvonne Drechsler<sup>1</sup>

<sup>1</sup> College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, United States

<sup>2</sup> North Illinois University, Dekalb, IL, United States

<sup>3</sup> College of Veterinary Medicine, University of California, Davis, CA, United States

<sup>4</sup> College of Veterinary Medicine, University of Georgia, Athens, GA, United States

The avian coronavirus, infectious bronchitis virus (IBV), causes an acute, highly contagious respiratory disease of chickens, resulting in economic losses worldwide. The degree of clinical illness associated with viral infection has been correlated with the B locus. In this study, the B2 chicks infected at 6 days of age were found to be more resistant to clinical illness than B19 chicks. Furthermore, they had fewer histopath lesions in the lungs and trachea than the B19 chicks. Because the differences in pathogenesis were observed early in infection, innate functions in the context of *ex vivo* activation of monocytes/macrophages from birds of each line were examined. Poly I:C was used as a surrogate of replication of RNA coronavirus replication. *Ex vivo* activation of cultured monocytes/macrophages of B2 chickens with poly I:C was consistently several fold greater than activation of B19 cells. This study suggests that innate immunity, in particular monocytes, is at least partially responsible for the greater resistance of the B2 chicks to IBV pathogenesis. Differences in cellular functions are being further examined in B19 and B2 macrophages stimulated with synthetic and viral RNA.

**O-14****EVALUATION OF INTRACELLULAR CYTOKINE STAINING BY FLOW CYTOMETRY FOR ASSESSMENT OF VACCINE OUTCOMES IN CHICKENS**

Tina S. Dalgaard<sup>1</sup>, L. R. Norup<sup>1</sup>, E. Wattring<sup>2</sup>, H. R. Juul-Madsen<sup>1</sup>

<sup>1</sup> *Department of Animal Health and Bioscience, Aarhus University, Tjele, Denmark*

<sup>2</sup> *Department of Virology, Immunobiology and Parasitology, National Veterinary Institute, Uppsala, Sweden*

The cellular immune system is essential for the control of viral infections and plays a key role also in vaccine-induced antiviral immunity. Thus, quantification of specific T cell responses has become a focus of interest in order to understand protective immunity in many infectious diseases. Studies of antigen-specific T cells are often based on functional analysis *ex vivo* after activating the cells with recall antigen. The lymphocyte activation can then be evaluated by different methods using functional read-outs such as proliferation, cytokine production or simply expression of surface activation markers.

Intracellular cytokine staining (ICS) to assess cytokine production profiles has been used extensively in human medical research to address antigen-specific T cell responses in settings such as experimental vaccination. The purpose of this study was to optimize and evaluate an ICS method for assessment of T cell responses in chickens vaccinated against Newcastle disease virus.

Chickens from one inbred white leghorn line containing the two MHC haplotypes B19 and B21 were divided into 3 experimental groups; one group was kept as naïve controls; one group was vaccinated intramuscularly with commercial inactivated NDV vaccine at 4 and 7 weeks of age and the last group was vaccinated orally with commercial live attenuated NDV vaccine at 4 and 7 weeks of age. Peripheral blood mononuclear cells were activated *ex vivo* polyclonally (ConA) or with recall antigen (NDV antigen) and the ICS method was used to determine intracellular IFN- $\gamma$  content. Several issues regarding the usefulness of the method to assess immunogenicity and protective immunity in chicken vaccination experiments will be discussed.

**P-15****FIVE-COLOR FLOW CYTOMETRY USED FOR IMMUNOPHENOTYPING OF PERIPHERAL CHICKEN T CELLS IN AN ATTEMPT TO DEFINE CORRELATES OF VACCINE-INDUCED PROTECTION**

Tina S. Dalgaard<sup>1</sup>, L. R. Norup<sup>1</sup>, A. R. Pedersen<sup>2</sup>, K. J. Handberg<sup>3</sup>, P. H. Jørgensen<sup>3</sup>, H. R. Juul-Madsen<sup>1</sup>

<sup>1</sup> *Department of Animal Health and Bioscience, Aarhus University, Tjele, Denmark*

<sup>2</sup> *Department of Genetics and Biotechnology, Aarhus University, Tjele, Denmark*

<sup>3</sup> *DTU National Veterinary Institute, Division of Poultry, Fish and Fur Animals, Aarhus, Denmark*

Vaccines play an increasing role in disease control of poultry and it is evident that better understanding of mechanisms, involved in protective immunity in the chicken is needed in order to develop new vaccines and to improve existing vaccine regimes. As in mammals cell-mediated immunity plays a crucial role in vaccine-induced protective immunity in chickens and techniques to elucidate T cell responses both quantitatively and qualitatively are important for future disease control. Flow cytometry has been proven as one of the most valuable tools in these studies as it offers a unique ability to investigate the expression of multiple antigens per cell and thus the possibility to clearly distinguish between T cell subsets.

The objective of the study was to use 5-color flow cytometry for immunophenotyping of peripheral T cell subsets in a vaccine/challenge experiment. Two inbred genetic chicken lines (L130 and L133) were subjected to two times vaccination against Newcastle disease (ND) and a subsequent challenge by ND virus (NDV) infection. The immunophenotypic profiles of the two lines were found to be very different as peripheral blood from L133 had a significantly lower CD4/CD8 ratio and a lower frequency of  $\gamma\delta$  T cells as compared to L130. Furthermore, peripheral lymphocytes from L133 exhibited a significantly higher expression of CD44 and CD45. A vaccine-induced effect on CD45 expression was observed, but only in L133, where immune chickens had a significantly higher CD45 expression on their lymphocytes than the naïve controls. After NDV challenge, L133 appeared to be better protected by the vaccine than L130 as determined by the presence of viral genomes. We hypothesize that CD45 expression is a parameter that may correlate with successful development of antigen-specific memory T cells in the chicken, but further investigations are required.

In the future, identification of new phenotypic markers for immunological memory will be important in order to unravel the mechanisms involved in protective cellular immunity in the chicken. Thus, simultaneous discrimination of expression patterns of phenotypic markers combined with activation markers or functional parameters like cytokine production and proliferation is required to further elucidate chicken T cell biology.



**O-16****THE BIOLOGICAL EFFECTS OF THE AVIAN MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) ON IMMUNE CELL FUNCTION**

Sungwon Kim<sup>1</sup>, Kate B. Miska<sup>2</sup>, Mark C. Jenkins<sup>2</sup>, Ray H. Fetterer<sup>2</sup>, Chasity M. Cox<sup>1</sup>,  
Lindsay H. Sumners<sup>1</sup>, Rami A. Dalloul<sup>1</sup>

<sup>1</sup> *Avian Immunobiology Laboratory, Animal & Poultry Sciences, Virginia Tech, Blacksburg, VA, United States*

<sup>2</sup> *Animal Parasitic Diseases Laboratory, Agricultural Research Service, USDA, Beltsville, MD, United States*

The lymphocyte-derived soluble factor - macrophage migration inhibitory factor (MIF) - inhibits the random migration of macrophages. Recent research shows a more prominent role of MIF as a multi-functional cytokine mediating both innate and adaptive immune responses. This study describes the expression and functional characterization of recombinant chicken MIF (rChMIF) in an effort to better understand its biological effect on immune system and potential in poultry health applications. To express rChMIF, the full-length avian MIF gene was amplified from stimulated chicken lymphocytes and cloned into a prokaryotic expression vector. The confirmed 115 amino acid sequence of avian MIF has 71% identity with human and murine MIF. The endotoxin-free rChMIF was purified using size exclusion high performance liquid chromatography (SEC-HPLC), and various *in vitro* biological tests were performed including chemotactic and proliferation assays. To determine the inhibition of random migration of macrophages, a chemotactic assay was performed using a Modified Boyden Chemotaxis chamber followed by Diff-Quick staining, resulting in sharply decreased migration of macrophages in the presence of 10 ng/ml rChMIF. Further, the presence of rChMIF resulted in enhanced proliferation of Con A-stimulated lymphocytes. This effect was abolished by addition of anti-rChMIF polyclonal antibody. Additionally, the expression of various cytokines was measured in PBMCs or splenocytes using quantitative real-time PCR (qRT-PCR). Immune cells were cultured in the presence or absence of rChMIF, with or without LPS or Con A for 6 or 12 h. qRT-PCR analysis revealed that rChMIF alone did not induce transcription of IL-1 $\beta$  or iNOS. However, the presence of rChMIF enhanced levels of IL-1 $\beta$  and iNOS during PBMCs stimulation with LPS. Similarly, there was no effect of rChMIF alone on splenocytes; however, the Con A-stimulated lymphocytes showed enhanced IFN- $\gamma$  and IL-2 transcripts in the presence of rChMIF. To our knowledge, this study represents the first report for the functional characterization of avian MIF, which inhibits migration of macrophages similarly to mammalian MIF, and it also mediates inflammatory responses during antigenic stimulations.

**Keywords:** *Avian, Macrophage Migration Inhibitory Factor (MIF), Inflammatory response, chemotaxis, Cytokines*



**P-17****DIETARY BETA-GLUCAN EFFECTS ON THE T HELPER CYTOKINE BALANCE IN THE INTESTINAL TRACT OF BROILER CHICKS**

Chasity M. Cox<sup>1</sup>, Lindsay H. Sumners<sup>1</sup>, Sungwon Kim<sup>1</sup>, Audrey P. McElroy<sup>1</sup>, Mike R. Bedford<sup>2</sup>, Rami A. Dalloul<sup>1</sup>

<sup>1</sup> *Avian Immunobiology Laboratory, Animal & Poultry Sciences, Virginia Tech, Blacksburg, VA, United States*

<sup>2</sup> *AB Vista Feed Ingredients, Marlborough, United Kingdom*

Immunomodulators like  $\beta$ -glucans have attracted considerable attention as potential alternatives to the prophylactic use of antibiotics. Despite increasing research, little is known about their regulatory influence on immune function in poultry. Two studies were conducted to evaluate the effects of a yeast derived  $\beta$ -glucan (Auxoferm YGT) on gene expression of T helper cytokines in the intestines. Day old chicks were fed a diet containing 0, 0.02, or 0.1%  $\beta$ -glucan. For the first study, small intestinal sections were collected on day 7 and 14 to evaluate gene expression by quantitative real-time PCR. On day 7, IL-18 expression was upregulated in the jejunum but decreased on day 14 in the duodenum of the 0.02%  $\beta$ -glucan birds. Expression of IL-18 also decreased on day 14 in the ileum of both  $\beta$ -glucan groups when compared to control. On day 7, IL-4 expression was downregulated in both  $\beta$ -glucan treated groups in the duodenum and in the 0.1% treated group in the jejunum and ileum. In contrast, IL-4 was upregulated in the duodenum of treated birds and in the ileum of 0.1% fed birds on day 14. IL-13 was similarly downregulated in all intestinal sections of 0.1%  $\beta$ -glucan fed birds on day 7. The second study included a mixed *Eimeria* infection on day 8 and samples collected on days 10, 14, and 21 post-hatch. In 0.1% YGT fed birds, IL-18 expression was highest in the jejunum, intermediate in duodenum and lowest in the ileum while expression in 0.0% YGT fed birds remained constant across intestinal sections. Interferon- $\gamma$  was upregulated in the duodenum of the control fed birds due to the *Eimeria* challenge while levels remained constant in 0.1% YGT fed birds. In the jejunum, interferon- $\gamma$  was upregulated due to the *Eimeria* challenge in the 0.1% YGT fed group. IL-4 expression was downregulated in non-challenged birds on the 0.1%  $\beta$ -glucan diet. On day 14, mucin-2 expression was decreased due to the *Eimeria* infection in 0.1%  $\beta$ -glucan fed birds. Taken together, the data provided from these trials strongly suggest that  $\beta$ -glucans downregulate T helper type 2 cytokines and thus favor a T helper type 1 cell response.

**Keywords:**  $\beta$ -glucan, poultry, cytokines, immunity, T helper cells

**P-18****IN VITRO UPTAKE OF ANTIGEN BY CHICKEN BM-DC AND EFFECT ON PHENOTYPE**Eveline D. de Geus<sup>1</sup>, B. Tefsen<sup>2</sup>, I. van Die<sup>2</sup>, L. Vervelde<sup>1</sup><sup>1</sup> Dept Inf. Dis. & Immunol., Faculty of Veterinary Medicine, Utrecht, The Netherlands<sup>2</sup> Dept. of Molecular Cell Biology & Immunology, VU University Medical Center, Amsterdam, The Netherlands

Immature dendritic cells (DC) are very well equipped for the uptake of antigen, while they possess low antigen-presenting capacity. Upon encounter and uptake of an antigen, DC start to mature: they downregulate receptors involved in uptake of antigen and upregulate expression of costimulatory molecules and MHC II. Mature DC are potent antigen presenting cells and are involved in priming of naïve T cells. In vitro cultured chicken DC have recently been described and they resemble mammalian DC in morphology. They readily ingest polystyrene microspheres and FITC-dextran. After stimulation with LPS or CD40L, maturation is induced with upregulation of costimulatory molecules.

In this experiment, DC were given beads and LPS at the same time and then cultured for 24h. When LPS-coated beads or beads with free LPS are given, uptake of beads was reduced compared to beads without LPS. LPS-coated beads induce upregulation of CD40 after 24h culture. We are now testing uptake kinetics of microspheres with different coatings, kinetics of cell surface marker expression and also cytokine expression upon uptake of the coated microspheres. Beads coated with inactivated avian influenza virus (AIV) and LPS will be compared. We hypothesize that culturing DC with microspheres with different coatings will result in differences in maturation status and cytokine profile of the DC. In previous experiments we found that DC cultured with LPS upregulate IL-2, IL-10 and IL-12α mRNA, whereas IFN-β mRNA is upregulated after culturing with AIV. Cytokine expression profiles of DC cultured with LPS-coated beads or AIV-coated beads are under investigation.

The receptors on the cell surface of chicken DC that are involved in uptake of pathogens are poorly characterized, although DEC205 and CD209 have been identified based on sequence homology. Using a panel of biotinylated polymeric glycan probes we found Ca<sup>2+</sup>-independent binding to αGalNAc and Ca<sup>2+</sup>-dependent binding to Galα1-3Gal, suggesting the presence of two different lectin receptors on chicken BM-DC that have specificity for different glycan moieties. Differentially-coated microspheres will be used to mimic uptake of different pathogens and to identify different receptors on chicken DC.

*This research was sponsored by the Program "Impulse Veterinary Avian Influenza Research in the Netherlands" of the Dutch Ministry of Agriculture, Nature and Food Quality*



## O-19

### **INTRATRACHEALLY ADMINISTERED ANTIGENS ARE TAKEN UP BY CHICKEN RESPIRATORY TRACT PHAGOCYTES THAT ACQUIRE A MATURE DC-LIKE PHENOTYPE**

Eveline D. de Geus<sup>1</sup>, J. M. Rebel<sup>2</sup>, L. Vervelde<sup>1</sup>

<sup>1</sup> *Dept Inf. Dis. & Immunol., Faculty of Veterinary Medicine, Utrecht, The Netherlands*

<sup>2</sup> *Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands*

In chickens it is unknown where and by which cells a respiratory antigen is taken up and presented to T cells. In mammals after antigen uptake the APC migrate to the draining lymph nodes and interact with T cells. In the chicken, lacking the draining lymph nodes, the respiratory antigen-presenting cells could migrate to bronchus-associated lymphoid tissue (BALT) or to the spleen. In chicken, BALT is constitutively present and is thought to compensate for the lack of draining lymph nodes.

We want to unravel the uptake of inhaled antigen and subsequent induction of immune responses against it. To determine where and by which cells the antigen is taken up, we intratracheally administered 1 µm fluorescent LPS-coated or uncoated control beads and sacrificed the chickens 2, 6 and 24 hours post inoculation. Lung, spleen and blood were collected for FACS analysis and immunocytochemistry.

In lung, for both types of beads ~80% of the bead+ cells were CD45+ leukocytes. Remaining bead+ cells were probably epithelial cells. Uptake of LPS-coated beads was increased compared to control beads and peaked at 6 hrs p.i. At 24 hrs p.i. the percentage LPS-bead+ cells was back at control level, suggesting migration of bead+ cells. Cells that ingested LPS-coated beads had a more activated phenotype, based on increased expression of CD40. In sections of lung tissue, LPS-bead+ cells were observed in BALT areas at 24 hrs p.i., which could suggest local induction of immune responses. Control beads were detected in and near the primary bronchus.

In blood of chickens inoculated with control beads, no bead+ cells were detected. In contrast, when LPS-coated beads were given, bead+ cells were detected in blood of 2 out of 11 chickens. The PBMC of birds given LPS-coated beads acquired an activated phenotype regardless of presence of bead+ cells in blood.

The drop in percentage bead+ cells in the lung at 24 hrs p.i. suggests migration of cells, possibly to the spleen. Analysis of spleen to detect whether bead+ cells migrate to the spleen and if certain subpopulations of APC are migrating is ongoing.

*This research was sponsored by the Program "Impulse Veterinary Avian Influenza Research in the Netherlands" of the Dutch Ministry of Agriculture, Nature and Food Quality*

**P-20****CD45 HEMOPOIETIC CELLS IN THE EARLY CHICKEN EMBRYO**

Dávid Dóra, Dávid Molnár, Imre Oláh, Nándor Nagy

*Semmelweis University, Department of Human Morphology and Developmental Biology  
1094 Budapest, Tűzoltó u.58, Hungary  
E-mail: nagy.nandor@med.semmelweis-univ.hu*

Peripheral blood fibrocytes (PBF) are present in the blood of the embryo and post hatched chicken. Leaving the circulation the PBF can settle down in the different tissues and contribute to the stromal, residential cell population: i.e. splenic reticular cells of the ellipsoid. The aim of this study was to determine the embryonic appearance of the PBF in the different embryonic tissue and their differentiation in loco. The first CD45 positive cells emerge in the blood island of the yolk sac at 48 hours of incubation, which is followed by their accumulation in the intraaortic cells clusters about 12-16 hours later. The overwhelming numbers of the CD45 positive cells are round or ovoid shaped, but in the mesenchyme few, scattered, stellate-shaped, CD45 positive cells also occur. By 120 hours of incubation stellate-shaped CD45 positive cells colonized all organ rudiments, even they appear in the mesenchyme of the limb buds. The round or ovoid-shaped CD45 positive cells are concentrated around the aorta and in the splanchnic mesenchyme they are getting to form a "sheath" around the splanchnic arteries. In the mesenchyme, the CD45 hemopoietic cells express MHC class II antigen, which makes them capable for antigen presentation. These CD45 and MHC class II double positive cells possibly the precursors of lymphoid and tissue dendritic cells, indicating their common origin. The fate of the round-shaped CD45 positive cells is unknown, but we assume, that some of them will colonize the hemopoietic organs (spleen, bone marrow) and initiates hemopoiesis. A pilot experiment seems to support this hypothesis, because around day 5 of incubation Bu-1, and CD3, B and T cell markers appear on the CD45 positive cells.

*Grant sponsor OTKA; K 69061.*

**O-21****SYNOVIAL FLUID OF MYCOPLASMA SYNOVIAE INFECTED CHICKENS CONTAINS PRO-INFLAMMATORY CYTOKINES AND AUTOANTIBODIES**

Daliborka Dušanić, Saša Kastelic, Dušan Benčina, Mojca Narat

*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Chair for Genetics, Animal Biotechnology and Immunology, Groblje 3, 1230 Domžale, Slovenia*

The link between joint inflammation and joint destruction is poorly understood both in humans and animal models. A complex network of tissue and immune cells, immune mediators and antibodies is being studied in humans. Infectious synovitis caused by *Mycoplasma synoviae* in chickens shows resemblance to human arthropaties. The condition is manifested as swelling of joints, inflammation, accumulation of macrophages, B and T lymphocytes in the synovial liquid.

In our previous studies, we demonstrated a strong effect of *M. synoviae* on gene and protein expression of macrophages. Preliminary experiments showed elevated expression of several genes, including cytokines and citrullinase also in infected chondrocytes and several cytokines were detected in supernatants of infected chondrocytes. Using phenotype microarrays, we demonstrated that chondrocytes exposed to *M. synoviae* respond to a higher number of immune mediators compared to non-infected cells. Cytokines are known to significantly reduce the expression of anabolic genes, up-regulate various catabolic genes and intercellular mediators in chondrocytes. These mechanisms lead to cell death, tissue destruction and persistence of inflammation and can also lead to exposure of new antigens and formation of autoantibodies.

In this study, synovial fluids of infected chickens were analysed for presence of local autoantibodies against cartilage proteins. Protein profiles of chondrocytes and cartilage from three healthy animals were obtained using PAGE in reducing and non-reducing conditions. Following blotting to membranes, profiles were incubated in samples of synovial fluids and the reactions detected using peroxidase-conjugated anti-chicken antibodies. Additionally, two serum samples obtained from infected chickens were used to allow discrimination between locally and systemically present antibodies. Preliminary results show presence of local autoantibodies against several proteins of chondrocytes and cartilage.

Additionally, we obtained a cytokine profile in synovial fluids of infected chickens. Cytokines were detected on protein profiles of synovial fluids using cytokine-specific monoclonal antibodies.

Our study shows *M. synoviae* has a profound impact on chicken chondrocytes. It causes alterations in gene expression and induces production of cytokines. Local autoantibodies that are present in synovial fluids of infected animals target proteins of chondrocytes and cartilage. Understanding how *M. synoviae* interacts with chondrocytes could help elucidate the pathogenesis of infectious synovitis.



## O-22

### CHARACTERIZATION OF NOVEL TYPE I CHICKEN INTERFERONS

Ibrahim Eldaghayes<sup>1,2</sup>, Jean-Remy Sadeyen<sup>1</sup>, Lisa Rothwell<sup>1,3</sup>, Zhiguang Wu<sup>1,3</sup>, Zhengxing Lian<sup>1,4</sup>, Changlin Chen<sup>5</sup>, Peter Russell<sup>5</sup>, Pete Kaiser<sup>1,3</sup>

<sup>1</sup> *Institute for Animal Health, Compton RG20 7NN, United Kingdom*

<sup>2</sup> *Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Al-Fateh University, Tripoli, Libya*

<sup>3</sup> *The Roslin Institute and R(D)SVS, University of Edinburgh, EH25 9RG, United Kingdom*

<sup>4</sup> *College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China*

<sup>5</sup> *The Royal Veterinary College, Royal College Street, London NW1 0TU, United Kingdom*  
*E-mail: ibrahim.eldaghayes@vetmed.edu.ly*

Research on interferons started over half a century ago with the discovery of a virus-induced factor in embryonated eggs that interfered with influenza virus replication in chorioallantoic membranes of chicken embryos, hence the name interferon (IFN). Since then, it has become apparent that there are at least two, arguably three, types of IFN. Type I IFNs in mammals include a multigene family of IFN- $\alpha$ , a single IFN- $\beta$ , IFN- $\kappa$ , IFN- $\omega$  and IFN- $\tau$ . The single type II IFN is IFN- $\gamma$ , although this cytokine would be better reclassified as an interleukin. In mammals, there are three IFN- $\lambda$  genes, which are sometimes classified as type III IFNs, sometimes as type I IFNs.

The chicken IFN repertoire to date includes a multigene family of IFN- $\alpha$ , a single IFN- $\beta$ , IFN- $\gamma$  and a single IFN- $\lambda$ . Here we report the cloning and sequence of two novel chicken type I IFNs, IFN- $\kappa$  and IFN- $\omega$ , and compare and contrast their biological activities with those of the other chicken IFNs.



**O-23****IMMUNOPATHOLOGY IN AUTOIMMUNE VITILIGO IN SMYTH LINE CHICKENS: A CENTRAL ROLE OF INTERLEUKIN-21?**

Fengying Shi, Gisela F. Erf

*Department of Poultry Science, University of Arkansas, Division of Agriculture, Fayetteville, Arkansas, United States*

*E-mail: gferf@uark.edu*

Vitiligo is a pigmentation disorder characterized by post-natal loss of pigmentation in the skin due to loss of melanocytes (pigment cells). The Smyth line (SL) chicken model for vitiligo exhibits spontaneous, autoimmune loss of melanocytes in growing feathers (1,2). Onset of SL vitiligo occurs between 6 and 20 weeks of age and affects 85-95% of SL chickens. Multiple factors contribute to melanocyte death, including genetic susceptibility to vitiligo and environmental factors that trigger autoimmune loss of SL melanocytes in susceptible individuals. The spontaneous nature and the predictably high incidence of autoimmune SL vitiligo, together with the easy access to the target tissue and the ability to visually monitor vitiligo expression in the same individual, make the SL chicken an excellent model for autoimmune diseases. To examine the etiology and immunopathology of SL vitiligo, we evaluated pigmentation and collected growing feathers from SL chickens at weekly intervals from ages 4- to 20-weeks and stored the samples until use. Once feather collection was completed, an individual's samples were assigned to three groups reflecting the stage of vitiligo: early (just before visual onset), active (visual pigmentation loss) and complete (no pigmentation) vitiligo. Age-matched samples from SL chickens that did not develop vitiligo served as no-vitiligo controls. Samples were used for gene-expression analysis at the transcriptome level by microarray and real-time RT-PCR and for cell population analysis by immunohistochemistry. The data confirmed the cell-mediated nature of this autoimmune response. In addition to IFN-gamma (Th1 cytokine), the expression of IL-21 was greatly ( $P<0.05$ ) elevated during vitiligo, but did not appear to be accompanied by IL-17 (Th17 cytokine) expression. The expression of IL-10, generally known as an anti-inflammatory cytokines, was also greatly elevated ( $P<0.05$ ) in vitiligo, while IL-4 (Th2 cytokine) was not. Histological analysis of affected feathers revealed infiltrating B cells and macrophages in addition to the previously reported T cell populations, but not heterophils. Based on current literature, IL-21 may play a central role in the onset and progression of SL autoimmune vitiligo, driving both innate and adaptive immune activities in this chronic response.

Funding: NIH grant R15AR052670-01 and the Tyson Endowed Professorship in Avian Immunology

**Keywords:** *immunopathology, autoimmune disease, cytokines, lymphocytes, macrophages*

**References:**

1. Erf, G. F. 2008. Autoimmune diseases of poultry. Pages 339-358 in: *Avian Immunology*, Davison F., Kaspers, B., and K. Schat editors. Elsevier, London, San Diego, CA.
2. Erf, G. F. 2010 Animal models. Pages 205-218 in: *Vitiligo*, Picardo, M and Taieb, A, editors. Springer-Verlag GmbH, Berlin Heidelberg, Germany.

**P-24****AVIAN DENDRITIC CELL EXPRESS  $\gamma$  ACTIN**Balázs Felföldi<sup>1,3</sup>, Nándor Nagy<sup>2</sup>, G. T. Pharr<sup>3</sup>, Imre Oláh<sup>2</sup><sup>1</sup> Ceva-Phylaxia Zrt., 1107 Budapest, Szállás u. 7, Hungary, [balazs.felfoldi@ceva.com](mailto:balazs.felfoldi@ceva.com)<sup>2</sup> Semmelweis University, Department of Human Morphology and Developmental Biology  
1094 Budapest, Tűzoltó u. 58, Hungary<sup>3</sup> Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University,  
Mississippi State, MS 39762, United States

Monoclonal antibody (mAb), designated GIIF3, was raised against splenic cell suspension of guinea fowl. The GIIF3 mAb recognized avian myogenic cell specific molecule (Nagy et al. 2001). Mass spectrometry analysis and immunohistochemical studies indicated that the GIIF3 positive antigen is  $\gamma$  actin, which in the muscle cells is associated with myosin filaments. One of the fundamental properties of the dendritic cells is their ability to migrate. The precursors of the bursal secretory dendritic cells (BSDC) induce bud formation by entering the surface epithelium of the bursa of Fabricius (BF), and after IBDV infection the uptake of the virus induces their migration through the cortico-medullary border into the follicular cortex and connective tissue. In the spleen the precursors of the FDC are the ellipsoid associated cells (EAC), which after antigen uptake migrate to the T dependent regions and germinal centers (GC). Similarly, the Langerhans cells of the skin migrate to the regional lymph nodules. The migratory ability of the dendritic cells is a complex process that involves cell to cell and cell to extracellular matrix contacts. The adhesion of the cell requires rearrangement in the intermediate filaments, like vimentin in the BSDC and FDC. Therefore we tested if the GIIF3 antigen is present in the precursors of the BSDC and splenic FDC of the turkey. GIIF3 positive cells appear in the mesenchyme of the BF at day 14 of incubation. By day 16 they accumulate under the surface epithelium and several cells already entered the epithelium inducing bud formation. After hatching they are scattered over the medulla. The EAC(s) express the GIIF3 positive antigen, like the FDC. Beta galactosidase is taken up by the EAC and two days later the galactosidase containing cells appear in the GC indicating their migration from the ellipsoid to the GC. An actin is associated with the cytoskeleton of the cells unlike the  $\gamma$  actin, which is joined to the myosin filaments of the muscle cells. To clarify the function of  $\gamma$  actin in the migration and/or polarization of the precursors and mature BSDC and FDC further studies are needed.

*The first two authors contributed equally.*

*This work was sponsored partially by OTKA grant number 69061.*



**P-25****CLONING OF THE CHICKEN INTEGRIN ALPHA4 GENE**

Dorte R. Fink, G. Jungersen

*National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark*

*Background:* Integrin alpha4 is a member of the integrin gene superfamily in humans. Integrins are heterodimeric membrane-spanning proteins composed of an alpha and a beta chain, and they function as cell adhesion receptors that recognize extracellular matrix ligands and cell-surface ligands. In humans, integrin alpha4 dimerizes with either integrin beta1 or beta7. Integrin alpha4 beta7 dimers are involved in leukocyte homing to the gut mucosal tissues via interaction with Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1). In chickens, the existence of the integrin alpha4 gene has been predicted from computational analysis of the genomic sequence, and the finding of several expressed sequence tags supports the notion that the gene is transcribed into mRNA. However, the gene has not yet been cloned.

*Methods:* Total RNA was purified from chicken peripheral blood leukocytes and converted into cDNA, which was used as template in PCR amplification with full length primers designed from the predicted integrin alpha4 cDNA sequence. The PCR product was isolated and TA-cloned into the pCR4-TOPO vector, before transformation into *E. coli* TOP10 cells. Multiple colonies were picked and cultured, plasmids were purified, and the inserts were sequenced in both directions.

*Results:* The integrin alpha4 cDNA was successfully cloned using primers located in the 5' and 3' untranslated regions. The cDNA sequence contains 3755 nucleotides including an open reading frame encoding a polypeptide of 1021 amino acids. Two nucleotides in the open reading frame (T321 and A567) differ from the database sequence (Ensembl transcript Q9PSD7\_CHICK, ENSGALT00000014607), but in both cases the nucleotide substitution does not change the encoded amino acid. The integrin alpha4 protein has a large extracellular region consisting of aa 1-967 (of which the first 26 aa constitute a signal peptide), and this is followed by a short transmembrane region (aa 968-990) and a cytoplasmic tail (aa 991-1021). The calculated molecular mass is 114 kDa.

*Conclusions:* mRNA encoding the integrin alpha4 protein is expressed in the chicken, where the protein presumably dimerizes with integrin beta1 or beta7 as in humans. However, further investigations are needed to determine whether the function is similar to that of the human integrin alpha4 protein.

**P-26****EXPRESSION OF DUCK CHEMOKINE RECEPTOR CCR7 AND LIGANDS IN LYMPHOID TISSUES AND INFLUENZA INFECTION**

Ximena Fleming, Craig Brusnyk, Katie Ross, Jianguo Xia, Deb Moon, Katharine Magor

*Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada*

Ducks, the natural reservoir for influenza viruses, typically have asymptomatic infections. Ducks have poor immune responses to low pathogenic avian influenza infection, often leading to re-infection and perpetuation of the virus in nature, and similarly poor responses to agricultural vaccines. In contrast, ducks generate good immune responses to H5N1 highly pathogenic avian influenza. To investigate lymphocyte trafficking in ducks, we have identified orthologues of the homeostatic CC chemokines CCL19 and CCL21, and their receptor CCR7. Mammalian CCL19/21 are involved in homing of naïve lymphocytes and dendritic cells (DC) to secondary lymphoid organs, and CCL19 initiates a proinflammatory Th1 type immune response effective against viruses. To track homeostatic chemokine signaling and contribution of duck tissues to lymphocyte maturation, we studied the gene expression profile in lymphoid and mucosal tissues by reverse-transcription-PCR and real-time PCR. CCR7 and CCL19 expression is highest in spleen, lung and kidney, followed by duodenum and bursa. CCL21 has significant expression in all tissues examined. Although duck lymph nodes have been described, the expression of the chemokines and CCR7 in lymphatic tissue was poor or absent, suggesting they do not significantly contribute to lymphocyte maturation. In the mouse, CCL19/21 recruits lymphocytes and DCs to establish tertiary lymphoid tissue at the site of infection, which contributes to viral clearance. We examined the expression of CCL19/21 in lung or intestine tissues of ducks infected with low pathogenic (A/mallard/BC/500/05) or highly pathogenic avian influenza (A/Vietnam/1203/04) by real-time PCR. We observed a strong up-regulation of CCL19 and intermediate response of CCL21 in VN1203 infected duck lungs at 1 day post-infection, whereas the expression of both chemokines in response to BC500 was not significant. These data suggest that initiation of the adaptive immune response depends on influenza strain in ducks.

**O-27****IMMUNE RESPONSES IN CHICKS FOLLOWING VACCINATION WITH LIVE INFECTIOUS BRONCHITIS VACCINES**

Kannan Ganapathy<sup>1\*</sup>, Lisa Rothwell<sup>2</sup>, S. Lemiere<sup>3</sup>, Pete Kaiser<sup>2</sup>, R. C. Jones<sup>1</sup>

<sup>1</sup>University of Liverpool, Leahurst Campus, Neston, South Wirral, CH64 7TE, United Kingdom

<sup>2</sup>The Roslin institute, University of Edinburgh, Midlothian EH25 9RG, Scotland, United Kingdom

<sup>3</sup>Meril SAS, 13b Avenue Albert Einstein, 69100 Villeurbanne, France

\*E-mail: [gana@liverpool.ac.uk](mailto:gana@liverpool.ac.uk)

Infectious bronchitis (IB) caused by a coronavirus, is an economically important viral disease in chickens, mainly affecting the respiratory and urogenital systems. For the prevention of the disease, live and inactivated vaccines have been used for many decades but due to the ability of the virus to mutate or to form recombinants, occasionally novel variant viruses can penetrate through the protection conferred by existing vaccines. To overcome this, it has been shown that when chicks are vaccinated with two different serotypes/genotypes, there is a higher and broader protection against a range of unrelated IBV challenge viruses. Despite the widespread use of this protocol, the underlying immune mechanisms conferring such wide protection are not known. The present study was undertaken to examine the *in vitro* protection, humoral immune responses and levels of cytokine expressions in chicks vaccinated first with Massachusetts (H120) vaccine followed by a 793B type (CR88) vaccine. SPF chicks were divided into five groups and apart from one group (unvaccinated controls); the other four were vaccinated with H120 or CR88, or both. Chicks were vaccinated at one or 13, or both days. They were monitored for clinical signs and at intervals a number for birds from each group were killed for sampling. Oropharyngeal swabs and samples of tissues (trachea, lungs, spleen and kidneys) were collected for virus detection and for levels of various cytokines by real-time RT-PCR. Blood samples were collected for HI antibodies against common IB viruses. Also at 26 days old, tracheal rings harvested from each group were challenged with five different IBV genotypes.

Humoral ELISA and HI (against M41 and 793B) levels were highest in the group that received H120 at day old followed by CR88 at 13 days old compared to other vaccinated groups. Antibody levels against IBV QX, IT-02 and D1466 were variable. Cytokines expression in trachea and spleen was somewhat variable. It was found that for trachea, IFN- $\gamma$  and IL-1 $\beta$  was continuously up-regulated but other cytokines (eg. IL-6, IL-13) were either up-regulated or down-regulated depending on the sampling days. The tracheal challenge showed a strong homologous protection against M41 and 793B, and a variable degree of protection against IBV QX, IT-02 and D1466 depending on the vaccination regimes. This and other results will be discussed.

**Keywords:** chicks, IBV, vaccination, protection, humoral antibody, cytokines

**O-28****A GENETIC AND FUNCTIONAL ANALYSIS OF THE CHICKEN INTERLEUKIN-1 GENE FAMILY**

Mark Gibson<sup>1</sup>, Mark Fife<sup>1</sup>, Steve Bird<sup>2</sup>, Pat Woo<sup>3</sup>, Pete Kaiser<sup>4</sup>

<sup>1</sup> *Institute for Animal Health, Compton, Berkshire RG20 7NN, United Kingdom*

<sup>2</sup> *Scottish Fish Immunology Research Centre, Department of Zoology, University of Aberdeen, Aberdeen AB24 2TZ, United Kingdom*

<sup>3</sup> *Division of Infection and Immunity, UCL, 46 Cleveland Street, London W1T 4JF, United Kingdom*

<sup>4</sup> *Current address: The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9PS, United Kingdom*

In human, the interleukin-1 (IL-1) family contains 11 genes in three loci. IL-1 $\alpha$ , IL-1 $\beta$ , IL-1RN and IL-1F5-10 are present in a single locus on human chromosome 2, whereas IL-18 and IL-33 (IL-1F11) lie on chromosomes 11 and 9, respectively. There are only two known orthologues in the chicken – IL-1 $\beta$  and IL-18, which lie on chicken chromosomes 22 and 24, respectively.

Two novel putative chicken IL-1 family members have been identified from chicken EST libraries which appear to be orthologues of human IL-1 receptor antagonist (IL-1RN) and IL-1F5. In mammals, IL-1RN inhibits IL-1 activity by physically occupying the IL-1RI receptor and the balance between IL-1 and IL-1RN can critically determine disease severity. Little is known about the function of IL-1F5; however, it appears to act as an anti-inflammatory cytokine through interactions with the IL-1Rrp2 and SIGIRR receptors.

Full-length chicken IL-1RN and IL-1F5 cDNAs have been cloned and sequenced. Two further putative splice variants of IL-1RN have also been isolated. In human, alternative splicing of IL-1RN gives rise to 4 different transcripts, which correspond to secretory and intracellular protein isoforms. Although chicken IL-1RN also appears potentially to have secretory and intracellular isoforms, based on the predicted chIL-1RN gene structure the precise nature of the splice variants differ from those found in human.

There is no conserved synteny in the chicken genome for the large IL-1 family gene cluster found on human chromosome 2. Initial attempts to determine the genomic location of chicken IL-1RN and IL-1F5 have been unsuccessful. To address this problem, fluorescence *in situ* hybridisation (FISH) to chromosome spread preparations will shortly be carried out. Work is ongoing to characterize the biological function and mRNA expression profile of both chicken IL-1RN and IL-1F5.

**O-29****THE CHICKEN IG-LIKE RECEPTOR FAMILY: COMPLEXITY, STRUCTURE AND FUNCTION**

Birgit Viertlboeck, Thomas Göbel

*Institute for Animal Physiology, Department of Veterinary Sciences, LMU Munich, Germany*

The chicken leukocyte receptor complex is located on microchromosome 31 and encodes a large family designated chicken Ig-like receptors (CHIR) that are related to KIR and LILR. Like in mammals, the CHIR can be grouped according to several structural features into the prototypic inhibitory (CHIRB) and activating receptors (CHIRA). In addition, so called CHIRAB combine the charged transmembrane residue with a long cytoplasmic tail containing one to two ITIM, and may therefore reflect bifunctional receptors. Although the entire genomic locus has not been sequenced, preliminary analyses indicate that there are several hundred CHIR present in the genome. The CHIR diversity is further increased by haplotypic differences and allelic variations. It is suggested that the entire microchromosome 31 only encodes CHIR genes and therefore provides a unique locus where a strong selection of CHIR does not compromise unrelated genes. CHIR can be expressed by a wide variety of leukocytes, including macrophages, granulocytes, lymphocytes and natural killer cells. Most CHIR ligands remain elusive, however, the bifunctional CHIR-AB1 functions as a high affinity Fc receptor for the chicken immunoglobulin IgY that is an ancestral molecule of mammalian IgG and IgE. The crystal structure of CHIR-AB1 is most homologous to several LRC encoded proteins, especially KIR, CD89 and NKp46. Mutational analyses on CHIR-AB1 and IgY have shown that the CHIR-AB1 IgY interaction is similar to that of CD89 and IgA. CHIR-AB1 is expressed on several innate immune cells such as granulocytes, macrophages and NK cells, but it is also expressed by B cells. Depending on the cell type and the expression of adaptor molecules such as the common gamma chain, CHIR-AB1 may either activate cells as demonstrated by calcium influx upon CHIR-AB1 crosslinking or inhibit cellular reactions by recruiting SHP-1. Taken together CHIR-AB1 represents a primordial Fc receptor that combines several features of mammalian Fc receptors. Further genomic analyses comparing the Fc receptor carrying loci suggest that the primordial Fc receptor was located within the LRC, and that the mammalian Fc receptor locus has been expanded after the separation of birds and mammals.

**O-30****FROM GENES TO MARKERS: CHARACTERIZATION OF PUTATIVE CHICKEN NK CELL MARKERS**

Marie-Luise Neulen, Birgit C. Viertlboeck, Thomas W. Göbel

*Institute for Animal Physiology, Department of Veterinary Sciences, LMU Munich, Germany*

---

Mammalian NK cells have been intensively characterized utilizing markers that can be divided into three groups. The Ig-like receptors encoded by the leukocyte receptor complex resemble markers such as KIR or NKp46, whereas the second group consists of C-type lectins encoded by the natural killer complex, including Ly49, NKR-P1 or CD94/NKG2 genes. The third group is heterogeneous with genes spread across the genome such as CD56, CD122 or 2B4. Most of these molecules are not entirely specific for NK cells, but have overlapping reactivity with T cell subsets. The generation of chicken NK cell specific markers has been restricted by their low frequency and the minimal knowledge regarding gene orthologues of mammalian NK cell markers.

The chicken genome information now allows to specifically identify some of these genes and to examine their relevance as NK cell markers. One prototypic chicken Ig-like receptor (CHIR) family member CHIR-AB1 as homologue of the LRC genes that functions as a high affinity Fcγ receptor was characterized as a very useful marker for chicken NK cells in combination with a mab against the CD11c/CD18 molecule. The chicken NKC locus consists of only two genes that may resemble CD69 and CD94 homologues. We have generated mabs against CD94 and found that it is mainly expressed on chicken thrombocytes, but may also be a marker of NK cells. CD56 has been the marker for human NK cells, but it is not expressed on NK cells of various other species such as mice, rats or cattle. The specific mab generated against chicken CD56 are reacting with a small subset of cells in PBL that is CD3 negative and partially coexpresses CD8. Moreover, CD56 reactive cells are also found in the embryonic spleen, a rich source of NK cells. The careful analyses of these new markers in combination with qPCR assays that we have established for amplification of CD122, 2B4, perforin and the NK cell specific transcription factor NFIL3 should enable a more detailed characterization of chicken NK cells in normal or diseased tissues.

**O-31****THE CHICKEN IFN- $\lambda$  RECEPTOR COMPLEX**

Kate E. Goossens<sup>1,2</sup>, Alister C. Ward<sup>2</sup>, Andrew G. D. Bean<sup>1</sup>

<sup>1</sup> CSIRO, Australian Animal Health Laboratories, Geelong, Victoria, Australia

<sup>2</sup> School of Medicine, Deakin University, Geelong, Victoria, Australia

The control of inflammatory processes, like those observed during H5N1 avian influenza (AI) infection, is critical for the beneficial outcome of disease. Moreover, the recent emphasis on 'cytokine storm' as a critical factor contributing to H5N1 AI pathology suggests new strategies are needed for the control of cytokine inflammatory responses. Cytokines and their receptors play critical roles in the response to pathogens and the resolution of infection. The IL10R2 chain is one of the most versatile receptor subunits within the class II cytokine receptor family (CRF2), representing a common subunit of the receptors for interferon (IFN)- $\lambda$ , interleukin (IL)-10 and the IL-10 related cytokines, as well as, IL-22 and IL-26. At present, there is paucity of information about IL10R2 in the chicken, however, recent identification of IFN- $\lambda$  and its antiviral therapeutic potential in chickens highlights the need for further information regarding IL10R2. Here, we have characterized the chicken IL10R2. Chicken IL10R2 shows 79% and 74.6% amino acid sequence conservation with the human and mouse IL10R2, respectively. Furthermore, chicken IL10R2 possesses the two extracellular fibronectin type III (FN3) domains, a transmembrane domain and a short intracellular domain, like mammalian CRF2 molecules. Analysis of the expression of chicken IL10R2 showed it was detected across a wide range of tissues, with the highest levels observed in both lung and spleen. Similarly, chicken cell lines, such as DF1 fibroblast, HD11 macrophage-like cells and LMH liver epithelial cell line, were also positive for IL10R2 expression. To investigate IL10R2 function RNAi mediated gene knock-down was used to target IL10R2 in these cell lines. This gene silencing approach lead to 80% knockdown of IL10R2 mRNA *in vitro*. Future work will focus on manipulation of chicken IL10R2 to assess the role of this receptor complex molecule in the signaling response of cytokines, such as IFN- $\lambda$ .



## O-32

### AUTOMATED DIFFERENTIAL BLOOD COUNT IN THE CHICKEN

Sonja Härtle, Christian Seeliger, Bernd Kaspers

*Dept. of Veterinary Sciences, Institute for Animal Physiology, LMU Munich, Germany*

---

The most frequently used screening system for the analysis of the immunological status of animals is the differential blood count. While this procedure is well established and fully automated in mammalian medicine and research, differential blood counts in birds have proven to be difficult due the presence of nucleated erythrocytes and thrombocytes. Thus, currently used procedures relay on the semiquantitative counting under the microscope which is very time consuming and highly error prone.

We have established a new flow cytometry based method which allows the rapid quantification of blood leukocytes and thrombocytes and the analysis of absolute and relative cell numbers of leukocyte subpopulation. Through the use of directly conjugated chicken leukocyte specific monoclonal antibodies in combination with problem based gating strategies T-cells, B-cells, heterophilic granulocytes as well as monocytes and thrombocytes can be analysed in a one step procedure. If required, additional lymphocyte subsets can be included in the protocol.

EDTA blood samples can be collected by the researcher or clinician at virtually any location in the world. Samples are prepared for shipment through the addition of a fixative and should arrive in the analytic laboratory within 3 days. Blood samples are then subjected to a 4- or 5-colour flow cytometry staining applying a single-tube- no lyse-no wash strategy thereby avoiding changes in sample composition which could result from additional purification and treatment procedures.

This technology has proven to be highly reliable and intra- and interassay coefficients of variation in the range of 5-10%. We propose that this automated differential blood counting method can be used for a variety of applications including poultry flock monitoring and vaccine safety testing as well as basic research in avian immunology.

**O-33****EFFECTS OF CD40L ON CHICKEN B-CELL DEVELOPMENT AND DIFFERENTIATION**

Sonja Härtle<sup>1</sup>, Magdalena Schumacher<sup>1</sup>, Katharina Schenk-Weibhauser<sup>1</sup>, John Young<sup>2</sup>, Bernd Kaspers<sup>1</sup>

<sup>1</sup> Dept. of Veterinary Sciences, Institute for Animal Physiology, LMU Munich, Germany

<sup>2</sup> Institute for Animal Health, Compton, United Kingdom

The CD40/CD40L system of humans and mice is essential for the initiation of Germinal center (GC) reactions leading to class switch recombination and affinity maturation of B cells followed by the differentiation of B cells into antibody secreting plasma cells.

Recently homologues of CD40L and CD40 were identified in the chicken. We showed that both immature B cells in the bursa of Fabricii as well as mature peripheral B cells do express CD40 and that massive proliferation can be induced in these cells by stimulation with recombinant chCD40L. The addition of chIL-10 further increased the proliferative activity in a synergistic way. Analysis by flow cytometry and electron microscopy of B cells cultured in the presence of soluble CD40L revealed a large number of cells with a plasmablast like phenotype. These blasts upregulate expression of CD80 and loose expression of B cell surface markers chB6 and IgM while the amount of cytoplasmic IgM increases. Elispot analysis demonstrated that the presence of chCD40L alone is not sufficient to induce *in vitro* class switch to IgY and IgA while stimulation with chCD40L and chIL10 does produce IgY and IgA secreting cells.

By means of quantitative RT-PCR we were able to show that stimulation of spleen B cells with soluble CD40L leads to a decreased expression of Pax-5 and Bcl-6 while an increased expression of AID and Blimp-1 mRNA was detectable. These data suggest that CD40L induces *in vitro* differentiation of chicken B cells which is regulated by lineage specific transcription factors, as such corresponding to the established system in mice.

For *in vivo* studies the extracellular domain of CD40 was expressed in chickens as a soluble decoy receptor using the retroviral gene transfer system RCAS. First experiments show that chickens expressing the decoy receptor have significantly decreased amounts of total plasma IgM while amounts of IgY were only marginally reduced or unaffected. Interestingly, in these birds the size of the bursa as well as relative and absolute B cell numbers in the blood were significantly reduced indicating an additional role of CD40/CD40L for early B cell maturation in the bursa of Fabricius.

*This work is supported BMBF program FugatoPlus.*

**O-34****IDENTIFICATION OF REGULATORS IN EARLY CHICKEN B CELL DEVELOPMENT BY COMBINED DIFFERENTIAL TRANSCRIPTOME AND PROTEOME ANALYSIS OF THE BURSA OF FABRICIUS**

Julia Korte<sup>1</sup>, Stefan Schmieder<sup>1</sup>, Stefan Bauersachs<sup>2</sup>, Georg J. Arnold<sup>2</sup>, Bernd Kaspers<sup>1</sup>, Sonja Härtle<sup>1</sup>

<sup>1</sup> *Institute for Animal Physiology, Department for Veterinary Sciences, LMU Munich, Germany*

<sup>2</sup> *Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Germany*

Like in mammals antibody producing B cells form an essential part of the adaptive immune system in the chicken. However, in chickens early B cell differentiation and diversification of the antibody repertoire do not take place in the bone marrow but in a specialised gut associated lymphoid (GALT) tissue, the Bursa of Fabricius. While the molecular mechanisms of gene conversion, the chicken's way to generate B cell diversity, is comparatively well understood, very little is known how immigration of B cell precursors into the Bursa, massive proliferation of early B cells and subsequent emigration of mature B cells into the periphery are regulated.

To identify possible regulators of bursal B cell development we analysed the bursa transcriptome at relevant developmental stages (embryonic day 10 / 18, day 2 / 28 post hatch) using a customized Agilent 4x44K chicken genome array, complemented with a multitude of genes with known relevance for murine and human B cell development. In addition, corresponding samples were subjected to a differential proteome analysis using two-dimensional difference gel electrophoresis (2D-DIGE), which identified about 200 differentially abundant proteins during Bursa development. A first principal component analysis of the obtained data sets showed a strong discrimination of gene and protein expression profiles between embryonic day 10 and all other time points as well as clear differences between the immature (ed18, d2) and mature (d28) organ. Detailed examination of significantly expressed genes over time revealed several gene clusters with characteristic expression profiles including cytokines, chemokines and adhesion molecules. IL-7 e.g. is upregulated in the embryonic bursa and downregulated after hatch, while lymphotaktin (XCL1) is strongly upregulated after hatch. Taken together our studies identified many candidate genes which can now be further characterized to provide a better insight into the regulation of B cell development in the chicken and gives first information on chemokines involved in the migration of pre-bursal and post-bursal B cells.

*This work is supported by BMBF program FugatoPlus.*

**O-35****CHARACTERIZATION OF THE GERMINAL CENTRE REACTION IN CHICKEN**

Susanne Hainke, Bernd Kaspers, Sonja Härtle

*Institute for Animal Physiologie, LMU, Munich, Germany*

Processes in the germinal centre (GC) like affinity maturation and somatic hypermutation are essential to mount an effective humoral immune response in mammals. In order to characterize the development and the regulation of GC-formation in chicken we first established an immunization system which allows us to induce and detect antigen-specific GCs. Analysis of the kinetics of GC formation showed, that 3 days post immunization antigen-immune-complexes were deposited on follicular dendritic cells (FDCs) in GCs in the spleen. As soon as 1 week post immunization GCs with antigen-specific B-cells were detectable. The number of antigen-specific GCs remained constant until two weeks post immunization but strongly decreased by 4 weeks.

The typical organisation of mammalian GCs in a dark and a light zone is absent in avian GCs. However, immunohistological analysis showed that FDCs with immune complexes are concentrated in the GC centre. In addition, BrdU pulse labelling for 3h revealed a ring of BrdU+ proliferating B-cells in the border area of chicken GCs, suggesting that the GC periphery corresponds to the mammalian dark zone which contains rapidly dividing centroblasts. Interestingly, when organ samples were taken 20 hours after BrdU pulse labelling proliferating B-cells were equally distributed over the whole GC, strongly indicating centroblast migration from the border into the centre of the GC.

To characterize the influence of the B-cell-cytokine chBAFF on the GC reaction chBAFF was overexpressed in chicken by the retroviral gene transfer system RCASBP(A). RCAS based chBAFF-over-expression led to a strong increase in peripheral B-cell-numbers and total serum IgM levels. Interestingly, preliminary data show that the number of total and antigen-specific GCs in these birds is reduced while the size of GCs is unaffected.. Hence, chBAFF does not seem to support but rather to block B cell entrance into the GC reaction.

**P-36****THORACIC AORTA REGION COLONIZES LYMPHO-MYELOID ORGANS**

Krisztina Minkó<sup>1,2</sup>, Arianna Caprioli<sup>2</sup>, Karine Bollérot<sup>2</sup>, Françoise Dieterlen-Lievre<sup>2</sup>, Thierry Jaffredo<sup>2,3</sup>, Nándor Nagy<sup>1</sup>

<sup>1</sup> *Semmelweis University, Department of Human Morphology and Developmental Biology 1094 Budapest, Tűzoltó u. 58, Hungary, minko.krisztina@med.semmelweis-univ.hu*

<sup>2</sup> *Institut d'Embryologie cellulaire et moléculaire, Nogent-sur-Marne, France*

<sup>3</sup> *present address: Université Pierre et Marie Curie-Paris 6, Centre National de la Recherche Scientifique UMR7622, Laboratoire de Biologie du Développement, Paris, France*

During embryonic development stromal hematopoietic organ rudiments are colonized by extrinsic, blood-borne precursors in order to become functional. This dogma has been accepted from the 60's, when Moore and Owen (1965) demonstrated by parabiotic bird embryos, that in lymphatic organs hematopoietic cells do not emerge in situ from the mesenchyme. They proposed that the yolk sac is the only source of the seeding cells. Yolk-sac chimeric experiments turned the attention to the intraembryonic source of hematopoietic progenitors. However, the origin of seeding cells was not determined. In higher vertebrates the first signs of the intraembryonic hematopoiesis are associated to the developing aorta region. We have investigated the developmental potential of the embryonic chicken aorta region at the stage, when intraaortic cell clusters emerge and hematopoietic cells express early markers, such as the SCL/tal-1, Lmo2, GATA-2 transcription factors. On the 3rd day of development thoracic part of the aorta of quail embryos was grafted into the coelom of age-matched chicken embryos. After incubation the graft derived cells colonized the chicken lympho-myeloid organs (thymus, bursa of Fabricius, bone marrow and spleen), which were analyzed by flow cytometry and immunocytology using quail hematopoietic and endothelial cell specific antibody, QH1. Both flow cytometry and QH1 immunocytochemistry indicated that the thymus was preferentially colonised by aorta derived cells.

**O-37*****SALMONELLA ENTERICA* SEROVAR ENTERIDIS (SE) INFECTION IN 5 COMMERCIAL WHITE EGG AND 3 COMMERCIAL BROWN EGG STRAINS OF LAYING HENS: COMPARISON OF SE COLONIZATION WITH IMMUNE RESPONSIVENESS**

Peter S. Holt<sup>1</sup>, Lara E. Vaughn<sup>1</sup>, Richard K. Gast<sup>1</sup>, Kenneth E. Anderson<sup>2</sup>

<sup>1</sup> USDA/ARS Egg Safety and Quality Research Unit, Athens, Georgia, United States

<sup>2</sup> Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, United States

Different chicken breeds have been shown to exhibit varying susceptibility to infection with paratyphoid salmonellae. The current study was undertaken to compare the course of infection with SE in crops and intestines of 5 commercial white egg strains (W1-W5) and 3 commercial brown egg strains (B1-B3) of laying hens and attempt to correlate these infections with crop antibody responses and the ability of lymphocytes from the infected hens to respond *in vitro* to SE lipopolysaccharide (LPS) and SE flagella. Hens were orally challenged with 10<sup>8</sup> SE and crop lavage and fecal samples were collected weekly. Weeks 2 and 4 post infection (PI), 5 hens from each strain were bled via the jugular vein, lymphocytes purified and incubated with LPS or flagella, and proliferation measured 72 hours later. All of the white egg hens were 80-100% crop SE positive at one and two weeks PI and then levels decreased thereafter. This is in contrast to hen strain B3 and B1 which were 20% and 40% crop positive, respectively. However, examination of fecal shedding showed that all strains of hens were similar, with 80-100% of all the hens exhibiting fecal SE at week 1 PI. Crop IgA anti-SE antibodies were similar from all hen strains indicating that the lower SE levels in B3 and B1 crops was not due to higher antibody levels. Lymphocyte response to LPS and flagella were generally low at week 2 PI and strongly increased at week 4. Hen strain W4, however, exhibited high lymphocyte activity to both antigens prior to infection and this increased 2 weeks PI before decreasing at week 4. This high activity did not appear to impact crop or intestinal SE colonization as both sites were 100% SE+ at 1 week PI. The hen strain that exhibited low crop SE colonization, B3, responded strongly *in vitro* to both antigens and exhibited the highest response of all the strains at week 4 PI. These results indicate that different hen strains vary in their crop colonization by SE but this cannot be extended to gut colonization. The highest specific *in vitro* response to SE antigen was observed in lymphocytes taken from the hen strain that exhibited the lowest crop SE colonization but more studies need to be conducted before any conclusions can be drawn from these observations.

**O-38****ENHANCED ACTIVATION OF CHICKEN DENDRITIC CELLS UPON INFECTION WITH HIGH PATHOGENIC AVIAN INFLUENZA VIRUS**

Christine Jansen<sup>1</sup>, Sylvia Reemers<sup>1</sup>, Jacob Post<sup>2</sup>, Annemarie Rebel<sup>2</sup>, Lonneke Vervelde<sup>1</sup>

<sup>1</sup> *Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

<sup>2</sup> *Central Veterinary Institute, Wageningen UR, Lelystad, The Netherlands*

Infection with high pathogenic influenza virus has been reported to induce a cytokine storm caused by uncontrolled activation of the host's innate immune system in humans, mice and macaques. We hypothesize that dendritic cells (DC) may play a role in this process, since DC play a key role in regulating the immune response against influenza. DC produce pro-inflammatory cytokines and chemokines upon influenza infection. These may have a direct anti-viral effect or activate other players of the innate or adaptive immune system.

We investigated possible differences in DC activation upon infection with low pathogenic avian influenza virus (LPAI) and high pathogenic avian influenza virus (HPAI) using chickens, a natural host.

Bone marrow was isolated from 3 week old Lohman Brown chickens, and cells were cultured for 6 days in the presence of recIL-4 and recGM-CSF. At day 6, DC were infected with the LPAI strain H7N1 (A/chicken/Italy/1067/99) or the HPAI strain H7N1 (A/turkey/Italy/4580/99). DC were harvested at 4, 16 and 24 hours post infection and mRNA levels of the viral M1 protein, a panel of pro-inflammatory cytokines, chemokines and toll like receptors (TLR) were analysed by qPCR. Flowcytometry was performed to determine expression of DC activation markers (CD40, CD80, CD86) after LPAI infection.

Higher viral RNA levels were observed after infection with HPAI compared to LPAI (4 hrs post infection: 45-Ct 15.5 versus 12.1). Viral RNA levels after HPAI infection increased over time, while viral RNA levels after LPAI infection did not change (24 hrs post infection 45-Ct 24.3 versus 11.5). BM-derived DC infected with HPAI showed higher levels of IL-18, iNOS, IFN $\alpha$ , IL-12 $\beta$  and TLR21. Less IL-1 $\beta$  was observed after HPAI infection. Interestingly, infection with LPAI did not affect DC activation: similar levels of cytokines, chemokines, TLRs and activation markers were found in LPAI infected and uninfected DC.

In conclusion, infection of BM-derived DC with LPAI does not affect DC activation or function. Infection with HPAI results in enhanced production of some pro-inflammatory cytokines and chemokines, but in decreased levels IL-1 $\beta$ . We are currently investigating if these results are specific for the H7N1 viruses, or represent a more general difference between LPAI and HPAI.

*This work is financially supported by EU 6th framework Flupath (grant 044220) and Program "Impulse Veterinary Avian Influenza Research in the Netherlands", Dutch Ministry of Agriculture, Nature and Food Quality, and the Netherlands Organisation for Scientific Research (NWO, veni scheme)*



**O-39****ENHANCED NK-CELL FUNCTION AFTER INFECTION WITH AVIAN INFLUENZA VIRUS: STUDIES IN A NATURAL HOST**

Christine Jansen<sup>1</sup>, Peter van de Haar<sup>1</sup>, Daphne van Haarlem<sup>1</sup>, Willem van Eden<sup>1</sup>, Thomas Göbel<sup>2</sup>, Lonneke Vervelde<sup>1</sup>

<sup>1</sup> *Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

<sup>2</sup> *Institute for Animal Physiology, Department of Veterinary Sciences, Ludwig Maximilians University Munich, Munich, Germany*

Natural killer (NK) cells are important in the early defence against viruses. Mice that lack NK cells or the influenza-specific activating NK-cell receptor NKp46 died after influenza infection, suggesting a role for NK cells in influenza-specific immunity. As mice are not a natural host for influenza, it is not possible to distinguish between low and high pathogenic influenza viral infections.

We investigated the NK-cell response after influenza infection in chickens, which are a natural host. Chicken NK cells have been described as a population of CD3-CD8 $\alpha$ <sup>+</sup> cells. Previously, we identified 5 new markers for chicken NK cells and developed assays to measure NK-cell degranulation (reflected by CD107 expression on the cell surface) and killing. These tools were used to study chicken NK cells after infection with the low pathogenic avian influenza strain H9N2.

Three-week old Lohman Brown chickens were infected and sacrificed daily between 0 and 6 days post infection (dpi). Viral load was detected in lung, PBMC and to a lesser extent in spleen between 1 and 5 dpi. Infection with influenza resulted in an increase in CD3-CD8 $\alpha$ <sup>+</sup> cells in the lung that peaked at 1 and 3 dpi. Differences in frequencies of marker<sup>+</sup> cells were observed: for 3 markers the highest percentage of cells was observed at 1 dpi, for 2 markers frequencies peaked at 3 dpi. Enhanced NK-cell degranulation in the lung was observed at 1 and 4-6 dpi.

In conclusion, infection with AI results in infiltration of specific populations of NK cells in the lung and in enhanced activation of chicken NK cells. More studies are warranted to investigate the existence of different NK-cell populations and their contribution to differences in clinical outcome between low and high pathogenic influenza viral infections.

*This work is financially supported by EU EU 6th framework Flupath (grant 044220) and Program "Impulse Veterinary Avian Influenza Research in the Netherlands", Dutch Ministry of Agriculture, Nature and Food Quality, and the Netherlands Organisation for Scientific Research (NWO, veni scheme)*

**O-40****CD8+ T-CELL RESPONSES AGAINST AVIAN INFLUENZA VIRUS:  
THE IDENTIFICATION OF INFLUENZA SPECIFIC CD8+ T-CELL  
EPITOPES IN CHICKENS**

Sylvia Reemers, Christine Jansen, Alice Sijts, Lonneke Vervelde

*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine,  
Utrecht University, Utrecht, The Netherlands*

Avian influenza virus (AIV) infection is a continuing threat to both humans and poultry. In order to control outbreaks, effective vaccines are warranted. Current vaccines against AIV mainly induce antibodies directed towards highly variable viral surface proteins, and cross protection against other strains is limited. CD8+ T-cell inducing vaccines are targeted towards conserved viral proteins and may provide better protection against newly arising virus strains. Studies in humans and mice have shown an important role for CD8+ T cells in the clearance of influenza infection. However, in chickens the role of influenza-specific CD8+ T cells is less clear. In this study we set out to identify influenza-specific CD8+ T cell epitopes.

Prediction of CD8+ T-cell epitopes in the AIV proteins NP and M1 resulted in 33 B12- restricted, 14 B19-restricted, 4 B4-restricted, 1 B15-restricted and 6 B21-restricted peptides. MHC I inbred chickens were infected with the low pathogenic AIV strain H7N1 (A/chicken/Italy/1067/99) and sacrificed at 5, 7, 10 and 14 days post infection (dpi). Lymphocytes isolated from lung, spleen and blood were stimulated *ex vivo* with influenza-specific pooled (B4, B12, B19, B21) or individual (B15) peptides and the production of IFN $\gamma$  was determined by Elispot. Frequencies of CD4, CD8 $\alpha$  and CD8 $\beta$  T cells were analysed by flowcytometry.

Infection with AIV resulted in an increase in CD8 $\alpha$ , CD8 $\beta$  and CD4+ T-cells in the lung. Influenza-specific B12-restricted CD8+ epitopes were found in lungs of infected chickens at 7 (7 pools), 10 (7 pools), and 14 dpi (4 pools). At 10 dpi, 1 B4-restricted epitope was observed, while no B15-, B-19 and B21-restricted epitopes were found. No IFN $\gamma$  production was observed in lung cells from uninfected chickens. The individual peptides from each positive pool are currently screened to identify single epitopes.

In conclusion, we have for the first time identified influenza-specific CD8+ T-cell epitopes in chickens. This knowledge can be used to study the role of CD8+ T cells against influenza infection in more detail, and may be important for vaccine development.

*This work is financially supported by the Program "Impulse Veterinary Avian Influenza Research in the Netherlands", Dutch Ministry of Agriculture, Nature and Food Quality*

**P-41****GENETIC DIVERSITY OF CHICKEN INTERLEUKIN-17 CDNA FROM CHINESE NATIVE AND WESTERN BREEDS**

Wen Zhao, Zhiming Pan, Shizhong Geng, Qiang Fang, Xinan Jiao

*Laboratory of Zoonosis and Immunology, Yangzhou University, Yangzhou, Jiangsu 225009, China*

Interleukin-17 (IL-17) is a proinflammatory cytokine produced by activated T cells, enhances T cell priming and stimulates fibroblasts, endothelial cells, macrophages, and epithelial cells to produce multiple proinflammatory mediators, including IL-1, IL-6, TNF- $\alpha$ , metalloproteases, and chemokines, resulting in the induction of inflammation. Chicken IL-17 (chIL-17) shared 37%–46% amino acid sequence identity to the mammalian homologs. A part of the Interleukin-17 (IL-17) gene which is about 510bp was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from the total RNA of concanavalin A (ConA)-stimulated splenic lymphocytes. The IL-17 cDNAs of six chicken breeds, including Langshan chicken, Longxima chicken, Anka chicken, Yinxingbaiyu chicken, Shiqiza chicken and specific pathogen-free white Leghorn chicken, were amplified. Sequencing of positive clones showed that interleukin 17F (IL-17F) from all of the six strains exhibits more than 99% homology with the mRNA sequence of *Gallus gallus* referred in NCBI. By comparing the sequences, we found that a mutation G-A occurred in the 477 point in white Leghorn chicken and Yinxingbaiyu chicken; sites were in 17, 81, 189, 222, 477 occurred A-G, A-C, C-G, C-T, G-A mutation in Longxima chicken and Shiqiza chicken, one of which leads to an amino acid changing Tyr-6-Cys in the Shiqiza chicken; T-C (Leu-50-Pro), A-G (Ile-80-Val), G-A mutations occurred in Anka chicken in the sites of 149, 238, 477 and A-G (Tyr-6-Cys), T-C (Val-16-Ala), A-C, C-G, C-T mutations in Langsha chicken in the sites of 17, 47, 81, 189, 222 separately. After subcloned into prokaryotic expression vector, the recombinant plasmid pGEX-6P-1-IL17 was obtained. Then the recombinant plasmid was transformed into *E.coli* BL21, and the recombinant was designated as BL21 (pGEX-6P-1-IL17). The result of SDS-PAGE verified that a desired recombinant protein rGST-IL17 with molecular weight of 46000 expressed in BL21 (pGEX-6P-1-IL17). Western blot analysis further indicated this expressed product had the immunogenicity of chicken IL17 protein.

**Keywords:** *Chicken, Chinese Native Breeds, IL-17 cDNA, Genetic Diversity*

**P-42****DEVELOPMENT AND APPLICATION OF AN MAB-BASED SANDWICH ELISA IN THE ASSESSMENT OF CHICKEN INTERFERON- $\gamma$  RESPONSE TO THE LASOTA VACCINE OF NEWCASTLE DISEASE VIRUS**

Hua Dai, Junhua Chen, Zhiming Pan, Xinan Jiao

*Laboratory of Zoonosis and Immunology, Yangzhou University, Yangzhou, Jiangsu 225009, China*

The purpose of this study is to establish and evaluate an mAb-based ELISA for the measurement of chicken IFN- $\gamma$  (ChIFN- $\gamma$ ) response in chickens vaccinated by Lasota vaccine of Newcastle disease virus (NDV). Using lymphocyte hybridoma technique, four hybridoma cell lines secreting mAbs against ChIFN- $\gamma$  were obtained and named as 1G10, 2C3, 3E3, 3E5. Dot-ELISA, IFA and Western-blot results showed that they only react with ChIFN- $\gamma$ . And then, an mAb-based sandwich ELISA was developed. Then the efficiency of this sandwich ELISA was compared with standard chicken IFN- $\gamma$  CytoSet™. The results also indicated that double mAb sandwich ELISA is suitable to measure ChIFN- $\gamma$  in a variety of formats, it could be used as a tool to measure the in vitro release of ChIFN- $\gamma$  by T cells in response to special recall antigen. In the experiment infection of NDV, 4-week-old SPF chickens were divided into 2 groups, one group was immunized with NDV LaSota vaccine intranasally, the other group was the control. At the 7th, 14th, 21th, 28th day post immunization, 4 chickens per group were killed and their splenocytes were stimulated by recombinant NDV F protein. The stimulated splenocytes were measured by ChIFN- $\gamma$  production. The IFN- $\gamma$  response induced by NDV LaSota vaccine has been evaluated sequentially by antigen-specific ChIFN- $\gamma$  production from immunized chickens. The data showed that NDV LaSota vaccine is capable of stimulating CMI responses to NDV in chickens as measured by the ChIFN- $\gamma$  sandwich ELISA, the vaccinated chickens produced ChIFN- $\gamma$  after the stimulation of recombinant NDV F protein as the recall antigen during the 4 weeks of the study. The serum HI titers of immunized chickens showed that the vaccine also induce high level of protective antibodies in this period studied. It revealed that antigen specific ChIFN- $\gamma$  production can be used as a good indicator of immunity to NDV, and the sensitivity range of the sandwich ELISA could measure the ChIFN- $\gamma$  in different origins.

**Keywords:** Newcastle Disease Virus, MAb-based Sandwich ELISA, Interferon- $\gamma$  Response

**P-43****MANNOSE-BINDING LECTIN AFFECTS PHENOTYPE AND FUNCTIONS OF HD11 CHICKEN MACROPHAGES**

Hong Shen<sup>1,2</sup>, Liselotte R. Norup<sup>2</sup>, Tina S. Dalgaard<sup>2</sup>, Helle R. Juul-Madsen<sup>2</sup>

<sup>1</sup> *Animal Science and Technology College, Beijing University of Agriculture, Beijing 102206, China*

<sup>2</sup> *Department of Animal Health and Bioscience, Aarhus University, 8830-DK Tjele, Denmark*

Mannose-binding lectin is an innate immune receptor that recognizes structurally conserved pathogen-associated molecular patterns. Mannose-binding lectin has a high affinity for binding to mannose and other sugar residues present on the cell surface of bacteria, viruses and parasites. Upon binding to its targets, mannose-binding lectin activates the complement cascade through the 'lectin pathway' resulting in cell lysis or opsonization with complement factor 3 fragments. Mannose-binding lectin can also act directly as an opsonin and dramatically increase phagocytosis mediated by neutrophils, monocytes, and macrophages which express receptors for mannose-binding lectin.

Avian macrophages perform similar functions as mammalian macrophages serving as the first line of immune defense as an important cellular component in both innate and adaptive immunity. Upon stimulation the macrophages generate reactive oxygen radicals nitric oxide and secrete pro-inflammatory cytokines and chemokines that subsequently promote the adaptive immune responses necessary for the control of an infection. Mannose-binding lectin affects this innate immune response either positively or negatively depending on the microbe and the concentration of mannose-binding lectin in serum.

In this study, we will show that a high concentration of chicken mannose-binding lectin enhances microbial phagocytosis by increasing the proportion of HD11 chicken macrophages able to recognize microbes and decreases the production of nitric oxide. Furthermore, the expression of four different surface markers (CD44, CD45, KUL 01, and MHC II) was measured. Two of the markers may be decreased in the presence of a high concentration of mannose-binding lectin.

*This work was granted by Scientific Research Program of Beijing Municipal Commission of Education, and supported by CIRIUS of Denmark and China Scholarship Council.*

**P-44****THE EFFECT OF *ASCARIDIA GALLI* ORAL AND INTRA-MUSCULAR IMMUNISATION ON CHICKEN IMMUNE RESPONSE, AS WELL AS ON PARASITE EGG EXCRETION AND WORM LOAD FOLLOWING INFECTION**

Janne Pleidrup, Liselotte R. Norup, Tina S. Dalgaard, Rikke M. Kjærup, Helle R. Juul-Madsen

*Department of Animal Health and Bioscience, Aarhus University, 8830-Tjele, Denmark*

---

Parasite infections in poultry are common in deep-litter systems and in flocks with outdoor access. These infections cause a welfare problem to the hens and great economic losses for the farmers. In the EU, a ban on hens kept in ordinary cages will be a fact in 2012 and will lead to even more flocks in alternative production systems. *Ascaridia galli* (*A. galli*) is a helminth parasite found in the avian intestine. Parasite infections are found with flock prevalences up to 100%, hence the demand for a vaccine is high.

No avian roundworm vaccines have yet been developed. Development and production of an *A. galli* vaccine requires detailed knowledge of the functions and mechanisms of the chicken immune response to the parasite. Only little research on avian parasitic infections and immune response has been performed so far, therefore the objective of this study was to evaluate the chicken immune response to *A. galli* immunisation and infection.

*A. galli* crude extract was used as an immunisation candidate. Three different groups of chickens were tested; 1) a control, 2) an orally immunised and 3) an intra-muscularly immunised. Cholera toxin B-unit was used as an adjuvant. Chickens at the age of 4 weeks, were immunised 3 times over a 10-week period and later infected with *A. galli*. Samples were collected throughout the experimental period.

The chicken immune response was measured as *A. galli* specific immunoglobulin titres. Furthermore, immune cell phenotyping of different white blood cell subtypes was performed in different immunological tissues. Finally, after the infection the chickens were examined for parasite egg excretion in faeces and for worm load.

*Results from the study will be presented.*

**O-45****INTRANASAL APPLICATION OF ALPHA INTERFERON REDUCES MORBIDITY ASSOCIATED WITH LOW PATHOGENIC AVIAN INFLUENZA INFECTION**

Darrell R. Kapczynski<sup>1</sup>, Hai Jun Jiang<sup>2</sup>

<sup>1</sup> Southeast Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Athens, Georgia 30605, United States

<sup>2</sup> Division of Animal Molecular Virology and Immunology, China Agricultural University, Beijing, China

E-mail: darrell.kapczynski@ars.usda.gov

Type I interferons, including interferon alpha (IFN- $\alpha$ ), are expressed rapidly after viral infection, and represent a first line of defense initiated by the innate immune response. Following infection of chickens with avian influenza virus (AIV), transcription of IFN- $\alpha$  is quickly up regulated along with a myriad of other immune-related genes. In these studies, we determined the protective potential of IFN- $\alpha$  applied to birds prior to exposure to the H6N2 strain A/Chicken/California/K0301417/2003 and H7N2 strain A/Turkey/Virginia/4259/2002 low pathogenic AIV. Intranasal application with IFN- $\alpha$  prior to and during active AIV infection reduced clinical signs of disease, including weight loss and fever, compared to PBS treated controls. In addition, the incidence of viral shedding and viral titers from oral swabs was significantly reduced in IFN- $\alpha$  treated birds. Taken together, these studies show that IFN- $\alpha$  can protect chickens from disease associated with low pathogenic AIV and reduce the risk of transmission through decreased shedding. While this is a first step in determining innate protection against AIV in chickens, the effector molecules responsible for enhanced protection as well as scope of strains susceptible to IFN- $\alpha$  treatment remains to be determined.

**Keywords:** Interferon alpha, influenza A virus, chickens





## O-46

### THE USE OF HIGH THROUGHPUT SEQUENCING FOR AVIAN TRANSCRIPTOME ANALYSIS

Calvin L. Keeler Jr.<sup>1</sup>, Cynthia Boettger<sup>1</sup>, Michele N. Maughan<sup>1</sup>, John K. Rosenberger<sup>2</sup>, Carl Schmidt<sup>1</sup>

<sup>1</sup> *Dept. of Animal and Food Sciences, University of Delaware, Newark, Delaware, 19716-2150, United States*

<sup>2</sup> *Aviserve, Newark, Delaware, 19711, United States*

---

Microarrays have proven to be useful tools for studying the avian transcriptional response to infection by bacterial, viral, or protozoan pathogens. However, microarrays are limited by the need to identify elements to place on the array and by several technical limitations. We are currently evaluating the utility of using next generation high throughput sequencing to measure avian transcriptional activity. Initial studies using control chicken spleen RNA have shown that transcriptional activity can be detected in >90% of the predicted genes. As expected for the spleen, the most abundant transcripts were clustered in genetic elements involved in protein translation, immunoglobulin production, and the MHC. Three of the most abundant transcripts were found to be ferritin (7,238 counts/million/kb), elongation factor 1 (5,036 counts/million/kb), and the MHC Class II invariant chain CD47 (5,871 counts/million/kb). This report will focus on an examination of the transcriptional response in the spleen, thymus, and bursa of commercial broilers to infection with infectious bursal disease virus.

**P-47****STRUCTURAL GENE VARIANTS OF CHICKEN MANNOSE-BINDING LECTIN AND ASSOCIATION WITH SERUM CONCENTRATIONS**

Rikke M. Kjærup, Tina S. Dalgaard, Liselotte R. Norup, Janne Pleidrup, Helle R. Juul-Madsen

*Department of Animal Health and Biosciences, Aarhus University, 8830-Tjele, Denmark*

Mannose-binding lectin recognises a number of structural oligosaccharide components on the surface of pathogens. By binding to a pathogen mannose-binding lectin can act as an opsonin or activate the lectin complement pathway, thereby promoting the killing of the pathogen and playing a major role in the innate immune defence. Human individuals deficient in mannose-binding lectin have been found to be more susceptible to viral and bacterial infections as well as to autoimmune diseases, and the mannose-binding lectin deficiency has been accepted as having a genetic basis. Like humans, chickens produce mannose-binding lectin and upregulate the production during acute stages of virus infections. Results from our lab have shown that the level of mannose-binding lectin in serum is associated with the impact of infections caused by the pathogens Infectious Bronchitis Virus, *E. coli*, *Pasteurella multocida* and *Ascaridia galli*.

In this study selection for low or high mannose-binding lectin concentration in serum has been made for several generations to investigate the functional role of chicken mannose-binding lectin in the innate immune response. This resulted in two distinct lines of chickens. Earlier studies revealed that the differences in the serum concentrations of mannose-binding lectin were due to differences at the transcriptional level, why the sequences of the mannose-binding lectin promoter from the two chicken lines were analysed. Several single nucleotide polymorphisms have been discovered, which has resulted in the identification of two different genotypes. These two genotypes and others were found in commercial chickens. Mapping of the regulatory elements in the promoter region was performed. All together these results will make it possible to perform a “quality” analysis of different promoter alleles for use in selective breeding for disease resistant chickens.

**P-48****PREVENTIVE ADMINISTRATION OF *ENTEROCOCCUS FAECIUM* 55 TO *SALMONELLA* ENTERITIDIS INFECTED CHICKS AND IMMUNE RESPONSE**

Martina Kolesárová<sup>1</sup>, V. Revajová<sup>1</sup>, Z. Ševčíková<sup>1</sup>, A. Lauková<sup>2</sup>, V. Stropfová<sup>2</sup>, V. Spišáková<sup>1</sup>, M. Levkut<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine and Pharmacology, Košice, Slovak Republic

<sup>2</sup>Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic

We studied the protective effect of *Enterococcus faecium* 55 (EF) administrated in dry powder (lyofilisate) mixed with food to chicks during 22 days, on immune system and bacterial count in caecum and faeces together with single per os *Salmonella enterica* subspecies Enteritidis PT4 (SE) infection on 4 day. The experiment was carried on 80 female chicks ISA BROWN breed divided into 4 groups: control (C), EF, SE and combined EFSE. The samples were taken twice, at day 3 (1<sup>st</sup> sampling) and 14 (2<sup>nd</sup> sampling) post infection (pi). Haematologically we found in 1<sup>st</sup> sampling the significant increase of leukocytes and heterophils in EFSE group in comparison with EF and SE groups. Immunophenotypization of peripheral blood lymphocytes done by flow cytometry showed in 1<sup>st</sup> sampling significant improve of CD4, CD8, and IgM positive cells in EFSE group compared to EF and SE group. Caecal immunocompetent cells CD4+, CD8+, and Mf+ determined by immunohistochemistry were higher significantly in 1<sup>st</sup> and insignificantly in 2<sup>nd</sup> sampling in EFSE group in comparison with EF and SE groups. Microbiological examination showed in general higher counts of EF55 strain in caecum than in faeces of chicks after 21 days administration. Counts of SE PT4 strain in faeces of EFSE group were improved after 21 days, but in caecum were not present. It seems that under these conditions, EF55 strain administration during 21 days had beneficial effect by the improvement of immunocompetent cells and reduction the counts of SE PT4 strain in caecum, but do not tend to the decrease of mentioned bacteria in faeces. We supposed the important role of bacteriocin-production by EF55 strain in this process.

**Keywords:** *Enterococcus faecium* 55, chicks, *Salmonella enterica* subspecies Enteritidis PT4, immune response, immunocompetent cells

**P-49****IMMUNOMODULATORY EFFECT OF MYCOTOXINS STUDIED BY FLOW CYTOMETRY IN CHICKENS**

Z. Slaminková<sup>1</sup>, L. Grešáková<sup>2</sup>, Martina Kolesárová<sup>1</sup>, V. Revajová<sup>1</sup>, M. Levkut<sup>1</sup>

<sup>1</sup> *University of Veterinary Medicine and Pharmacology, Košice, Slovak Republic*

<sup>2</sup> *Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic*

Mycotoxins may influence all types of immune cells on various levels of immune response. Numerous studies that have been conducted on host resistance, antibody responses and cell-mediated immunity in animals showed that also trichothecenes can be either immunostimulatory or immunosuppressive depending on dose, exposure frequency, and timing of functional immune assay.

In our experiment we used flow cytometry for study the effect of diets containing low and high dose of DON and ZEA on white blood cells, phagocytic activity of granulocytes, T and B cell numbers in the peripheral blood and duodenal intraepithelial lymphocytes of Ross 308 hybrid broiler chickens. Contaminated diets of experimental chickens included low mycotoxin dose of DON and ZEA, both at levels 3.4 mg.kg<sup>-1</sup>, while high mycotoxin dose at level 8.2 DON and 8.3 mg.kg<sup>-1</sup> ZEA. Both doses caused the decrease of total count of leukocytes and lymphocytes. In spite of higher number of heterophiles, their phagocytic activity was lower in comparison to control chickens ( $P < 0.05$ ). Determination of lymphocyte subpopulations in the peripheral blood showed decrease of T cells values (CD3, CD4, CD8, CD44) and MHC II cells, but increase of IgG cells in both doses. Duodenal intraepithelial lymphocyte (IEL) subpopulations showed decrease values of CD3 and CD4 cells in groups fed with both contaminated diets. Percentage of CD8 and CD44 in group fed with high dose exceeded the values of control. MHC II cells were significantly improved, and IgA cells were significantly decreased in both experimental groups comparing to controls. Two weeks mycotoxins consumption in our experiment showed immunomodulatory effect in chickens. This may results in impaired immunity and decreased resistance to infectious diseases.

**Keywords:** *mycotoxins, chickens, immunomodulatory effect*

**P-50****DUST AND ITS COMPONENTS CAN MODULATE BROILER'S IMMUNE SYSTEM**

Huong Thi Lan Lai<sup>1,2,\*</sup>, André J. A. Aarnink<sup>1</sup>, Henk K. Parmentier<sup>2</sup>

<sup>1</sup> Wageningen UR Livestock Research, P.O. Box 65, 8200 AB Lelystad, The Netherlands

<sup>2</sup> Adaptation Physiology Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

\*Corresponding author: [Thi.lai@wur.nl](mailto:Thi.lai@wur.nl)

Intensive housing of poultry causes high emissions and concentrations of airborne particles, which can lead to respiratory diseases of human and animals. Deposition in the respiratory tract, and transportation of airborne particles depend on their sizes. We studied concentration and size distribution of airborne particles inside livestock houses. Dust concentrations in the different particle size ranges were substantially higher both in counts and mass, in poultry houses ( $3.43 \pm 0.9$  mg) than in pig houses ( $1.29 \pm 0.25$  mg), and also than in cattle ( $0.047 \pm 0.01$  mg) and mink houses ( $0.0163 \pm 0.0003$  mg). Pathogen-associated-molecular-patterns (PAMP), such as lipopolysaccharide (LPS), lipoteichoic acid (LTA),  $\beta$ -glucans (BGL), and probably many others are important components of (fine) dust in poultry houses. Broilers were intratracheal (i.t.) challenged with these PAMP. Furthermore, they were challenged with heat-inactivated dust particles and  $\text{NH}_3$ . Primary and secondary (total) systemic antibody (Ab) responses and (isotype) IgM and IgG responses to the concurrently i.t. administered model antigen, human serum albumin (HuSA), were determined. Growth rate and heart morphology of the broilers were measured, as well. This indicated that airborne dust constituents such as PAMP may lead to an enhanced status of immune reactivity. In the future we will study the effects of the particle's size range on chicken health and immune responses.

**P-51****SUSTAINED EFFECTS OF EARLY-LIFE ORAL COLISTIN TREATMENT ON IMMUNE REACTIVITY TO INTRATRACHEALLY ADMINISTERED LPS AND HUSA IN CHICKEN**

Aart Lammers, Linda J. W. van Zutphen, Ger de Vries Reilingh, Henk K. Parmentier

*Adaptation Physiology Group, Department of Animal Sciences, Wageningen University,  
P.O.Box 338, 6700 AH, Wageningen, The Netherlands*

*E-mail: aart.lammers@wur.nl*

Immediately after hatch the chicken gut is colonized by a wide variety of bacterial species. The composition of the microbiota changes over time. In mammals disturbance of the microbial ecosystem in early life, e.g. due to antibiotic treatment, influences immune competence in later life. In the present study we investigated long term effects of early life changes in the composition of the intestinal microbiota on immune reactivity of laying hens after a concurrent intratracheal (i.t.) challenge with lipopolysaccharide (LPS) and human serum albumin (HuSA).

Laying hens obtained polymyxin E (PE) via the drinking water (200 mg/l) from day 1-21 (early PE group), day 21-42 (late PE group), or no PE (control group). At days 49 and 50 birds were intratracheally challenged with LPS (0.5 mg/kg) HuSA (0.1 mg/kg) or HuSA alone.

PE treatment did not influence BW gain in the period before challenge. However, LPS challenge caused BW loss ( $p < 0.001$ ), which was higher in the early PE treated group. Chickens in the early PE group continued to lose BW from 24-48 h. post challenge, whereas chickens in the other groups returned to gain weight. Total antibody titers to HuSA were reduced by PE treatment ( $p < 0.05$ ). In addition, LPS challenge enhanced antibody titers to HuSA ( $p < 0.001$ ). Overall, PE tended to decrease IgA and IgG titers to HuSA, but no effect on IgM titers was found. Total and isotype specific titers to LPS were not influenced by PE treatment. Our results suggest that treatment effects are different for T dependent and T independent antibody responses. Overall KLH binding natural antibody titers were decreased by PE treatment, but enhanced by LPS challenge. Our data suggest that early life colonization with gram-negative bacteria reduces sensitivity to LPS lung challenge and stimulates T cell dependant adaptive humoral immune responsiveness in later life.

**O-52****VACCINAL POTENCY OF A RECOMBINANT NDV-H5 VACCINE AGAINST AN ASIAN HIGHLY PATHOGENIC CLADE 1 H5N1 VIRUS IN DAY-OLD-CHICKENS WITH OR WITHOUT MATERNAL-DERIVED-ANTIBODIES**

Lardinois Amélyne<sup>1</sup>, Mieke Steensels<sup>1</sup>, Bénédicte Lambrecht<sup>1</sup>, Dierk Rebeski<sup>2</sup>, Thierry van den Berg<sup>1</sup>

<sup>1</sup> *Veterinary and Agrochemical Research institute (VAR), Ukkel, Belgium*

<sup>2</sup> *Lohmann Animal Health (LAH), Cuxhaven, Germany*

Avian Influenza viruses are enveloped RNA-viruses belonging to the family of Orthomyxoviridae. Highly pathogenic avian influenza virus infection gives rise to a highly contagious and fatal disease in poultry resulting in high economic losses. Since its emergence in 1997, the Asian HPAI H5N1 virus spread throughout South-East Asia to Europe, Africa and the Middle East. In Europe, the presence of the Asian HPAI H5N1 viruses remained limited to sporadic local introductions. Efficacious vaccination strategies are thus desirable to help control more efficiently HPAI H5N1 infections and spread.

In this study, the vaccinal potency of a recombinant NDV-H5 vaccine against the Asian clade 1 HPAI A/crested\_eagle/ Belgium/01/ 2004 H5N1 strain was evaluated. Different vaccination strategies were tested in SPF chickens with or without maternal-derived-antibodies (MDA).

First, the protective efficacy of the recombinant vaccine NDV-H5 was demonstrated to be dose-dependent in SPF chickens. The highest dose ( $10^7$ EID<sub>50</sub>/dose) of the rNDV-H5 vaccine induced complete clinical protection and prevented the viral excretion after challenge with  $10^6$ EID<sub>50</sub> of the Asian clade 1 HPAI H5N1 strain. Besides, different routes of vaccination, by drinking water, oculonasal were tested and gave comparable results. Nevertheless, some interference with the vaccine-induced protection was detected in SPF chickens possessing both H5- and NDV-MDA. However, in the presence of NDV-MDA alone, a better protection was seen when compared to the SPF chickens without MDA and those with both NDV- and H5-MDA. These results suggest a stronger interference of the H5 than the NDV MDA with the efficacy of the recombinant NDV-H5 vaccine.

The recombinant NDV-H5 vaccine under evaluation looks very promising to protect against Asian HPAI H5N1 infection. Further investigation will be performed to determine the range of HPAI H5 viruses for which this rNDV-H5 vaccine can offer protection. Moreover, the bivalent potency of the vaccine will also be evaluated by challenging with a velogenic NDV strain.



**P-53****IMMUNOGENICITY OF TRANSGENIC *EIMERIA* AND *TOXOPLASMA* VACCINE VECTORS IN THE CHICKEN MODEL**

Xianyong Liu, Xiaoxi Huang, Jun Zou, Guangwen Yin, Huali Su, Jianan Li, Xun Suo\*

*College of Veterinary Medicine, China Agricultural University, Beijing, 100193, China*

*\*Corresponding author: Xun Suo*

*Fax: + 86 10 62734325*

*E-mail address: suoxun@cau.edu.cn*

Every year infectious diseases cause huge economic losses to the poultry industry; and more importantly, highly pathogenic avian influenza is high risk to the health of human being. Currently available vaccines for such diseases can not meet the increasing demand, so the urgency of development of novel vaccines is especially great. Within two decades, genetically engineered bacteria and viruses have being widely studied as vaccine vectors for both human and animal use. Here we expect to expand the category of vaccine vehicles by developing protozoa-based vector.

In recent study, we established transgenic system for eimerian parasites and obtained several transgenic *Eimeria* lines. Based on the finding that the transgenic eimerian parasite maintains its immunogenicity as live anticoccidial vaccine, we carried out further assay to detect its immunogenicity against exogenous proteins. We found that compartmentalization of yellow fluorescent protein (YFP) could affect the type of YFP-specific immune response induced by different transgenic lines (manuscript submitted by Huang et. al.). At the same time, the measurement of immune response against *Eimeria*-expressed AIV nucleoprotein (NP) is underway.

In another research, we tested the immunogenicity of transgenic *Toxoplasma gondii* as vaccine vectors in the chicken model. Data showed that NP protein expressed by transgenic *T. gondii* elicited significant humoral and cellular immune response, especially after the chicken was primed with transgenic parasites, while boosted with YFP protein (manuscript submitted by Zou et. al.). At the same time, the measurement of immune responses against *Eimeria*-expressed AIV nucleoprotein (NP) is underway.

Taking together, our results demonstrate that *Eimeria* and *Toxoplasma gondii* are immunogenic when genetically modified to express foreign gene(s). When the mechanisms concerning spatial and temporal expression are deciphered, it will promise the potential of using *Eimeria* and other protozoa based vaccines for disease control not only in poultry, but also in livestock.

*This study was supported by the National Natural Science Foundation of China (No. 30871862 and 30671579), the National High Technology Research and Development Program of China (2006AA02Z458), and Chinese Universities Scientific Fund (2009-2-02).*



## O-54

### CHICKEN INTERFERONS

John W. Lowenthal, Adam Karpala, Kate Goossens, Audrey Baule, Kristie Jenkins, Mary Broadway, Andrew G. D. Bean

*CSIRO Livestock Industries, Australian Animal Health Laboratory, Private Bag 24, Geelong, Victoria, 3220, Australia*

---

The interferons (IFN) represent a family of immune proteins that share the common characteristic of viral replication inhibition. Although it has been over 50 years since the discovery of IFN activity, initially discovered in the chicken, there is still a paucity of information around a number of interferons and how they interfere with virus propagation. In addition to their anti-viral properties, IFNs also share a range of other biological functions including the activation of immune cells, regulation of cell surface recognition molecules, induction of inflammation and anti-proliferative effects. There is an ever growing membership for this family, which at present is represented by three distinct IFN types, which adds to the diversity of their anti-viral activity. It has taken some 4 decades from the initial discovery of IFN activity before the chicken type 1 IFN (alpha and beta) genes were identified and the proteins individually characterised, this was followed shortly by the type 2 IFN (IFN-gamma) and, more recently, the type 3 IFN (IFN-lambda). We have investigated the activity of a number of IFNs in the antiviral response to assess their potential to be harnessed as anti-viral therapeutics. The results of this has implications for the development of new therapeutics against increasingly pathogenic infections important to poultry industries worldwide.

**O-55****ALLELIC DIVERSITY OF MHC CLASS I IN WILD MALLARD DUCKS**

Shawna Jensen, Christine Mesa, Julie Parks-Dely, Deb Moon, Kathy Magor

*Department of Biological Sciences, University of Alberta, Edmonton Alberta, T6G 2E9, Canada,  
E-mail: kmagor@ualberta.ca*

We are examining antiviral defenses of ducks to determine why they serve as the reservoir for influenza viruses. Immune responses to low pathogenic influenza are weak and ducks can be re-infected, leading to perpetuation of the virus in nature. MHC class I proteins mediate a variety of functions in antiviral defense, in particular, NK recognition and presentation of antigens to cytotoxic T cells. In the White Pekin mallard we showed that a single MHC class I gene was predominantly expressed in the duck (*Anas platyrhynchos*), despite the presence of five genes in the locus. The dominant MHC class I gene was adjacent to the genes encoding the transporters for antigen processing, the *TAP1* and *TAP2* genes. The close proximity of the genes involved in peptide transport and presentation may have led to co-evolution of the proteins. Additional MHC genes, presumably unable to load peptides transported by TAP, were selectively inactivated in the locus. To examine diversity in a natural population of ducks, we have sequenced the expressed MHC class I alleles in 38 wild mallards. In each animal, one gene is predominantly expressed, and usually one allele is more abundant. The additional alleles cluster with the more abundant alleles, suggesting they are highly similar. Allelic diversity is vast and primarily located in alpha 1 and alpha 2 domains in the residues known to interact with peptide in mammalian MHC class I, suggesting the diversity is functional. We are sequencing genomic fragments spanning MHC and *TAP2* from three wild mallards, to determine whether the highly expressed gene is the one adjacent to *TAP2*, as seen in domestic ducks. This genomic organization may place constraints on antigen presentation and has implications for the ease with which influenza can adapt to this host.

**O-56****THE ANTIVIRAL ROLE OF CHICKEN TYPE 1 INTERFERONS (IFN) AGAINST IN VITRO FOWLPOX VIRUS (FPV) INFECTION**Heba Mahgoub<sup>1</sup>, Mark Fife<sup>1</sup>, Colin Butter<sup>1</sup>, Michael Bailey<sup>2</sup>, Pete Kaiser<sup>3</sup><sup>1</sup> Institute for Animal Health, Compton, United Kingdom<sup>2</sup> School of Clinical and Veterinary Science, University of Bristol, United Kingdom<sup>3</sup> Roslin Institute, University of Edinburgh, United Kingdom

Poxviruses have multiple evasion strategies to counteract the antiviral host defence. They encode for a number of cytokine-binding proteins, including tumour necrosis factor (TNF)- $\alpha$ , TNF- $\beta$ , IL-1 $\beta$ , IL-2, IL-18, IFN- $\alpha/\beta$ , IFN- $\gamma$ , and granulocyte macrophage-colony stimulating factor (GM-CSF). The IFN-modulating effect of FPV was studied in chicken embryo fibroblast (CEF) following *in vitro* infection with fpIBD1 (FPV expressing VP2 from IBDV/strain F52/70) (Bayliss et al., 1991). fpIBD1 up-regulated the transcription of all type I IFNs mRNA (IFN- $\alpha$ , - $\beta$ , - $\lambda$ , and - $\omega$ ) except for IFN- $\kappa$  which was down-regulated following fpIBD1 infection. Moreover, toll-like receptor (TLR) 3 and Mx1 mRNA levels were down-regulated. We then investigated the antiviral role of type I IFNs on fpIBD1 in *in vitro* infected CEF. For this, CEF were treated with different doses of recombinant IFN-  $\alpha$ , - $\beta$ , - $\lambda$ , - $\omega$ , and - $\kappa$ . Only IFNs- $\alpha$  and - $\beta$  down-regulated the transcription of fpIBD1 mRNA, coincident with up-regulation of TLR3 and Mx1 mRNA levels. However, the culture supernatant from CEF treated with IFN- $\alpha$ , - $\beta$ , - $\lambda$ , and - $\omega$  lacked infectivity (as measured by cytopathic effect (CPE)) when tested in fresh CEF cultures. Only the culture supernatant from CEF treated with IFN- $\kappa$  showed CPE on fresh CEF, which titrated out. Such CPE was consistent across the mock-treated infected controls. Hence, we can conclude that all type I IFNs in the chicken showed antiviral activity against FPV *in vitro* infection.

Bayliss, C. D., Peters, R. W., Cook, J. K., Reece, R. L., Howes, K., Binns, M. M., & Boursnell, M. E. 1991. A recombinant fowlpox virus that expresses the VP2 antigen of infectious bursal disease virus induces protection against mortality caused by the virus. *Arch Virol*, 120(3-4): 193-205.

**P-57****MICROGLIAL CELL POPULATION EXPRESSES B CELL SPECIFIC ANTIGEN**

Dávid Molnár, Dávid Dóra, Imre Oláh, Nándor Nagy

*Semmelweis University, Department of Human Morphology and Developmental Biology  
1094 Budapest, Tűzoltó u. 58, Hungary  
E-mail: molnar.david@med.semmelweis-univ.hu*

Cogburn and Glick (1981) identified a temporary lymphoid burst in the chicken pineal gland. Later we studied the lymphoid burst by transmission electron microscope and found lymphocytes, macrophages, granulocytes and secretory dendritic cells not only in the pial septae, but inside the pineal parenchyma (Olah and Glick 1980). Recently, we have used different cell specific markers to identify the phenotypes of these hemopoietic cells in the pineal gland and central nervous system (CNS). In the pineal parenchyma and the CNS we found a highly ramified CD45 positive cell. Ricinus communis agglutinin I (RCAI) lectin provided evidence, that the CD45 positive, highly ramified cells in the pineal parenchyma and CNS are microglia, and express MHC class II and CD44 antigens, but negative for chicken macrophage markers like 74.2, KUL01, 68.1, 68.2. Further immunocharacterization of the microglial cells showed Bu-1 antigen expression. It's widely accepted, that the Bu-1 antigen is characteristic for B-cell lineage, but flow cytometric studies indicated the presence of Bu-1 antigen on a small population of monocytes outside the CNS. Chick-quail chimeric studies provided evidence for the hemopoietic origin of the microglial progenitors. The microglial progenitor enters the CNS and their differentiation is timely regulated during their ramification. After homing in the CNS the antigens are sequentially expressed: CD45-CD44-MHC class II-Bu-1, on the microglial cells. These results indicate, that the microglial cells are either a unique monocyte / macrophage cell population, which express Bu-1 antigen, or raised the possibility, that microglial cells belong to B cell lineage.

*This work was supported by OTKA grant number: K-69061.*

**P-58****A NOVEL CELL TYPE OF HEMOPOIETIC ORIGIN IN THE AVIAN ENTERIC NERVOUS SYSTEM**Nándor Nagy<sup>1</sup>, G. Csikós<sup>1</sup>, Dávid Molnár<sup>1</sup>, A. M. Goldstein<sup>2</sup>, Imre Oláh<sup>1</sup><sup>1</sup> *Department of Human Morphology and Developmental Biology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. nagy.nandor@med.semmelweis-univ.hu*<sup>2</sup> *Department of Pediatric Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States*

The enteric nervous system (ENS) shares embryological, morphological, neurochemical, and functional features with the CNS therefore, the gastroenterologists and anatomists may be considered the ENS as the “brain in the gut”. Both CNS and ENS are structured by neuronal and supporting cells, namely glial and Schwann or satellite cells, respectively. In the CNS the micro- or mesoglia contributes to innate (phagocytosis) and acquire immunities. A cell with similar phenotype to the microglia has not been identified in the peripheral nervous system (PNS) including the ENS.

Recently we have recognized a cell type in the PNS, which share many features with the microglia of the CNS. For collecting information of this type of cells in the ENS we have used antibodies, which identify; neural crest derived cells, hemopoietic cells including B and T lymphocytes, macrophages and dendritic cells. Anti-MHC II antibody was also used to obtain preliminary information about immune function. In the enteric and sensory ganglia the hemopoietic marker CD45 recognized a highly ramified cell type. This cell type express B cell, but not T cell, macrophage and dendritic cell markers. The CD45+/Bu1b+/RCA (Ricinus communis agglutinin I) I+ phenotype strongly supports their non-neural crest, but hemopoietic origin. The presence of MHC class II antigen on their surface suggests their capability for antigen presentation. Their migratory capability and invasion into the intestine were proved by chick-quail chimeras: quail hindgut with nerve of Remak were isolated from 7 day old embryo and transplanted into the coelomic cavity of 3 days old chicken embryo. After 14 days of incubation the quail hindgut and nerve of Remak were tested for chicken specific (CD45, Bu1, MHC II, RCA I) antigens, which resulted in colonization of quail intestine and nerve of Remak by chicken blood-borne cells, evidencing the immigration of hemopoietic cells into the ENS. Our experiments indicate that there is a highly dendritic-like cell in the PNS with identical phenotype of the microglia.

*This work was supported by OTKA grant number: K-69061.*

**P-59****MYCOPLASMA SYNOVIAE INDUCED MODIFICATIONS IN RESPIRATORY ACTIVITY OF CHICKEN CHONDROCYTES ANALYZED BY PHENOTYPE MICROARRAYS**

Mojca Narat, Daliborka Dusanic, Dusan Bencina, Irena Oven

*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domzale, Slovenia*

*M. synoviae* has been detected in many internal organs, as well as in the synovial fluid of chickens with infectious synovitis (IS) where macrophages, T-lymphocytes and B-lymphocytes and cytokines, IL-1 $\beta$ , IL-18, IFN $\alpha$ , IFN $\gamma$  and NO were also found. The interplay between *M. synoviae*, immune cells and immune mediators must have an important impact on the phenotype of host cells and could determine the intensity of joint inflammation and local tissue destruction.

In this study, phenotype microarrays were used for the first time to study host-pathogen interaction *i.e.* to evaluate the influence of *Mycoplasma synoviae* on the global metabolic activity of chicken chondrocytes (CCH). CCH were cultured in the presence of 504 compounds, spotted in wells of 11 phenotype microarrays for eukaryotic cells (PMMs) and exposed to viable *M. synoviae* or its membranes. After incubation, the respiration of CCH was recorded during next 24 h. Metabolic and sensitivity profiles of CCH were obtained and compared to the profiles of *M. synoviae* infected CCH or membrane exposed CCH. For CCH, 25 h presence of 53 carbon/nitrogen sources, 14 ions, 69 cytotoxic chemicals and 4 hormones was found to increase the respiration. Longer incubation time (44 h) intensified the effect of hormones/immune mediators as the respiration was increased in the presence of 23 hormones/immune mediators. Metabolic profiles were significantly different in CCH treated with *M. synoviae*. In CCH exposed to membranes for 5 hours, 25 h presence of 42 carbon/nitrogen sources, 17 hormones/immune mediators and 32 cytotoxic chemicals increased the respiration, while it was decreased with 12 carbon/nitrogen sources, 2 cytotoxic chemicals and 14 ions. In CCH infected with viable *M. synoviae* for the last 14 h during 44 h incubation with hormones/immune mediators, 40 of them increased the respiration, while it was decreased with 9 compounds. Different respiratory kinetics indicates that the effect of compounds is time dependent and also differs upon type of antigen exposure of CCH.

We determined numerous metabolic factors (especially those involved in immune response) that affect the phenotype of CCH and we clearly demonstrated that the infection with *M. synoviae* changes the metabolic/sensitivity profile of CCH.



## P-60

### ASSESSMENT OF LOCAL ADAPTIVE IMMUNITY AFTER INFECTION WITH *ASCARIDIA GALLI* IN CHICKENS

Liselotte R. Norup<sup>1</sup>, Tina S. Dalgaard<sup>1</sup>, Janne Pleidrup<sup>1</sup>, Rikke M. Kjærup<sup>1</sup>, Gregers Jungersen<sup>2</sup>, Helle R. Juul-Madsen<sup>1</sup>

<sup>1</sup> Department of Animal Health and Bioscience, Aarhus University, 8830-Tjele, Denmark

<sup>2</sup> National Veterinary Institute, Technical University of Denmark, 1790-Copenhagen, Denmark

---

Very little work has been published on immune responses induced by gastrointestinal infections with the helminth parasites *Ascaridia spp.* and *Heterakis spp.* in chickens, and even less is known about the immune responses that correlate with total or partial resistance to these parasites. Therefore it is of importance to develop methods for assessment of local immune responses during a gastrointestinal infection with helminth parasites in this species.

The chicken lacks lymph nodes and little is known about the actual site of antigen presentation and lymphocyte activation in adaptive immune responses to avian infections. Also, the migration of activated lymphocytes is poorly characterized in this species. The purpose of this study was to use flow cytometry to analyze various lymphoid tissues for the *in vivo* presence of recently activated lymphocytes (lymphoblasts) during an experimental infection with *Ascaridia galli*.

Furthermore, lymphocytes from peripheral blood and spleen single cell suspensions were subjected to *in vitro* lymphocyte transformation by stimulation with mitogen or recall *Ascaridia galli* antigen followed by analysis of blastogenesis by flow cytometry in an attempt to identify the presence of antigen-specific cells.



## P-61

### IMMUNOGLOBULIN LEVELS IN CHICKEN SERUM AND BILE DURING AN *ASCARIDIA GALLI* INFECTION

Liselotte R. Norup<sup>1</sup>, Tina S. Dalgaard<sup>1</sup>, Janne Pleidrup<sup>1</sup>, Rikke M. Kjærup<sup>1</sup>, Torben W. Schou<sup>2</sup>, Anders Permin<sup>2</sup>, Helle R. Juul-Madsen<sup>1</sup>

<sup>1</sup> Department of Animal Health and Bioscience, Aarhus University, 8830-Tjele, Denmark

<sup>2</sup> Department of Human Health and Safety, DHI, 2970-Hørsholm, Denmark

Due to an increasingly larger proportion of poultry held in systems with access to outdoor areas, problems with parasite infections are also increasing. Some of these parasites are intestinal worms such as *Ascaridia galli*, *Heterakis gallinarum* and *Capillaria spp.*, which are found with flock prevalences up to 100%. The most pronounced injuries caused by the intestinal worms are intestinal haemorrhages leading to low body weight gain, low exploitation of feed, cannibalism and in more serious cases the infections may cause death.

We wanted to test whether the severity of an infection with *Ascaridia galli* is dependent on the chicken MHC haplotype, and to explore whether the development of antibodies has an effect on persistence of the *Ascaridia galli* infection.

Therefore, chickens of 8 different MHC haplotypes were infected experimentally. The following weeks faecal parasite egg counts and worm load were determined to follow the severity of infection during the experimental period. The development in *Ascaridia galli* specific antibody titres in serum and in bile was assessed to elucidate whether control of the infection was affected by this.

The experiment showed that the results of faecal egg counts were dependent on the MHC haplotype in the chickens. Likewise, development of *Ascaridia galli* specific IgG antibodies in serum showed large variations between chickens with different MHC haplotypes. Three MHC haplotypes with low faecal egg counts were among those with the highest *Ascaridia galli* specific antibody titres in serum, and the MHC haplotype with the highest faecal egg count was also among those with the lowest *Ascaridia galli* specific antibody titres in serum. No overall correlation between faecal egg counts and *Ascaridia galli* specific antibody titres in serum was seen.

**P-62****ELECTROPORATION-MEDIATED DELIVERY OF A DNA VACCINE AGAINST H5N1 AVIAN INFLUENZA LED TO AN ENHANCED MEMORY ANTIBODY RESPONSE**

Dele Ogunremi<sup>1</sup>, Drew Hannaman<sup>2</sup>, Sylvia van Drunen Little-van den Hurk<sup>3</sup>, Gary P. Kobinger<sup>4</sup>, John Pasick<sup>5</sup>, Yohannes Berhane<sup>5</sup>, Alfonso Clavijo<sup>5</sup>

<sup>1</sup> *Ottawa Laboratory (Fallowfield), Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, K2H 8P9, Canada*

<sup>2</sup> *Ichor Medical Systems, Inc., 6310 Nancy Ridge Dr., San Diego, CA 92121, United States*

<sup>3</sup> *Department of Microbiology & Immunology, and Veterinary Infectious Diseases Organization – International Vaccine Centre, University of Saskatchewan, 120 Veterinary Drive, Saskatoon, Sask., S7N 5E3, Canada*

<sup>4</sup> *National Microbiology, Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, R3E 3R2, Canada*

<sup>5</sup> *National Centre for Foreign Animal Disease, Winnipeg (Arlington) Laboratory, Canadian Food Inspection Agency, 1015 Arlington Street, Winnipeg, R3E 3M4, Canada*

The induction of immunological memory by a vaccine is a prerequisite for conferring long term immunity against a pathogen, but very little is known about the ability of DNA vaccines to induce a memory antibody response in chickens. To investigate the ability of a DNA vaccine to generate a potent antibody response against an H5N1 avian influenza (AI) virus and to induce a memory antibody response, two DNA vaccine constructs were prepared. One construct consisted of the hemagglutinin (HA) gene of A/goose/Germany/R1400/2007 (H5N1) virus inserted into the expression vector pSLKIA, while the second construct contained the HA gene of A/Hanoi/30408/2005 H5N1 virus inserted into the expression vector pCAG to generate pSLKIA-HA and pCAG-HA, respectively. Furthermore, we examined whether these DNA vaccines against AI are compatible with an existing commercially available anti-AI vaccine. Chickens were vaccinated with either the pSLKIA-HA or pCAG-HA both delivered by electroporation into the breast muscle. Ten weeks after the primary immunization, chickens were boosted with a commercially available water-in-oil emulsion of inactivated, recombinant H5N3 avian influenza virus in a proprietary adjuvant mixture. Antibodies against the hemagglutinin protein of H5N1 were measured by an indirect ELISA using a baculovirus expressed HA antigen of H5N1 and performed on serum samples obtained after the primary (DNA vaccine) and secondary (inactivated virus) vaccinations. When compared to a group of chickens injected with pCAG-HA vaccine intramuscularly using a syringe and hypodermic needle, electroporation resulted in a 10-fold enhanced anti-HA antibody response. Chickens that received 10 µg of pCAG-HA vaccine delivered by electroporation produced an antibody response that was superior to chickens that received 500 µg of the vaccine by syringe delivery. Thus, electroporation-mediated delivery of the DNA vaccine may result in up to a 50-fold increase in the efficiency of the immune response. A strong anamnestic anti-HA antibody response was observed among chickens that received the DNA vaccines by electroporation followed by boost with the commercial vaccine. These chickens had ELISA antibody titres that were significantly higher than those vaccinated with only the commercial vaccine (Mann Whitney Test,  $p < 0.05$ ). Thus, both DNA vaccines used in the study were able to induce a strong, memory antibody response following electroporation-mediated delivery, and were both compatible with a commercial inactivated virus vaccine.

**O-63****VACCINATION WITH A DNA CONSTRUCT ENCODING HEMAGGLUTININ GENE RESULTED IN PROTECTION AGAINST THE AVIAN INFLUENZA VIRUS IN CHICKENS**

Dele Ogunremi<sup>1</sup>, John Pasick<sup>2</sup>, Yohannes Berhane<sup>2</sup>, Gary P. Kobinger<sup>3</sup>, Drew Hannaman<sup>4</sup>, Alfonso Clavijo<sup>3</sup>

<sup>1</sup> *Ottawa Laboratory (Fallowfield), Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, K2H 8P9, Canada*

<sup>2</sup> *National Centre for Foreign Animal Disease, Winnipeg (Arlington) Laboratory, Canadian Food Inspection Agency, 1015 Arlington Street, Winnipeg, R3E 3M4, Canada*

<sup>3</sup> *National Microbiology, Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, R3E 3R2, Canada*

<sup>4</sup> *Ichor Medical Systems, Inc., 6310 Nancy Ridge Dr. #107, San Diego CA, 92121, United States*

Protection against the avian influenza (AI) H5N1 virus is conferred mainly by antibodies directed against the disease-inducing haemagglutinin (HA) protein of the virus. We investigated the induction of protective anti-HA antibodies in chickens vaccinated with a DNA vaccine construct encoding the HA gene of AI virus A/Hanoi/30408/2005 (H5N1) in the hybrid eukaryotic vector, pCAG (pCAG-HA). Three-week old chickens were immunized with 10 or 100 µg of pCAG-HA delivered intramuscularly with or without electroporation and were tested at day 56 post-immunization for the presence of anti-HA antibodies by hemagglutination inhibition (HI) and competitive enzyme-linked immunosorbent assay (cELISA). On day 72 post-immunization, birds were inoculated with 106 ELD<sub>50</sub> (50% egg lethal dose) of highly pathogenic A/chicken/Pennsylvania/1370/1/1983 (H5N2) via the oronasal route and were monitored for clinical signs and virus shedding. Seven of eight chickens vaccinated with 100 µg of pCAG-HA by electroporation, developed high levels of H5-specific antibodies. Following challenge with highly pathogenic H5N2 virus, none of the eight birds showed clinical signs. Six of the birds completely cleared their infection, while one of the birds shed low levels of the virus in the cloaca and another in the oropharynx as determined by real-time RT-PCR. In contrast, none of the five birds immunized with 100 µg of the pCAG control vector developed detectable anti-hemagglutinin antibodies, three developed clinical signs following H5N2 challenge and two died of the infection. In the group of eight birds immunized with 10 µg of pCAG-HA by electroporation, five developed antibodies, showed no clinical signs following challenge, and survived infection. Three of the five seropositive birds were completely cleared of their viral load while the other two had low levels of virus in the cloaca or the oropharynx. Two of the low-dose immunized birds which did not seroconvert died from AI infection while the other bird recovered and completely cleared the virus. In contrast, all 5 control birds that received 10 µg of control pCAG by electroporation developed clinical signs, 2 died from the infection while the remaining birds still harboured virus in their cloaca (3 out of 5) or oropharynx (2 out of 5). Birds receiving 10 µg (n=3) or 100 µg (n=3) of the pCAG-HA vaccine administered without electroporation did not develop detectable H5-specific antibodies, exhibited clinical signs after virus challenge (5 out of 6) and died from infection (2 out of 6). The four surviving birds shed virus from their oropharynx or cloaca. In conclusion, electroporation delivery of AI DNA vaccine conferred protection which is mainly associated with the induction of an anti-HA antibody response.

**O-64****IFN $\gamma$  DOES NOT APPEAR TO CONTRIBUTE TO THE IMMUNITY CONFERRED BY DNA VACCINATION AGAINST AVIAN INFLUENZA**

Dele Ogunremi, Hilary Kelly, Qigao Fu

*Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Ottawa, K2H 8P9, Canada*

Humoral antibody response directed against the H5N1 avian influenza (AI) virus, particularly hemagglutination inhibition (HI) antibody appears to be a reliable predictor of protection in chickens. However, protection has been observed in the absence of anti-AI antibody prompting the conclusion that a cell-mediated immune response may contribute to immunity. We have investigated the role of interferon-gamma (IFN $\gamma$ ) a major mediator of cellular immune response, in protection against AI. Chickens were immunized intramuscularly with DNA vaccine encoding the hemagglutinin (HA) gene in the hybrid eukaryotic plasmid vector, pCAG (pCAG-HA) delivered either by means of electroporation or with a syringe and hypodermic needle. Control chickens were similarly injected with the empty eukaryotic vector pCAG (i.e., without HA insert). Serum HI antibodies were determined from blood samples obtained from the chickens. Single cell suspensions prepared from the spleen or bursa of Fabricius of vaccinates and controls were cultured in the presence of concanavalin A or hemagglutinin protein. IFN $\gamma$  present in the culture supernatant was measured by a bioassay relying on IFN $\gamma$ -induced nitrite production by HD 11 cells while IFN $\gamma$  messenger RNA in the cells was determined by RT-PCR. We observed the production of HI antibodies, as well as early and sometimes sustained IFN $\gamma$  production by splenic and bursa cells, from chickens immunized with pCAG-HA delivered by electroporation. However, chickens immunized with the empty pCAG vector delivered by electroporation also produced IFN $\gamma$ . In addition, chickens immunized with a single intramuscular injection of pCAG-HA (100  $\mu$ g) administered with syringe and needle were able to produce IFN $\gamma$  but not HI antibodies and remained susceptible to virus challenge. Only chickens immunized with pCAG-HA delivered by electroporation were protected against AI virus challenge. Our experiments demonstrated that under conditions favouring the generation of IFN $\gamma$  but not HI antibodies, vaccinated chickens remained unprotected from AI virus challenge. Thus, any protective role of the cell-mediated immune response against AI is probably conferred through an IFN $\gamma$ -independent mechanism. We hypothesize that the induction of IFN $\gamma$  may be deleterious for anti-AI immunity in chickens.

**P-65****IN VITRO ASSESSMENT OF POTENCY OF A DNA VACCINE BY MEASURING GENE EXPRESSION**Dele Ogunremi<sup>1</sup>, Hyun Lillehoj<sup>2</sup>, Hilary Kelly<sup>1</sup>, Qigao Fu<sup>1</sup><sup>1</sup> Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Ottawa, K2H 8P9, Canada<sup>2</sup> U.S. Department of Agriculture, Agricultural Research Service Building 1040, BARC-East Beltsville, MD 20705, United States

The regulatory assessment of veterinary biologicals is essential in ensuring the availability of high quality veterinary products for producers and animal health practitioners. Biotechnological advancement continues to result in the development of new products for which existing regulatory precedents are inadequate and thus demand an innovative approach. DNA vaccines are an example of a new biotechnology advancement for which traditional methods of assessing vaccine potency are inadequate. There is also the societal pressure to use fewer animals for testing. For these reasons, we have developed an *in vitro* test for assessing the potency of a candidate DNA vaccine containing the hemagglutinin (HA) gene of the H5N1 avian influenza (AI) virus, which has been shown to confer protection against AI in chickens. Two chicken cell lines, macrophage cell line HD 11 and fibroblast cell line OU-2, and the African green monkey kidney cell line, VERO, were transfected with DNA vaccine constructs encoding the HA gene of H5N1 avian influenza and assessed over time for the expression of messenger RNA of the HA gene. All three cell lines expressed the mRNA of the HA gene within 24 hours of transfection. We have correlated the *in vitro* expression of the hemagglutinin gene with muscle biopsies obtained from chickens 3 days after immunization with DNA vaccine and with the expression of the HA gene in the lung 1-4 days following *in ovo* immunization of day 18 embryos. We conclude that transfection of an appropriate cell line could provide reliable data about the *in vivo* potency of a DNA vaccine. This approach may serve as a rapid regulatory tool for conducting laboratory assessment of the potency of a DNA vaccine without the use of live animals.





## O-66

### **INNATE IMMUNE RESPONSES TO INFECTION WITH H5N1 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS IN DIFFERENT DUCK SPECIES**

Mary Pantin-Jackwood, Caran Cagle, Jamie Wasilenko

*Southeast Poultry Research Laboratory, USDA.ARS, Athens, United States*

Ducks have been implicated in the dissemination and evolution of H5N1 highly pathogenic avian influenza (HPAI) viruses. Differences in pathogenicity and response to vaccination have been observed between different duck species. The innate immune system is responsible for controlling viruses during the initial stages of infection, which is crucial in determining disease resistance or susceptibility. The objective of this study was to examine the differences in innate immune responses among two domestic ducks species, Pekin (*Anas platyrhynchos*, var. *domestica*) and Muscovy (*Cairina moschata*), and a wild type species, Mallard (*A. platyrhynchos*) infected with a H5N1 HPAI virus. All challenged ducks died, but Muscovy ducks died 2 days earlier than Pekin and Mallard ducks. However, high virus titers were present in all duck tissues, with a significant higher titer only observed in the heart of the Muscovy ducks. On the other hand, Pekin and Mallard ducks had higher body temperatures after infection than Muscovy ducks, indicating differences in the innate immune response between the ducks. Despite being genetically more closely related, no obvious gene expression pattern emerged that was shared by both domestic Pekin and wild Mallard duck species, but lacking in the more susceptible Muscovy ducks. However, higher levels of IL-6, IL-18, and chemokine SicA19 gene expression were observed in the Muscovy ducks. RIG1, MHC-I, and IFN $\gamma$  gene expression were also upregulated in all three duck species. In contrast, MHC-II was down regulated. Differences were also found in TLR7 and IFN $\alpha$  gene expression. In conclusion, the differences observed in the expression of innate immune related genes between these duck species could explain in part the differences observed in pathogenicity.

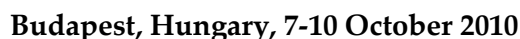


**O-67****POST TRANSLATIONAL POLYMORPHISM OF NATURAL ANTIBODIES: RAPID PROTECTION TO INFECTION?**

Henk K. Parmentier, M. G. B. Nieuwland, T. Bergstra, Aart Lammers

*Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, The Netherlands*

Natural antibodies (NAb), i.e. antibodies present in individuals without prior immunization or infection, perform many important functions in various immune responses, and are often polyreactive of nature with low binding affinity. Also natural auto-antibodies (N(A)Ab) binding at least one auto-antigen were found in various species including the chicken. Natural auto-antibodies might provide regulation in various other physiological systems. In poultry, levels of NAb are highly heritable, and were related with health and survival. Levels of NAb not only increase with age, but dietary pro- and antibiotics, and immunizations with innate antigens like lipopolysaccharide (LPS) at least temporarily enhance NAb levels. Polyreactivity of NAb may rest on a change of the three-dimensional structure of the immunoglobulin F(ab)<sub>2</sub> fragment caused by various locally present oxidizing agents, salts and lower or higher pH, as a result of the activation of inflammatory cells. We evaluated by Western blotting effects of subcutaneously administered LPS and lipoteichoic acid (LTA), respectively, on binding characteristics of chicken N(A)Ab towards the 'auto-antigen' chicken-liver-cell-lysate (CCL) *in situ* prior to (day 0) and 3 days after subcutaneous challenge, as well as the effect of different *in vitro* maltreatments in the form of oxidizing agents: hydrogen peroxide, low pH, and aqua dest on chicken N(A)Ab polymorphism. Prior to, and at 3 days after challenge, plasma N(A)Ab bound to CCL. Differences in the staining patterns of individual CCL molecular weight-identified fragments (MWIF) were found as was true for the extinction intensity of these fragments after LPS or LTA challenge. Staining of CCL by plasma samples was prone to *in vitro* maltreatment of the plasma samples. The results suggest that chicken N(A)Ab are prone to irreversible post translational polymorphism *in vitro*, which can be initiated by PAMP-induced inflammatory agents *in situ*, and which may provide a rapid humoral defense to be mobilized after infection.



# AVIAN BETA-DEFENSIN 2 IS WEAKLY ACTIVE AGAINST ENTERIC BACTERIAL PATHOGENS BUT IS CHEMOTACTIC TOWARDS LEUKOCYTES

INRA, UR 1282 Infectiologie Animale et Santé Publique, 37380 Nouzilly, France

Avian beta-defensins (AvBD) belong to the innate immunity components that may constitute the first line arsenal of mucosal host-pathogen interface. They have been pointed out as important for the resistance/susceptibility of chicken to intestinal colonization by bacterial pathogens. Some member of this antimicrobial peptides family can be isolated from the bone marrow and exhibits discrepancies in their activity spectrum. AvBD2, as well as AvBD7, is revealed highly active against Gram+ bacteria. However AvBD2 does not show a strong antibacterial activity towards Gram- enteric bacterial strains, by contrast to AvBD7. We were thus interested in the exploration of another function for AvBD2 such as the recruitment of leukocytes, which is documented for various defensins in mammals. Interestingly, AvBD2 was shown to attract lymphocytes and granulocytes more efficiently than AvBD7. Taken together, these data suggest that AvBD7 could compensate a defect in AvBD2-mediated antimicrobial activity and AvBD2 could be mainly involved in the recruitment of immune cell populations in the gut.



## P-69

# ANTIBODY RESPONSE TO H5N9 INACTIVATED VACCINE AND MHC DIVERSITY IN THE CHICKEN

Pascale Quéré<sup>1</sup>, Pierre Sibille<sup>1</sup>, Magali Bergès<sup>1</sup>, Nicolas Bruneau<sup>2</sup>, Tran Xuan Toàn<sup>3</sup>, Jean-Charles Maillard<sup>3</sup>, Bertrand Bed'Hom<sup>2</sup>

<sup>1</sup> INRA, UR1282 Infectiologie Animale et Santé Publique, Nouzilly, France

<sup>2</sup> INRA, UMR1313 Génétique Animale et Biologie Intégrative, Jouy-en-Josas, France

<sup>3</sup> CIRAD, UR22 gestion Intégrée de la Faune, Hanoi, Vietnam

The MHC (Major Histocompatibility Complex) locus contains genes that are involved in antigen presentation via expressed class I and class II molecules on the chicken cells. The first aim was to study the efficiency of antigen presentation and level of specific antibody response to Influenza in the context of various MHC haplotypes. The marker LEI0258 was chosen for MHC typing in different genetic backgrounds: Leghorn and Fayoumi inbred lines from INRA, commercial broiler lines and native Vietnam breeds. The vaccine test was conducted with a H5N9 vaccine commercially available both in France and Vietnam. Antibody response has been measured at 2, 4 and 8 weeks after vaccination. The results showed that the Leghorn lines homozygous for different MHC alleles exhibited a different clear-cut antibody response, ranked as follows: B13 > B21 > B12 > B19 throughout all the post-inoculation survey. Other Leghorn lines, selected respectively for high antibody response to Newcastle Disease Virus and for carbon clearance during 13 generations, and native Vietnam breeds did not show much difference between lines and breeds: antibody titers increasing up to 8 weeks post-inoculation. For Leghorn selected lines, the animals homozygous for allele B21 exhibited higher antibody titer than those homozygous for alleles B15 and then B124. Moreover an overdominance effect was observed in B15 / B21 heterozygotes. In Fayoumi, the allele called [311] towered above the others. On the contrary, commercial broiler lines from France and Vietnam were characterised by very low antibody responses. The second aim was to compare vaccine efficiency in the highest (B13/B13) and the lowest (B19/B19) antibody responder inbred lines. Both chicken lines were able to demonstrate efficient reduction of intra-tracheal inoculated H5N9 virus replication after homologous vaccination, and reduction of histological lesions compared to naive chickens (day 1 to day 3 survey). However B13/B13 vaccinated chickens developed lung lesions by day 6, possible glimpse of excessive immune reactivity. In conclusion, a strong correlation was demonstrated between MHC alleles and response to H5N9 vaccine in the Leghorn background. Moreover, a negative correlation was observed between the level of antibody response and zootechnical performances, leading to low response in broiler lines.

**P-70****MACROPHAGE ACTIVATION-INDUCED THYMOSIN BETA 4 PRODUCTION: A TISSUE REPAIR MECHANISM**

Narayan C. Rath, Lakshmi Kannan, Rohana Liyanage, Jackson O. Lay

*USDA/ARS, Fayetteville, United States*

Macrophages play significant role in immunity which not only kill pathogens, produce cytokines but also clear dead tissues at the site of inflammation and stimulate wound healing. Much less is known how these cells contribute to tissue repair process. In course of our studies comparing the peptide profiles of chicken monocytes and heterophils, we found monocytes rich in thymosin beta 4 (Tb4), a peptide with multiple biological functions such as angiogenesis and wound healing. Intracellularly Tb4 binds to cytoskeletal actin to regulate cell motility and phagocytosis. We hypothesized that Tb4 production may be dynamically regulated by the factors which activate macrophage and facilitate their tissue repair function. Using toll-like receptor (TLR) activating ligands we evaluated their effects on Tb4 production by a transformed chicken macrophage cell line HTC at 6 and 24h after stimulation. Stable isotope labelling of amino acids in cell culture (SILAC) and mass spectrometry was used to monitor the changes in cellular and conditioned media (CM) Tb4. Real time PCR and metabolite measurements were used to determine macrophage activation. None of the TLR agonists showed any significant change in either cellular or CM Tb4 levels at 6h but some agonists such as LPS, peptidoglycan (PGN), and CpG induced the expression of interleukins-1beta, -6, and nitric oxide synthase genes but not Tb4 at that time point. Nitrite accumulation was discernible at both time points supporting gene expression results. At 24 h time point however, the same agonists caused significant depletion of cellular Tb4 with its concomitant detection in the CM. The CM also contained Tb4 sulfoxide, a metabolite with anti inflammatory efficacy. Incongruity between the Tb4 gene expressions along with its release into the CM at 24 h without the possibility of its replenishment, suggested that only macrophage death could cause its externalization. To verify we determined the percentage of trypan blue positive cells and lactate dehydrogenase (LDH) activity of the CM as indicators of cell death. The results showed that the TLR agonists which induced depletion of intracellular Tb4 also increased macrophage death implying that these highly dynamic cells may contribute to wound healing process by their ultimate sacrifice.

**O-71****GUT-ASSOCIATED IMMUNOLOGICAL RESPONSES AFTER NEMATODE INFECTION OF CHICKENS**

A. Schwarz<sup>1</sup>, H. Abel<sup>2</sup>, M. Gauly<sup>2</sup>, G. Breves<sup>3</sup>, Silke Rautenschlein<sup>1</sup>

<sup>1</sup> University of Veterinary Medicine Hannover, Clinic for Poultry, Germany

<sup>2</sup> University of Goettingen, Department of Animal Sciences, Germany

<sup>3</sup> University of Veterinary Medicine Hannover, Institute for Physiology, Germany

Gallinaceous birds kept outdoors or in deep litter housing systems are likely to be exposed to endoparasites such as nematodes, which may affect their growth performance without other major clinical signs. Although worm infections have been known for many years, not much information is available on the gut-associated immune reactions after nematode infections. Studies in mammalian species have shown that the local gut-associated immunity plays a major role in the clearance of the worm infections and the ability of the animal to resist an infection or not. Our objectives were to investigate gut-associated immune reactions after infection of layer chickens with *Ascarida (A.) galli* and *Heterakis (H.) gallinarum*, which are known to have a high prevalence in alternative production systems. At two, three, and five to six weeks post infection (p.i.), chickens were necropsied, pathological and histopathological lesions, and worm burden was determined. Samples of duodenum and middle jejunum as well as cecum were investigated after *A. galli* and *H. gallinarum* inoculation, respectively, for different immune cell populations. Both worm infections induced mild to moderate infiltrations of different T cell populations into the lamina propria of the respective gut segments. These observations indicate stimulation of local cell-mediated immune reactions comparable to nematode infections in mammalian species. Furthermore, Th1 and Th2-related cytokine up-regulation was locally investigated. The up-regulation of IL-13 expression two weeks after *H. gallinarum* infection complements the observations made on the cellular level and provides circumstantial evidence for the stimulation of the Th2-response after inoculation of this layer chicken line with this nematode. Our studies provide new information regarding the gut-associated immune system of chickens and stimulation of cell-mediated immune responses after nematode infection.

**P-72****ASSESSMENT OF THE INFLUENZA SPECIFIC CELL-MEDIATED IMMUNE RESPONSE IN CHICKENS BY DETECTION OF CHICKEN INTERFERON-GAMMA AFTER *EX VIVO* ANTIGENIC STIMULATION ON LYMPHOCYTES**

Fabienne Rauw, Sofia Anbari, Thierry van den Berg, Bénédicte Lambrecht

Veterinary and Agrochemical Research Center (VAR), Groeselenberg 99, B-1180 Brussels, Belgium  
E-mail: farau@var.fgov.be

Avian influenza virus (AIV) causes serious disease in a wide variety of birds. Infections in poultry can be unapparent or cause respiratory disease, decreases in production or a rapidly fatal systemic disease known as highly pathogenic avian influenza (HPAI). Numerous vaccines against avian influenza (AI) have been described and experimentally shown to be efficacious for the prevention of the mortality and usually the morbidity. Only a relative few have received licensure for commercial use : inactivated vaccines and recombinant vectors expressing AI genes. Vaccine-induced protective immunity against AIV is primarily the result of the humoral immunity, including systemic as well as mucosal antibody production. Although our knowledge of avian cellular immunology has expanded rapidly in the last decade, very little is known about the importance of the cell-mediated immunity (CMI) against AIV in chickens. During recent years, several studies in poultry have shown that the CMI specific to a pathogen might be evaluated by the measurement of proliferation or ChIFN $\gamma$  release after *ex vivo* antigenic-stimulation of T lymphocytes from spleen or blood. This system has been used to measure the CMI after Newcastle disease vaccination. The purpose of the present study is to evaluate the use of this recall model for the measurement of the CMI specific to AIV in chickens. In this aim, concentrated AIV recall antigens were produced by concentrating whole virus and by dissociating viral proteins. They were characterized *in vitro* and evaluated as recall antigen on lymphocytes from spleen and blood of SPF chickens inoculated with a low pathogenic AIV strain (LPAI). Protocols to isolate the lymphocytes from the lung and from the trachea, which are the major target organs of influenza virus, were developed and allow the investigation of cellular immune response in the respiratory tract.

**P-73****IMMUNE REACTION IN CHICKENS AND DUCKS IN RESPONSE TO LOW PATHOGENIC AVIAN INFLUENZA**

J. B. W. J. Cornelissen<sup>1</sup>, J. Post<sup>1</sup>, B. Peeters<sup>1</sup>, H. Fijten<sup>1</sup>, L. Vervelde<sup>2</sup>, Johanna M. J. Rebel<sup>1</sup>

<sup>1</sup> Central Veterinary Institute of Wageningen UR, Postbus 65, 8200 AB Lelystad, The Netherlands

<sup>2</sup> Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Influenza infection in wild waterfowl is ubiquitous and nearly always asymptomatic. In chickens however the virus can cause mild disease when infected with low pathogenic avian influenza virus (LPAI) or mortality when infected with high pathogenic avian influenza virus (HPAI). Upon viral challenge with the same virus, the clinical outcome differs in ducks and chickens. Here we studied the immunological responses in the lung, of ducks and chickens within the first 14 days after infection with LPAI. The ducks and chickens were intranasally and intratracheally infected with LPAI H<sub>7</sub>N<sub>1</sub>, euthanized at 1, 2, 4, 7 and 14 days post infection. The amount of avian influenza A virus was measured by real time-PCR. The production of pro-inflammatory cytokines and type I and II interferons, and pattern recognition receptor was studied by real time-PCR. Viral RNA was detected in the lungs of chickens from day 1 to 7, whereas in the lungs of ducks it was mainly detected at day 1. In chickens the lung reacted with an induction of IFN- $\alpha$ , IFN- $\beta$ , IL-6 and IL-8 together with an significant induction of TLR3 at day 1 p.i., while in the lungs of the ducks an significant induction of IFN- $\gamma$  (day 1, 2 and 4) and TLR 7 (day 1) was observed

In conclusion ducks and chickens react with a different cytokine profile in the lungs and intestine to the same challenge.

*This research was - in part - sponsored by the Program "Impulse Veterinary Avian Influenza Research in the Netherlands" of the Dutch Ministry of Agriculture, Nature and Food Quality and in part by the EU*



**O-74****DIFFERENCES IN HOST RESPONSE AND VIRUS REPLICATION IN CHICKENS INFECTED WITH LOW PATHOGENIC OR HIGH PATHOGENIC AVIAN INFLUENZA VIRUSES**

J. Post<sup>1</sup>, J. B. Cornelissen<sup>1</sup>, D. Burt<sup>2</sup>, V. Broks<sup>1</sup>, D. van Zoelen<sup>1</sup>, B. Peeters<sup>1</sup>, Johanna M. J. Rebel<sup>1</sup>

<sup>1</sup> *Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands*

<sup>2</sup> *Roslin Institute, Midlothian, United Kingdom*

To study differences in disease development, we examined virus replication and host response in chickens infected with high- or low pathogenic H7N1 avian influenza virus. Both LPAIV H7N1 and HPAIV H7N1 spread systemically in chickens after combined intranasal/intratracheal inoculation. Both viruses were recovered from the brain, but the viral load was much higher in the brains of HPAIV-infected than of LPAIV-infected animals. Differences in host gene expression were seen in brains of HPAIV-versus LPAIV-infected chickens. In the brains of LPAI-infected chickens only 4 genes were induced, while in the brains of HPAI-infected chickens 4400 genes were induced within 16 hours pi, including genes in the Toll-like receptor pathway. In contrast, in the intestine the transcriptional response was comparable between HPAI- and LPAI-infected chickens. To further examine differences between the two H7N1 strains in neurovirulence, we infected primary chicken brain cell cultures. Both HPAIV and LPAIV could infect brain cells, but replication of LPAIV was limited to one replication cycle only.

In conclusion, both H7N1 LPAIV and HPAIV infected a broad range of tissues beyond the respiratory and gastrointestinal tract, indicating systemic infection. However, in the brain HPAIV replicated to higher titers and induced a much stronger transcriptional response than LPAIV. These observations may suggest that differences in virus replication in the brain are responsible for the differences in mortality between HPAIV and LPAIV.

**P-75****EFFECT OF MICROBIAL-NUTRITION INTERACTION ON CHICKEN IMMUNE SYSTEM AFTER THE EARLY ADMINISTRATION OF PROBIOTIC WITH ORGANIC ACIDS IN YOUNG CHICKS**

J. C. Rodríguez-Lecompte<sup>1</sup>, J. Brady, G. Camelo-Jaimes, S. Sharif, G. Crow, G. Ramirez-Yanez, W. Guenter, J. House

<sup>1</sup> Department of Animal Science, University of Manitoba, Winnipeg, Canada  
E-mail: JC\_Rodriguez-Lecompte@umanitoba.ca

The present study was conducted to characterize the effect of *Lactobacillus acidophilus*, *Streptococcus faecium*, and *Sacharomyces cerevisiae* in conjunction with organic acids such as sorbic acid and citric acid on intestinal morphology, immunological parameters (avian defensins, beta 3 (AvBD3) and AvBD6, and Cathelicidin B 1 (CTHLB1), endogenous microbicidal Angiogenin 4 (AvAng4) and cytokines IL-6, 10, 12 and interferon gamma (IFN- $\gamma$ )) and nutritional associate-genes (growth hormone inducer and food intake genes ghrelin (GHS), and GHS- receptor (GHSR), and folic acid transporters, reduced folate carrier (RFC) and proton couple folate transporter (PCFT). At 21 days experiment was conducted using one day-old chicks. Chicks were randomly allocate to 3 treatments; treatment 1 (T0) consisting of chicks that were not received probiotic or organic acids (PO), treatment 2 (T2) consisting of chicks that received both PO during 7 consecutive days and treatment 3 (T3) consisting of chicks that received both PO during 14 consecutive days. On days 11 and 21 old (4 and 7 after finished the respective treatments) intestinal sections from duodenum (distal), jejunum (proximal to Meckel's diverticulum), ileum (proximal to ileo-caecal junction) and cecal tonsil were collected and analyzed by histology, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), quantitative RT-PCR to determine the level of expression of cytokine and folic acid transporters. T2 and T3 decreased ( $P<0.05$ ) villous height and width, crypt depth in the jejunum area during the first 7 days. At day 22 T3 affected ( $P<0.05$ ) crypt depth and the number of goblet cell/mm<sup>2</sup> in the jejunum and ileum area. Regardless the treatment at day 0, 11 and 22 there was mRNA expression of just AvBD3 in crop, proventriculus, duodenum, jejunum, ileum, cecal tonsil and bursa; however CTHLB1 was only present in the bursa in all the treatments after day 7. Interesting AvAng4 was not affect either for treatment or time at all. In the ileum area just mRNA levels of IL-10 and IL-12 were affected ( $P<0.05$ ) for T2 and T3 at day 22. In cecal tonsils T2 and T3 affected ( $P<0.05$ ) IL-6 and 12 at day 11, however there was a significant ( $P<0.05$ ) down regulation of IL-12 and up regulation of IFN- $\gamma$  at day 22. T2 and T3 affected mRNA levels of PCFT in cecal tonsil at day 22; there were not changes ( $P>0.05$ ) in ileum of either RFC or PCFT anytime. GHR and GHSR mRNA expression was detected in all the groups at day 11 and 22. In conclusion, probiotic and organic acid effects on chicks' intestine include triggering sensor molecules of the innate immune system, which may produce antimicrobial proteins and peptides. Probiotics and their cellular debris may cause immuno-modulation, accelerating the colonization by lymphoid cells and immune response against pathogens and improving nutrient utilization.

**Keywords:** Probiotics, Organic acids, Defensins, Cytokines, ghrelin, Folic acid

**O-76****HUMORAL IMMUNE RESPONSES TO MYCOPLASMA GALLISEPTICUM INFECTION IN HOUSE FINCHES (CARPODACUS MEXICANUS)**

Karel A. Schat, Jessica Grodio, Dana M. Hawley, Keila V. Dhondt, Erik E. Osnas

*Cornell University, Microbiology and Immunology, College of Veterinary Medicine, United States*

---

A novel strain of *Mycoplasma gallisepticum* (MG) emerged in house finches (HOFI) (*Carpodacus mexicanus*) in 1994. Infections with MG cause conjunctivitis in HOFI and as a consequence the population of HOFI has drastically declined in the eastern USA. In order to determine the role of local and systemic antibody responses in the development of lesions we infected HOFI bilaterally in the conjunctiva with VA1994 (the index isolate), CA2006, or NC2006. Lesion scores, MG load, IgA and IgY antibodies to MG were measured between 0 and 8 weeks post infection (pi). Infection with NC2006 resulted in the most severe eye lesions and CA2006 caused the least severe eye lesions. Quantitative PCR assays demonstrated that the severity of the lesions correlated with pathogen load. MG antibodies were detected in lachrymal fluids and sera using the Idexx FlockChek MG antibody Elisa kit with modifications to allow the detection of house finch antibodies (Grodio et al., Vet Immunol Immunopathol 132:288, 2009). Specific IgY and IgA responses were detected with all three isolates at 2 weeks pi. Starting at 4 weeks pi, the IgY response was significantly higher after infection with NC2006 than with CA2006 or VA1994. The IgA titers in the lachrymal fluid were also higher between 4 and 7 weeks pi with NC2006 than with the other two isolates. Independently of the isolate, HOFI with severe lesions had significantly higher levels of IgY and IgA to MG antibodies than birds with mild lesions. Western blot analysis showed that the three isolates are antigenically similar. However sera from HOFI infected with CA2006 recognized fewer MG proteins than sera from HOFI infected with VA1994 or NC2006. In conclusion, antibody-mediated immunopathology likely contributes to the development of eye lesions. Immunopathology has also been reported for other *Mycoplasma* species (e.g., Bodhankar et al, J Infect Dis 202:39, 2010).

**O-77****SALMONELLA HAS IMPACT ON SPATIAL-TEMPORAL IMMUNOLOGICAL PROCESSES DURING CHICKEN JEJUNAL DEVELOPMENT**Dirkjan Schokker<sup>1</sup>, Mari A. Smits<sup>1</sup>, Johanna M. J. Rebel<sup>2</sup><sup>1</sup> Wageningen UR Livestock Research, Animal Breeding and Genomics Centre, P.O. Box 65, 8200 AB, Lelystad, The Netherlands<sup>2</sup> Central Veterinary Institute, Wageningen UR, P.O. Box 65, 8200 AB, Lelystad, The Netherlands

To study effects of *Salmonella enteritidis* on immunological changes in chicken jejunal development, we analyzed gene expression profiles at seven points post-infection in 1–21 day-old broiler chickens. Nine clusters with different gene expression patterns were identified, and the genes in each cluster were further analyzed by a functional annotation clustering method (DAVID). A clear difference between normal developing- and *Salmonella* disturbed jejunum was the higher expression of genes involved in cell turn-over at early stages in the infected jejunum. Surprisingly, we found no clustered immune related processes in the infected birds. To compare the immunological processes between control and *Salmonella* infected chickens, the gene expression data was superimposed on known immunological KEGG pathways. Furthermore an in-depth analysis on the immune gene level was performed. As expected, we did find immunological processes in the *Salmonella* infected jejunum. Several of these processes could be verified by immunohistochemistry measurements of different immunological cell types. However, the well-ordered spatial-temporal development of the immune system, as observed in control non-infected animals, was completely abolished in the infected animals. Several immunological processes started much earlier in time, whereas other processes are disorganized. These data indicate that normal morphological and immunological development of jejunum is changed dramatically by a disturbance due to *Salmonella* infection. Due to the disturbance, the well-organized spatial-temporal development of morphological processes are delayed, those of the immunological development are scattered, whereas metabolic functional processes are almost not affected. This demonstrates the flexibility of developmental processes in the broiler chicken intestine.

**O-78****LACK OF ANTIVIRAL ACTIVITY OF IFN AND THE IFN EFFECTOR PROTEIN MX DURING HPAIV INFECTION IN THE CHICKEN**

Benjamin Schusser<sup>1</sup>, Carsten Krohmann<sup>1</sup>, Nicola Penski<sup>2</sup>, Thomas Vahlenkamp<sup>3</sup>, Peter Staeheli<sup>2</sup>, Bernd Kaspers<sup>1</sup>, Sonja Haertle<sup>1</sup>

<sup>1</sup> *Dept. of Veterinary Sciences, Institute for Animal Physiology, LMU Munich, Germany*

<sup>2</sup> *Department of Virology, University of Freiburg, Germany*

<sup>3</sup> *FLI, Riems, Germany*

The chicken is a natural host of influenza A virus and the economic consequences and public health issues associated with avian influenza A virus infections (FLUAV) are well known. Host-pathogen interaction in influenza infection has been studied intensively in the murine system. From this work it became clear that influenza viruses are profoundly sensitive to interferon (IFN) and have therefore developed mechanisms to inhibit the IFN system. To investigate whether chickens produce IFN after influenza A virus infection birds were infected intratracheally with HPAIV (A/Cygnus Cygnus/Ruegen-Germany/2006 H5N1). Within 12h post infection biological active type I IFN was detected in lung, spleen and plasma the concentration of which further increased until the birds' death (at appr. 48 hours p.i.). Though, protective effects against FLUAV were observed in tissue culture, we found that treatment of chickens with high doses of recombinant chicken IFN-alpha prior to and during infection failed to confer protection against H5N1 virus challenge which is in clear contrast to the data obtained in mice.

One of the key players in antiviral defence against influenza A virus infection in mice is the IFN stimulated myxovirus resistance (Mx) gene which is also upregulated in IFN treated and H5N1 infected chicken. In the chicken, Mx was initially described as a cytoplasmic protein lacking antiviral activity. But recent studies revealed that the chicken Mx gene is highly polymorphic and suggested that an Asn/Ser polymorphism at amino acid position 631 might determine antiviral activity. To examine whether any Mx-isoform does mediate resistance against FLUAV infection chMx constructs differing only in AS631 were generated and overexpressed in vitro and in vivo with the retroviral vector system RCAS. While expression of huMxA and muMx1 conferred protection, neither in chicken embryo fibroblasts (CEF) nor in RCAS-transduced chicken embryos an antiviral activity of either chMx isoform was detectable. Moreover the IFN induced protection of CEFs against FLUAV was not abrogated, when chMx protein was knocked down using a siRNA approach. However, though the chicken's Mx protein does not confer antiviral activity we were able to show GTPase activity for the protein, which is an absolute requirement for the antiviral effects of human Mx.

Taken together, the chicken's IFN system reveals striking differences in both induction and effector mechanisms to paradigms established in mice and though IFN was initially discovered in the chicken its functional role during FLUAV infection is still not fully understood.

*This work was supported by the BMBF project FluResearchNet.*

**O-79****DEVELOPMENT OF A VIROSOME-BASED VACCINE AGAINST AVIAN INFLUENZA VIRUS**

Amirul Islam Mallick<sup>1</sup>, Payvand Parvizi<sup>1</sup>, Leah Read<sup>1</sup>, Éva Nagy<sup>1</sup>, Shahriar Behboudi<sup>2</sup>, Shayan Sharif<sup>1</sup>

<sup>1</sup> *Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G2W1, Canada*

<sup>2</sup> *The Institute of Hepatology, University College London, London, United Kingdom*

Several types of vaccines are currently available for control of avian influenza virus (AIV), including inactivated influenza virus and vectored recombinant vaccines. Subunit vaccines have also been used experimentally for conferring protection against AIV in chickens. However, subunit vaccines are generally poorly immunogenic, compared to conventional vaccines, unless they are administered using suitable delivery vehicles or adjuvants. In the present work, we have evaluated immunogenicity of fusion-active virosomes in terms of elicitation of antibody- and cell-mediated immune responses. Furthermore, we asked whether combining interferon-gamma or CpG ODN with virosomes would enhance immunogenicity of the virosome-based vaccine. Virosomes prepared from H4N6 avian influenza virus were used as an immunogen alone or in combination with purified baculovirus expressed recombinant chicken IFN- $\gamma$  or CpG ODN. Using a prime-boost regimen, birds were immunized with various vaccine formulations and sera were collected on a weekly basis to measure antibody responses. In addition, spleens were collected for measuring T cell responses to the virus or the virosome.

Results of our present experiment indicated that all birds immunized with various virosome preparations seroconverted. Moreover, our results demonstrated that combining CpG ODN with virosomes significantly increased haemagglutinin inhibition (HI) antibody titers compared to birds that were immunized with virosome alone or virosome in combination with recombinant IFN- $\gamma$ . Induction of higher cytokine response was also evident in the group that had received virosome+CpG ODN compared to the group that received virosome + IFN- $\gamma$  or virosome alone. In conclusion, our results demonstrate that a virosome-based vaccine is able to induce immune response against AIV antigen and this response is enhanced by incorporating CpG ODN into virosomes.

**P-80****CHARACTERIZATION OF IMMUNE RESPONSE TO A T CELL  
EPITOPE OF THE HAEMAGGLUTININ (HA) ANTIGEN OF AVIAN  
INFLUENZA VIRUS H5 SUBTYPE IN CHICKENS**Hamid R. Haghighi<sup>1\*</sup>, Leah R. Read<sup>1\*</sup>, S.M. Mansour Haeryfar<sup>2</sup>, Shahriar Behboudi<sup>3</sup>, Shayan Sharif<sup>1</sup><sup>1</sup> *Department of Pathobiology, University of Guelph, Ontario, Canada*<sup>2</sup> *Department of Microbiology and Immunology, University of Western Ontario, London, Canada*<sup>3</sup> *The Institute of Hepatology, University College London, London, United Kingdom**\*These two authors contributed equally to the work*

Highly pathogenic avian influenza viruses (HPAI) of H5N1 subtype has caused morbidity and mortality in human populations in Asia, Europe and Africa. Although migratory birds are the main reservoir of the virus, chickens can play an important role in transmission of the virus. Despite the importance of chickens as a host for this virus, little is known about host responses to H5N1 viruses in this species. Therefore, the objective of our study was to characterize these responses. In particular, we set out to identify the haemagglutinin (HA) epitopes recognized by chicken T cells. To accomplish this objective, chickens homozygous for the B19 major histocompatibility complex (MHC) haplotype were immunized with a recombinant fowlpoxvirus vector expressing the HA antigen of an avian H5 influenza virus. Subsequently, spleen cells of immunized birds were treated in vitro with 112 individual peptides within an overlapping peptide library covering the full length of the HA antigen. A 15-mer peptide was identified that could cause cell stimulation in an MHC-dependent manner. This epitope was found to activate both CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets, marked by proliferation and expression of interferon-gamma by these cells as well as expression of granzyme A by CD8<sup>+</sup> T cells. This is the first report of a T cell epitope of AIV recognized by chicken T cells. These studies should lead to the development of more efficacious strategies for control of H5N1 HPAI.



**O-81****CHICKEN DENDRITIC CELLS ARE INFECTED AND ACTIVATED BY AVIAN INFLUENZA VIRUS**

Karen Staines, Raul Ruiz-Hernandez, Colin Butter

*Institute for Animal Health, Compton, Newbury Berkshire, RG20 7NN, United Kingdom*

*E-mail: Karen.staines@bbsrc.ac.uk*

Infection of antigen presenting cells with viruses might benefit the host by increased opportunity for initiation of responses, or may interfere with the response by destruction of these cells. The balance of outcome will depend on the rate of destruction compared to the rate of effective maturation. To investigate this phenomenon in chickens, we have started to study the infection of chicken dendritic cells with Avian Influenza virus.

Ex-vivo chicken dendritic cells were used to study early activation responses using the previously described antibodies recognising dendritic cell markers DEC205 and CD83. Rapid mobilisation of the putative endocytic receptor DEC205 was observed in response to TLR signalling and was accompanied by changes in subcellular distribution of CD83.

Exposure of dendritic cells to influenza virus resulted in similar activation-related changes in these two molecules during productive viral replication in these cells. Macrophages also supported viral replication. This indicated that the replication in dendritic cells may be an important determinant of the immune response and the outcome of infection and immunisation with live virus.

**O-82****ACROSS-LINE SNP ASSOCIATION STUDY FOR (INNATE) IMMUNE TRAITS IN LAYING HENS**

Jan J. van der Poel<sup>1</sup>, Filippo Biscarini<sup>1</sup>, Johan A. M. van Arendonk<sup>1</sup>, Henk K. Parmentier<sup>2</sup>, Annemieke P. Jungerius<sup>2</sup>, Henk Bovenhuis<sup>1</sup>

<sup>1</sup> *Animal Breeding and Genomics Centre, Wageningen, The Netherlands*

<sup>2</sup> *Adaptation Physiology Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands*

<sup>3</sup> *Hendrix Genetics, Research & Technology Centre, Spoorstraat 69, 5830 AC Boxmeer, The Netherlands*

A population of about 600 hens was genotyped with 1022 SNP for an association study. The immune parameters measured in blood samples were natural and acquired antibody titers and complement activity. Natural antibodies (NAb) for keyhole limpet hemocyanin (KLH), Lipopolysaccharide (LPS) were measured at 20, 40 and 65 weeks, acquired antibodies (SpAb) to Newcastle disease virus vaccine at 20 weeks, and classical and alternative *Complement* activity measured at 20, 40 and 65 weeks. The association analysis was conducted across nine different lines of White Leghorn and Rhode Island Red origin. Across lines linkage disequilibrium is conserved at shorter distances than within lines; therefore, SNP significantly associated with traits across lines are expected to be closer to functional mutations. SNP having a significant across-line effect but no significant SNP-by-line interaction were identified, to test for consistency of association across lines.

For immune traits 59 significant SNP associations were detected, confirming previously identified QTL and identifying new QTL potentially involved in the immune function. *IL17A* (chromosome 3) is involved in NAb and SpAb titers as well as *Complement* cascade activation.

We found evidence for a role of *IL17A* (chromosome 3) in NAb and SpAb titers and in the classical and alternative pathways of complement activation. The major histo-compatibility complex genes on chromosome 16 showed significant association with NAb and SpAb titers and classical complement activity. The *IL12B* gene (chromosome 13) was associated with NAb titers and *IRF1* with NAb and SpAb titers and classical complement activity.

In addition feather condition score on the back, rump and belly of laying hens was performed as a measure of feather damage, which is closely related to feather pecking behavior in group-housed hens. The serotonin receptor 2C (*HTR2C*) gene on chromosome 4 was highlighted, supporting existing evidence of the prominent involvement of the serotonergic system in modulation of feather pecking behavior in laying hens. *HTR2C* also affected classical complement levels.

**O-83****UPREGULATION OF IMMUNE GENES IN DUCK LUNG AND INTESTINE DURING HIGH AND LOW PATHOGENIC AVIAN INFLUENZA INFECTION**Hillary Vanderven<sup>1</sup>, Robert Webster<sup>2</sup>, Kathy Magor<sup>1</sup><sup>1</sup> *Department of Biological Sciences, University of Alberta, Edmonton, AB T6E 2E9, Canada*<sup>2</sup> *Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, United States*

Ducks are the natural reservoir of influenza A virus and survive infection by most strains. Humans and chickens are more susceptible to disease. It is important to understand what constitutes a successful antiviral response in influenza's environmental host. Since influenza is an acute infection the innate immune system is likely critical in viral clearance and host survival. To elucidate the host-pathogen interactions that occur during influenza A infection we are examining host genes upregulated early in an immune response. Suppressive subtractive hybridization (SSH) was used to determine which genes are upregulated one day post infection with highly and low pathogenic avian influenza in ducks. RNA from lung or intestine tissue of ducks infected with either highly (H5N1/VN1203/04) or low (H5N2/BC2005/500) pathogenic strains of influenza A virus was compared to that of mock-infected animals by SSH. Both VN1203 and BC500-infected lung and intestine tissues showed increased expression of a wide variety of immune genes during influenza infection, including MHC class I, interferon-induced protein with tricopeptide repeats 5 (IFIT5), 2'-5' oligoadenylate synthetase (OAS), interferon-induced transmembrane proteins (IFITMs), activation-induced C-type lectins (AICLs), heat shock proteins, as well as several housekeeping genes. The differential expression of these genes during an immune response to influenza was confirmed by reverse transcription PCR (RT-PCR) and dioxigenin-dUTP (DIG) dot blotting. Real time qPCR analysis of OAS, IFIT5 and MHC-I showed greater than 1000-fold upregulation of these interferon stimulated genes in duck lung following VN1203 infection, and much less upregulation by BC500 infection. Our results suggest that ducks mount a more robust response to potentially lethal influenza viruses. Identifying genes involved in a successful antiviral response in the natural host may reveal new targets for therapeutic intervention in human and veterinary disease.

**P-84****ADAPTIVE IMMUNITY IN CONJUNCTIVA ASSOCIATED LYMPHOID TISSUE AFTER OCULAR IMMUNIZATION**

Frederik W. van Ginkel, Stephen L. Gulley, Aart Lammers, Haroldo Toro

*Auburn University, Pathobiology, Auburn, Alabama, United States*

Conjunctiva-associated lymphoid tissues (CALT) constitute along with the Harderian glands the chickens main para-ocular lymphoid system. These tissues are the first to be exposed to pathogens entering by the ocular or nasal routes and to provide adaptive immunity and, consequently, influence avian disease resistance. Although considerable knowledge has accumulated regarding the role of Harderian glands in ocular immunity, the role of CALT in generating antigen-specific ocular immune responses has not been well defined. It is assumed that CALT, like Harderian glands, have a sentinel role protecting mucosal surfaces of the eye. To test whether CALT plays an important role in generating protective ocular immunity, chickens were ocularly immunized with a human, replication-deficient adenovirus vector of serotype 5 (Ad5). Initially, the T cell composition of CALT was analyzed by fluorescence-activated cell sorting (FACS). Approximately 37% of the lymphocytes were CD3<sup>+</sup> of these CD3<sup>+</sup> lymphocytes about half of them were CD4 positive and ~20% CD8 positive. Thus, both T helper and cytotoxic T cells are present in CALT, which can drive humoral and cell mediated immune responses after ocular Ad5 immunization. The induction by Ad5-specific ocular immunity was supported by elevated levels of IFN-gamma in tears. In order for B cell responses to be effective at mucosal surfaces, transport of IgA across the epithelium by the polymeric immunoglobulin receptor (pIgR) would be required. Our RT-PCR and immunohistochemistry results indicated, that the pIgR is expressed by epithelial cells of the eyelid. Analyses by RT-PCR and ELISPOT demonstrated that ocular immunization with Ad5 resulted in increased production of IFN-gamma in both CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes as well as induction of Ad5-specific antibody secreting B cells in CALT. Thus, CALT are important mucosal inductive sites producing antigen-specific antibodies and activated cytotoxic T cells and T helper type 1 cells after ocular Ad5 immunization. The epithelium of the eyelid expressed the pIgR allowing active transport of IgA. The prevalence of IgA producing cells in CALT combined with the presence of pIgR demonstrates that CALT contributes to mucosal immune protection of the eye.

**P-85****IDENTIFICATION OF CORE ELEMENTS INVOLVED IN ANTIBACTERIAL AND IMMUNOMODULATORY ACTIVITIES OF CHICKEN CATHELICIDIN 2**Albert van Dijk<sup>1</sup>, E. Margo Molhoek<sup>2</sup>, Floris Bikker<sup>3</sup>, Edwin J. A. Veldhuizen<sup>1</sup>, Henk P. Haagsman<sup>1</sup><sup>1</sup> *Division Molecular Host Defence, Dept. of Infectious Diseases & Immunology, Faculty of Veterinary Medicine. Utrecht University, The Netherlands*<sup>2</sup> *TNO Defense, Security and Safety, Rijswijk, The Netherlands*<sup>3</sup> *Dept. of Oral Biochemistry, Academic Centre for Dentistry Amsterdam, University of Amsterdam, Amsterdam, The Netherlands*

Several host defence peptides (HDPs) have demonstrated to possess a broad spectrum of antimicrobial activity as well as immunomodulatory activities. Here we describe these activities for the HDP chicken cathelicidin 2 (CATH-2). Truncated peptides (10 to 26 aa) corresponding to different regions of the mature peptide were synthesized using fmoc chemistry to correlate CATH-2 domains to antibacterial and immunomodulatory activities. Compared to CATH-2, C-terminally shortened peptides maintained their antibacterial activity against *Salmonella enteritidis* and *Staphylococcus aureus*, but displayed a reduced hemolytic activity towards chicken erythrocytes. Other CATH-2-derived peptides displayed immunomodulatory activity as they were both able to induce cytokine production through direct interaction with chicken macrophages (HD11 cells), as well as to lower the LPS-induced IL-1 $\beta$  production of these cells. Both antibacterial and immunomodulatory activities could be even further improved by simple point mutations in the CATH-2 derived peptides. These results show that dissection of antibacterial and immunomodulatory activities of CATH-2 is possible, which may aid in the design of novel peptide antibiotics with tailor-made host specific biological properties.

**P-86****INVOLVEMENT OF AVIAN COLLECTINS IN HOST DEFENCE**

Edwin J. A. Veldhuizen<sup>1</sup>, Astrid Hogenkamp<sup>1</sup>, Lonneke Vervelde<sup>2</sup>, Sylvia S. Reemers<sup>2</sup>,  
Martin van Eijk<sup>1</sup>, Henk P. Haagsman<sup>1</sup>

*Utrecht University, The Netherlands*

<sup>1</sup> *Division Molecular Host Defence*

<sup>2</sup> *Division of Immunology, Dept. of Infectious Diseases & Immunology, Faculty of Veterinary Medicine*

---

Collectins are members of the family of vertebrate C-type lectins. They are important effector molecules of the mammalian innate immune system, and especially the role of surfactant protein A (SP-A) and SP-D in lung defense has been well studied. Contrary to mammals, SP-D seems to be absent in chicken, but recent studies have shown that a chicken SP-A homologue, and chicken lung lectin (cLL, an SP-A like protein lacking collagen) are highly expressed in the respiratory tract. In addition, three other chicken collectins (cCL1-3) homologous to human collectins CL-L1, CL-K1 and CL-P1, respectively, were also expressed in lung tissue. In order to elucidate the involvement of chicken collectins in innate defense we determined the effect of influenza A virus infection of chickens on collectin gene expression levels. Both upregulation and downregulation of collectins was observed depending on the location within the respiratory tract. In addition, recombinantly expressed cLL showed viral hemagglutination inhibition activity in vitro. These results indicate that collectins could be important in innate defense of the chicken lung against (viral) infections. Further elucidation of the role of chicken collectins could lead to strategies that prevent infectious diseases in poultry, which could also be beneficial for public health.

**P-87****TOLL-LIKE RECEPTOR REPERTOIRE IN GREY PARTRIDGE**

M. Vinkler, O. Tomasek, H. Bainova, P. Opatova, T. Albrecht, A. Bryjova, J. Bryja

*Charles University in Prague, Department of Zoology, Czech Republic*

Contrary to the rapidly growing amount of information available on Toll-like receptor (TLR) diversity across mammalian species, TLRs are only rarely studied in non-model species of birds. This unfortunate circumstance precludes us from understanding of avian TLR evolution. To improve the current knowledge of structural variability of these pattern-recognition receptors within Galliformes, i.e. the most intensively studied group of birds, we focused our investigation on identification of the TLR repertoire in Grey partridge (*Perdix perdix*). In our contribution we present preliminary structural data for 8 *ppTlr* genes (partridge orthologues of chicken *Tlrs*): *Tlr1LA*, *Tlr2A*, *Tlr3*, *Tlr4*, *Tlr5*, *Tlr7*, *Tlr15* and *Tlr21*. We compare further these newly described *ppTlrs* and their predicted products with known TLRs in other avian species (domestic chicken *Gallus gallus*, duck *Anas platyrhynchos*, turkey *Meleagris gallopavo* and Zebra finch *Taeniopygia guttata*). The SMART analysis of the amino acid sequences revealed a high similarity in the domain composition between the *ppTLRs* and their chicken counterparts. That is consistent with the results of the phylogenetic analysis showing close proximity of TLRs in Grey partridge to chicken TLRs. For the first time, we herein provide data originating from a systematic research of TLR repertoire in a free-living bird.

**Keywords:** *diversity, evolution, Grey partridge, pattern-recognition receptors, domain structure, TLRs, Toll-like receptors*



**O-88****B CELL CHEMOATTRACTANTS AND THE MAREK'S DISEASE VIRAL CHEMOKINE**Victoria Waters<sup>1</sup>, Dirk Werling<sup>2</sup>, Pete Kaiser<sup>3</sup><sup>1</sup> *Institute for Animal Health, Compton, Berkshire, RG20 7NN, United Kingdom*<sup>2</sup> *Royal Veterinary College, Hertfordshire, AL9 7TA, United Kingdom*<sup>3</sup> *The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, United Kingdom*

---

The chicken genome encodes 3 B cell chemoattractants, named CXCL13L1-3, in contrast to mammalian genomes which encode one. B cells rearrange their single canonical Ig H and L genes in the bone marrow, and then migrate to the bursa of Fabricius where the B cell receptor repertoire is established via gene conversion. B cells then seed to the periphery. The bursa involutes with age and it has been hypothesised that there is a "post-bursal stem cell". B cell biology in the chicken is therefore very different to that of mammals and it is hypothesised that the 3 B cell chemoattractants will have different roles and/or expression patterns at different stages of the bird's development. The mRNA expression patterns of the 3 chemokines have been analysed in a range of tissues from birds of different ages. Expression of CXCL13L1 mRNA is higher in the spleen and expression of CXCL13L3 mRNA is higher in the bursa and thymus. CXCL13L2 mRNA expression is generally low at all time points and may instead primarily be involved in inflammatory responses.

The presumed receptor for the three CXCL13s is CXCR5, as it is in mammals. Chemotaxis assays are also being performed to confirm the ligand-receptor relationships of the 3 chemokines.

Marek's disease virus also encodes a chemokine-like protein (vCXC). This has been described as a CXCL8-like protein; however, in phylogenetic analysis the vCXC groups with the chicken B cell chemoattractants. Chemotaxis assays were also used to attempt to show to which receptor vCXC binds.

**P-89****LOCAL PERFORIN MRNA EXPRESSION AT EXPERIMENTAL *EIMERIA TENELLA* INFECTION OF NAÏVE AND IMMUNE CHICKEN**

Sofia E. Magnusson, Per Thebo, Adrian L. Smith, Anna Lundén, Eva Wattrang<sup>1</sup>

<sup>1</sup> Dept. of Virology, Immunobiology and Parasitology, National Veterinary Institute, Uppsala, Sweden

The study aimed to compare the replication of protozoan parasite *Eimeria tenella* and host immune responses at experimental infection of naïve and immune chicken.

The experiment comprised 3 age matched groups of chickens; uninfected controls, primary infected: infected only at day 0 (6 weeks old, i.e. challenge) and immune: infected at 2 weeks of age, 4 weeks of age and at day 0 (challenge). Six birds from each group were euthanized and tissue samples for PCR-analyses collected at day 1, 2, 3, 4 and 10 after challenge. The infection dose was 1000 *E. tenella* oocysts/bird at all occasions and fecal oocyst excretion was monitored from day 5 to 10 after each infection. Parasite DNA and mRNA expression of immunological markers was quantified in tissue samples by real-time PCR.

At challenge infection, primary infected chickens excreted in mean  $7 \times 10^4$  oocysts/bird. Chickens in the immune group excreted in mean  $4 \times 10^2$  oocysts/bird after challenge compared a mean of  $9 \times 10^4$  oocysts/bird after their first infection. *E. tenella* DNA was detected in caecal tissue from primary infected and immune birds in at low levels in occasional samples at day 1 and 2 post challenge. At day 3, caeca from most primary infected birds were positive for parasite DNA while only 50 % of the samples from immune chickens were positive. At days 4 and 10 post challenge all samples from primary infected chickens were positive and contained >200 times more *E. tenella* DNA than positive samples from immune birds where only <50 % of samples were positive. Caecal tonsils from immune chickens displayed about 40 % higher levels of perforin mRNA expression at day 2, 3 and 4 after challenge compared to uninfected and primary infected birds. At day 10 post challenge primary infected chickens showed the highest perforin mRNA expression, about 40 % higher compared to uninfected and immune chickens. In conclusion, these results show that parasite replication was inhibited at the early stages of its life cycle in immune chickens. This inhibition coincided with increased perforin expression indicating involvement of activated cytotoxic T lymphocytes in protective immunity.

**O-90****FURTHER CHARACTERISATION OF CHICKEN BONE MARROW-DERIVED DENDRITIC CELLS**Zhiguang Wu<sup>2</sup>, Lisa Rothwell<sup>2</sup>, Colin Butter<sup>1</sup>, Pete Kaiser<sup>2</sup><sup>1</sup> *Institute for Animal Health, Compton, Berkshire RG20 7NN, United Kingdom*<sup>2</sup> *Current address: The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG, United Kingdom*

The function of dendritic cells (DC) is controlled, at least in part, by the cell surface receptors they express. Of these, some of the most important are Toll-like receptors (TLR) and chemokine receptors. The former control the ability of DC to respond to pathogens and direct their subsequent maturation and downstream adaptive immune responses. The latter control migration of DC to appropriate sites, including the site of infection and that of antigen presentation to the adaptive immune response.

LPS and CD40L were used to activate the maturation of chicken bone marrow-derived dendritic cells (chBM-DC), chicken bone marrow-derived macrophages (chBM-MØ), chicken blood monocyte-derived macrophages (chMo-MØ) and HD11 cells. The mRNA expression levels of all chicken TLRs, except TLR1LB and TLR3, were measured by real-time quantitative RT-PCR (qRT-PCR). In unstimulated cells, highest expression was seen for TLR4 and TLR15 in all cell types. Following maturation of chBM-DC with LPS and CD40L, TLR4 and TLR15 expression levels were increased, suggesting that both play a role in controlling Gram-negative bacterial infections. TLR2A, TLR2B, TLR5, TLR7 and TLR21 expression levels were decreased, suggesting a link between DC maturation and TLR expression. ChBM-MØ had similar TLR expression patterns to chBM-DC. Mo-MØ and HD11 showed similar patterns, except for TLR4 and TLR1LA. This suggests different antigen presenting cells (APC) express different TLR mRNA after stimulation.

The expression of both CXCR (CXCR1, CXCR4 and CXCR5) and CCR (CCR6 and CCR7) chemokine receptors has been measured on chBM-DC. After maturation with LPS or CD40L, CCR7 expression levels were significantly up-regulated.

**P-91****PHYLOGENY AND GENETIC VARIABILITY OF RED JUNGLEFOWLS AND NATIVE CHICKENS ASSESSED BY MEANS OF BLOOD GROUP SYSTEM IN SOUTHEAST ASIA**Yoshio Yamamoto<sup>1</sup>, Takao Namikawa<sup>2</sup><sup>1</sup> Graduate School of Biosphere Science, Hiroshima University, Japan<sup>2</sup> Graduate School of Bioagricultural Sciences, Nagoya University, Japan

One hundred forty two blood samples of red junglefowls (*Gallus gallus*) and 1,001 of native chickens from Japan and six Asian countries of Bangladesh, Bhutan, Indonesia, Laos, Myanmar and Vietnam were examined for four blood group systems of A, B, D and E by hemagglutination, and the gene frequencies were calculated by the direct counting. The numbers of reagents used for blood typing were three for the A, seven for the B, and two each for the D and E systems. The genetic variabilities within populations were measured by the proportion of polymorphic loci ( $P_{poly}$ , the criterion was the most common allele frequency  $<0.99$ ), and the average heterozygosity

( $\bar{H}$ ), to be compared among those of red junglefowl and native chicken population in the six Asian countries. For the  $P_{poly}$  estimates, most of red junglefowl and native chicken populations showed comparatively bigger measurements except for Vietnam and RJF-N of red junglefowl

populations. There is not so much difference in the  $\bar{H}$  values among tested populations. In order to estimate a position of each red junglefowl or native chicken populations in gene constitution, the genetic distance comparisons were carried out among red junglefowl populations and other three junglefowls species, grey junglefowl (*Gallus sonneratii*), Ceylon junglefowl (*G. lafayettii*) and green junglefowl (*G. varius*). The Nei's genetic distance (D) between every pairs of totally 12 Junglefowl populations were computed as shown in matrix form, and two dendrograms were drawn by the unweighted-pair-group (UPGMA) method and by the neighbor-joining (NJ) method of clustering. These two dendrograms show that RJF-BAN has a similar gene constitution for blood group systems to RJF-BHU (the both populations belong to *G. g. murghi*), but the gene constitution of RJF-VIE population (*G. g. gallus*) is a member of cluster involving RJF-MYA populations which seemingly belong to *G. g. spadiceus*. In order to estimate a relationship among of the red junglefowl and the Asian native chicken populations in gene constitution, the genetic distance comparisons were carried out among the red junglefowls and the Asian native chicken populations. The position of six Asian native chicken populations was the inside of third group of red junglefowl populations. Our result may suggest that *G. g. gallus* and *G. g. spadiceus* subspecies are the main origin for the Asian native chicken (examined in this study) domestication.

**P-92****EXPERIMENTAL EVIDENCE FOR THE ECTODERMAL ORIGIN OF THE EPITHELIAL ANLAGE OF THE BURSA OF FABRICIUS**

Nándor Nagy, Imre Oláh

*Department of Human Morphology and Developmental Biology, Faculty of Medicine,  
Semmelweis University, Budapest, Hungary*

---

The bursa of Fabricius is a central lymphoid organ of the birds. It is responsible for the B cell maturation and the antigen specific IgM-IgG switch, which depends upon functional bursal follicles of epithelial origin. Bursa arises in the tail bud, caudal to the cloaca in close association of cloacal or urodeal membrane, where the bottom of the anal invagination (anal sinus) of ectoderm and the caudal wall of the cloaca of endodermal origin are juxtaposed. These anatomical conditions give rise the possibility that both ectoderm and endoderm may also contribute to the epithelial anlage of the bursa of Fabricius. However, the anal sinus gradually transform to proctodeum and the bursal duct communicates with the amnion and cloacal cavity at embryonic day (ED) 7-8 and ED17, respectively. These observations have raised the possibility of ectodermal origin together with the expression pattern of sonic hedgehog, which is expressed in the endoderm but not in the ectoderm and the epithelial anlage of bursa of Fabricius. The aim of this study was to clarify the origin of bursal epithelial anlage. The homotopically transplanted quail tail bud ectoderm to ectoderm ablated chick resulted in bursal follicle formation from the quail ectoderm. This result was confirmed by a series of in vitro quail-chick tissue recombination experiments followed by chick coelomic cavity implantation. Follicle formation occurred only if tail bud ectoderm was the recombinant partner of chick mesenchyme. Chick-quail chimeric experiments provide experimental evidence for ectodermal origin of bursal epithelial anlage.

*Grant OTKA: K-69061*

**P-93****ELUCIDATING SPECIFIC RESPONSES AGAINST INFLUENZA IN VACCINATED AND/OR INFECTED CHICKENS USING ELISPOT ASSAYS**

Raul Ruiz-Hernandez, Connor Carson, Dave Balkissoon, Karen Staines, Debra Clayton, Sarah Gilbert, Colin Butter

*Institute for Animal Health, Compton, Berkshire RG20 7NN, United Kingdom*

*E-mail: raul-ruiz.hernandez@bbsrc.ac.uk*

*University of Oxford, Jenner Institute, Old Road Campus Research Building, Oxford, OX3 7DQ, United Kingdom*

A broadly acknowledged method for measuring the immune specific responses is the IFN $\gamma$  ELISPOT assay. We have developed this technology for detection of specific responses in chickens using our own or commercial available antibodies. In the first part of work, these antibodies were first validated in ELISA assays, a calibration for the best reagent and cell culture conditions was performed and a the equivalence of our own and commercial antibodies. Although previous studies have employed IFN $\gamma$  ELISPOT to measure specific responses in viral infectious diseases (ref 1), chicken IFN $\gamma$  ELISPOT has not been used to quantify the specific responses against avian influenza antigens. We vaccinated *in ovo* an inbred line of white leghorn chickens against influenza antigens, infecting then with MVA or Ad5 recombinant virus that expressed Np and M1 influenza proteins. After boosting, those birds vaccinated with this MVA or Ad5 recombinant virus presented specific responses against pools of peptides of Np and M1 proteins in comparison with birds with birds infected with a control MVA- GFP recombinant virus or that received PBS. Furthermore, we found that specific responses are mainly presented in splenocytes in comparison with peripheral blood mononuclear cells (PBMCs). In another experiment, vaccinated birds were infected with low pathogenic avian influenza strain H5N3 (A/strain/Duck/Singapore/1997). We were also able to detect specific responses against pools of peptides of Np and M1 proteins, proving the use of IFN $\gamma$  ELISPOTs to monitor immune responses to avian influenza infection.

**Keywords:** ELISPOT, IFN gamma, Avian influenza infection

**References:**

1. ELISPOT and intracellular cytokine staining: novel assays for quantifying T cell responses in the chicken. Ariaans, M. P. *Dev Comp Immunol.* 2008;32(11):1398-404. Epub 2008 Jun 11.

## P-94

# PATHOGEN-SPECIFIC RESPONSES ARE GENERATED IN THE AVIAN LYMPHOID LUNG

Colin Butter, Laura Fyfe, Karen Staines

*Institute for Animal Health, Compton, Berkshire RG20 7NN, United Kingdom*

*E-mail: colin.butter@bbsrc.ac.uk*

---

We have previously defined the immunological development of the avian lung.

In this study we present compelling evidence that the lung is critical in generating protective immune responses.

Following challenge with a test antigen administered by the intratracheal route the chicken produced a rapid systemic and mucosal humoral response. Vaccination with cell-associated herpesvirus of turkeys by the intratracheal route produced a more robust protective immunity to challenge with Marek's Disease Virus than conventional parenteral administration, particularly when the challenge was also given by the intra-tracheal route. To test whether the lung itself was instrumental in generating immune responses we extracted lymphocytes from spleen and lung of vaccinated animals and determined the number of specific antibody-producing cells (spot forming cells (SFC)) by B-cell elispot. SFC were found in spleen and lung but were seen to be much longer-lasting in the lung. This duration of immunity was found to be reliant on vaccination with live virus, possibly due to persistent infection of the lung with vaccine virus leading to continuous restimulation of the antibody response.

**Keywords:** *lung, antibody response, vaccination, protective immunity*





## O-95

# MULTI-PLATFORM NEXT GENERATION SEQUENCING OF THE DOMESTIC TURKEY (MELEAGRIS GALLOPAVO): GENOME ASSEMBLY AND ANALYSIS

The Turkey Genome Sequencing Consortium

Rami A. Dalloul<sup>1</sup>, Julie A. Long<sup>2</sup>, Aleksey V. Zimin<sup>3</sup>, Luqman Aslam<sup>14</sup>, Kathryn Beal<sup>15</sup>, Le Ann Blomberg<sup>2</sup>, David W. Burt<sup>5</sup>, Oswald Crasta<sup>1</sup>, Richard P.M.A. Crooijmans<sup>14</sup>, Kristal Cooper<sup>1</sup>, Roger A. Coulombe<sup>16</sup>, Supriyo De<sup>8</sup>, Mary E. Delany<sup>10</sup>, Jerry B. Dodgson<sup>11</sup>, Jennifer J. Dong<sup>12</sup>, Clive Evans<sup>1</sup>, Paul Flicek<sup>15</sup>, Liliana Florea<sup>3</sup>, Otto Folkerts<sup>1,6</sup>, Martien A.M. Groenen<sup>14</sup>, Tim T. Harkins<sup>9</sup>, Javier Herrero<sup>15</sup>, Steve Hoffmann<sup>17</sup>, Hendrik-Jan Megens<sup>14</sup>, Andrew Jiang<sup>10</sup>, Pieter de Jong<sup>13</sup>, Pete Kaiser<sup>18</sup>, Heebal Kim<sup>19</sup>, Kyu-Won Kim<sup>19</sup>, Sungwon Kim<sup>1</sup>, David Langenberger<sup>17</sup>, Mi-Kyung Lee<sup>16</sup>, Taeheon Lee<sup>19</sup>, Shrinivasrao Mane<sup>1</sup>, Guillaume Marçais<sup>3</sup>, Manja Marz<sup>17,20</sup>, Audrey P. McElroy<sup>1</sup>, Thero Modise<sup>1</sup>, Mikhail Nefedov<sup>13</sup>, Cédric Notredame<sup>21</sup>, Ian R. Paton<sup>5</sup>, William S. Payne<sup>11</sup>, Geo Pertea<sup>16</sup>, Dennis Prickett<sup>18</sup>, Daniela Puiu<sup>3</sup>, Dan Qioa<sup>1</sup>, Emanuele Raineri<sup>21</sup>, Steven L. Salzberg<sup>3</sup>, Michael C. Schatz<sup>3</sup>, Chantel Scheuring<sup>12</sup>, Carl J. Schmidt<sup>22</sup>, Steven Schroeder<sup>7</sup>, Edward J. Smith<sup>1</sup>, Jacqueline Smith<sup>5</sup>, Tad S. Sonstegard<sup>7</sup>, Peter F. Stadler<sup>17</sup>, Hakim Tafer<sup>17</sup>, Zhijian (Jake) Tu<sup>1</sup>, Curtis P. Van Tassell<sup>7</sup>, Albert J. Vilella<sup>15</sup>, Kelly Williams<sup>1</sup>, James A. Yorke<sup>3</sup>, Liqing Zhang<sup>1</sup>, Hong-Bin Zhang<sup>12</sup>, Xiaojun Zhang<sup>12</sup>, Yang Zhang<sup>12</sup>, Kent M. Reed<sup>4</sup>, Jim Biedler<sup>20 (PART OF 1)</sup>

<sup>1</sup>Virginia Tech, Blacksburg, VA 24061, USA; <sup>2</sup>USDA Agricultural Research Service, Beltsville, MD 20705, USA;

<sup>3</sup>University of Maryland, College Park, MD 20742, USA;

<sup>4</sup>University Minnesota, St. Paul, MN 55108, USA; <sup>5</sup>The Roslin Institute, Roslin, EH25 9PS, UK; <sup>6</sup>Chromatin Inc., 60 Hazelwood Drive Champaign, IL 61820, USA; <sup>7</sup>USDA ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA; <sup>8</sup>National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA; <sup>9</sup>Roche Applied Science, Indianapolis, IN 46250, USA; <sup>10</sup>University of California, Davis, CA 95616, USA; <sup>11</sup>Michigan State University, East Lansing, MI 48824, USA; <sup>12</sup>Texas A&M University, College Station, TX 77843, USA; <sup>13</sup>Children's Hospital and Research Center at Oakland, Oakland, CA 94609, USA; <sup>14</sup>Wageningen University, 6700 AH Wageningen, The Netherlands; <sup>15</sup>European Bioinformatics Institute, Hinxton Cambridge CB10 1SD, UK; <sup>16</sup>Utah State University, Logan, UT 84322, USA; <sup>17</sup>University of Leipzig, D-04107 Leipzig, Germany; <sup>18</sup>Institute for Animal Health, Compton, RG20 7NN, UK; <sup>19</sup>Seoul National University, Seoul 151-742, Korea; <sup>20</sup>Philipps-Universität Marburg, 35032 Marburg, Germany; <sup>21</sup>Centre for Genomic Regulation, E08003 Barcelona, Spain; <sup>22</sup>University of Delaware, Newark, DE 19716, USA;

Corresponding author: dave.burt@roslin.ed.ac.uk

Next-generation sequencing technologies were used to rapidly and efficiently sequence the genome of the domestic turkey. The current genome assembly (1.1 billion base pairs) includes 917 million base pairs of sequence assigned to specific chromosomes. Innate heterozygosity of the sequenced bird allowed the discovery of more than 600,000 high quality single nucleotide variants. Annotation pipelines predicted nearly 16,000 genes, with 15,093 recognized as protein coding and 611 non-coding RNA genes. Comparative analysis of turkey, chicken and zebrafish genomes, and comparisons with mammals, supports the notable stability of avian genomes and identifies genes unique to birds. Clear differences are seen in the number and variety of genes of the immune system where gene duplication events are less frequent in birds than gene losses. The turkey genome sequence provides resources to further understand the evolution of avian genomes and the reference to discover genetic variations underlying economically important quantitative traits.



**Budapest, Hungary, 7-10 October 2010**

---



---

## **Contact list of corresponding authors**





CODE	NAME	E-MAIL
P-01	BAINOVA, Hana	hanulay@gmail.com
P-02	BALKISSOON, Devanand	dave.balkissoon@bbsrc.ac.uk
O-03	BARBER, Megan	megan.macdonald@ualberta.ca
O-04	BEAN, Andrew	andrew.bean@csiro.au
P-05	BEAN, Andrew	andrew.bean@csiro.au
P-06	BENYEDA, Zsófia	zsbenyeda@prophyl.hu
P-07	BÍRÓ, Éva	biro.eva@med.semmelweis-univ.hu
P-08	BÓDI, Ildikó	bodi.ildiko@med.semmelweis-univ.hu
P-09	CARSON, Connor	connor.carson@ndm.ox.ac.uk
P-10	CHIMENO ZOTH, Silvina	schimeno@cnia.inta.gov.ar
P-11	CHIMENO ZOTH, Silvina	schimeno@cnia.inta.gov.ar
O-12	COLLISSON, Ellen	ecollisson@westernu.edu
O-13	COLLISSON, Ellen	ecollisson@westernu.edu
O-14	DALGAARD, Tina	Tina.Dalgaard@agrsci.dk
P-15	DALGAARD, Tina	Tina.Dalgaard@agrsci.dk
O-16	DALLOUL, Rami	RDalloul@vt.edu
P-17	DALLOUL, Rami	RDalloul@vt.edu
P-18	DE GEUS, Eveline	e.d.degeus@uu.nl
O-19	DE GEUS, Eveline	e.d.degeus@uu.nl
P-20	DÓRA, Dávid	david.dora@med.semmelweis-univ.hu
O-21	DUSANIC, Daliborka	daliborka.dusanic@bf.uni-lj.si
O-22	ELDAGHAYES, Ibrahim	ibrahim.eldaghayes@vetmed.edu.ly
O-23	ERF, Gisela	gferf@uark.edu
P-24	FELFÖLDI, Balázs	balazs.felfoldi@ceva.com
P-25	FINK, Dorte	dorof@vet.dtu.dk
P-26	FLEMING, Ximena	flemingc@ualberta.ca
O-27	GANAPATHY, Kannan	gana@liv.ac.uk
O-28	GIBSON, Mark	mark.gibson@bbsrc.ac.uk
O-29	GÖBEL, Thomas	goebel@lmu.de
O-30	GÖBEL, Thomas	goebel@lmu.de
O-31	GOOSSENS, Kate	kate.goossens@csiro.au
O-32	HAERTLE, Sonja	Sonja.Haertle@lmu.de
O-33	HAERTLE, Sonja	Sonja.Haertle@lmu.de
O-34	HAERTLE, Sonja	Sonja.Haertle@lmu.de
O-35	HAINKE, Susanne	susanne.hainke@tiph.vetmed.uni-muenchen.de
P-36	HERBERTH-MINKÓ, Krisztina	minko.krisztina@med.semmelweis-univ.hu
O-37	HOLT, Peter	pete.holt@ars.usda.gov
O-38	JANSEN, Christine	c.a.jansen@uu.nl
O-39	JANSEN, Christine	c.a.jansen@uu.nl
O-40	JANSEN, Christine	c.a.jansen@uu.nl
P-41	JIAO, Xinan	jiao@yzu.edu.cn
P-42	JIAO, Xinan	jiao@yzu.edu.cn
P-43	JUUL-MADSEN, Helle	Helle.JuulMadsen@agrsci.dk
P-44	JUUL-MADSEN, Helle	Helle.JuulMadsen@agrsci.dk
O-45	KAPCZYNSKI, Darrell	darrell.kapczynski@ars.usda.gov
O-46	KEELER, Calvin	ckeeler@udel.edu
P-47	KJAERUP, Rikke	rikkem.kjaerup@agrsci.dk



Budapest, Hungary, 7-10 October 2010

CODE	NAME	E-MAIL
P-48	KOLESÁROVÁ, Martina	kolesarova@uvm.sk
P-49	KOLESÁROVÁ, Martina	kolesarova@uvm.sk
P-50	LAI, Huong Thi Lan	Thi.Lai@wur.nl
O-51	LAMMERS, Aart	aart.lammers@wur.nl
O-52	LARDINOIS, Amélyne	amlar@var.fgov.be
P-53	LIU, Xianyong	liuxianyong@cau.edu.cn
O-54	LOWENTHAL, John	john.lowenthal@csiro.au
O-55	MAGOR, Kathy	kmagor@ualberta.ca
O-56	MAHGOUB, Heba	heba.mahgoub@bbsrc.ac.uk
P-57	MOLNÁR, Dávid	molnar.david@med.semmelweis-univ.hu
P-58	NAGY, Nándor	nagyn@ana2.sote.hu
P-59	NARAT, Mojca	mojca.narat@bf.uni-lj.si
P-60	NORUP, Liselotte	LiselotteR.Norup@agrsci.dk
P-61	NORUP, Liselotte	LiselotteR.Norup@agrsci.dk
P-62	OGUNREMI, Dele	Dele.Ogunremi@inspection.gc.ca
O-63	OGUNREMI, Dele	Dele.Ogunremi@inspection.gc.ca
O-64	OGUNREMI, Dele	Dele.Ogunremi@inspection.gc.ca
P-65	OGUNREMI, Dele	Dele.Ogunremi@inspection.gc.ca
O-66	PANTIN-JACKWOOD, Mary	mary.pantin-jackwood@ars.usda.gov
O-67	PARMENTIER, Henk	Henk.Parmentier@WUR.nl
P-68	QUERE, Pascale	quere@tours.inra.fr
P-69	QUERE, Pascale	quere@tours.inra.fr
P-70	RATH, Narayan	narayan.rath@ars.usda.gov
O-71	RAUTENSCHLEIN, Silke	Silke.rautenschlein@tiho-hannover.de
P-72	RAUW, Fabienne	farau@var.fgov.be
P-73	REBEL, Johanna	annemarie.rebel@wur.nl
O-74	REBEL, Johanna	annemarie.rebel@wur.nl
P-75	RODRIGUEZ-LECOMPTE, Juan	JC_Rodriguez-Lecompte@umanitoba.ca
O-76	SCHAT, Karel	kas24@cornell.edu
O-77	SCHOKKER, Dirkjan	dirkjan.schokker@wur.nl
O-78	SCHUSSER, Benjamin	schusser@tiph.vetmed.uni-muenchen.de
O-79	SHARIF, Shayan	shayan@uoguelph.ca
P-80	SHARIF, Shayan	shayan@uoguelph.ca
O-81	STAINES, Karen	karen.staines@bbsrc.ac.uk
O-82	VAN DER POEL, Jan	jan.vanderpoel@wur.nl
O-83	VANDERVEN, Hillary	hillary@ualberta.ca
P-84	VAN GINKEL, Frederik	vangifw@auburn.edu
P-85	VELDHUIZEN, Edwin	E.J.A.Veldhuizen@uu.nl
P-86	VELDHUIZEN, Edwin	E.J.A.Veldhuizen@uu.nl
P-87	VINKLER, Michal	vinkler1@natur.cuni.cz
O-88	WATERS, Victoria	victoria.waters@bbsrc.ac.uk
P-89	WATTRANG, Eva	eva.wattrang@sva.se
O-90	WU, Zhiguang	zhiguang.wu@roslin.ed.ac.uk
P-91	YAMAMOTO, Yoshio	yyama1940@yahoo.co.jp
P-92	NAGY, Nándor	nagyn@ana2.sote.hu
P-93	RUIZ-HERNANDEZ, Raul	raul-ruiz.hernandez@bbsrc.ac.uk
P-94	BUTTER, Colin	Colin.Butter@bbsrc.ac.uk
O-95	BURT, David W.	dave.burt@roslin.ed.ac.uk



---

## **Authors' index**







## A

Aarnink, A. J. A.	50
Abel, H.	71
Albrecht, T.	1, 87
Aldridge Jr., J. R.	3
Anbari, S.	72
Anderson, K. E.	37
Arnold, G. J.	34
Aslam, L.	95

## B

Bailey, M.	56
Bainová, H.	1, 87
Balkissoon, D.	2, 93
Banat, G. R.	13
Barber, M. R. W.	3
Bauersachs, S.	34
Baule, A.	54
Beal, K.	95
Bean, A. G. D.	4, 5, 31, 54
Bed'Hom, B.	69
Bedford, M. R.	17
Behboudi, S.	79, 80
Benčina, D.	21, 59
Benyeda, Zs.	6
Bergès, M.	69
Bergstra, T.	67
Berhane, Y.	62, 63
Berinstein, A.	10, 11
Biedler, J.	95
Bikker, F.	85
Bingham, J.	4, 5
Bird, S.	28
Bíró, É.	7
Biscarini, F.	82
Blomberg, L. A.	95
Bódi, I.	6, 8
Boettger, C.	46
Bollérot, K.	36
Bovenhuis, H.	82
Brady, J.	75
Breves, G.	71
Briles, W. E.	12, 13

Broadway, M.	54
Broks, V.	74
Bruneau, N.	69
Brusnyk, C.	26
Bryja, J.	1, 87
Bryjová, A.	1, 87
Burggraaf, S.	4, 5
Burt, D.	74, 95
Butter, C.	2, 56, 81, 90, 93, 94

## C

Cagle, C.	66
Camelo-Jaimes, G.	75
Caprioli, A.	36
Carballeda, J. M.	10, 11
Carrillo, E.	10
Carson, C.	93
Chen, C.	22
Chen, J.	42
Chimeno Zoth, S.	10, 11
Clavijo, A.	62, 63
Clayton, D.	93
Collisson, E. W.	12, 13
Cooper, K.	95
Cornelissen, J. B. W. J.	73, 74
Coulombe, R. A.	95
Cox, C. M.	16, 17
Crasta, O.	95
Crooijmans, R. P. M. A.	95
Crow, G.	75
Csikós, G.	58

## D

Dai, H.	42
Dalgaard, T. S.	14, 15, 43, 44, 47, 60, 61
Dalloul, R. A.	16, 17, 95
Dawes, M.	13
de Geus, E. D.	18, 19
de Jong, P.	95
de Vries Reilingh, G.	51
De, S.	95
Delany, M. E.	95
Dhondt, K. V.	76
Dieterlen-Lievre, F.	36
Dodgson, J. B.	95



Dong, J. J.	95
Dóra, D.	20, 57
Drechsler, Y.	13
Dušanić, D.	21, 59

## E

Eldaghayes, I.	22
Erf, G. F.	23
Evans, C.	95

## F

Fang, Q.	41
Felföldi, B.	24
Fetterer, R. H.	16
Fife, M.	28, 56
Fijten, H.	73
Fink, D. R.	25
Fleming, X.	26
Flicek, P.	95
Florea, L.	95
Folkerts, O.	95
Fu, Q.	64, 65
Fyfe, L.	94

## G

Ganapathy, K.	27
Gast, R. K.	37
Gaully, M.	71
Geng, S.	41
Gibson, M.	28
Gilbert, S.	93
Goldstein, A. M.	58
Gómez, E.	10, 11
Goossens, K. E.	31, 54
Göbel, T.	29, 30, 39
Gravisaco, M. J.	10, 11
Grešáková, L.	49
Griggs, L.	13
Grodio, J.	76
Groenen, M. A. M.	95
Guenter, W.	75
Gulley, S. L.	84

## H

Haagsman, H. P.	85, 86
Haertle, S.	78
Haghighi, H. R.	80
Hainke, S.	35
Handberg, K. J.	15
Hannaman, D.	62, 63
Harkins, T. T.	95
Härtle, S.	32, 33, 34, 35
Hawley, D. M.	76
Herrero, J.	95
Hoffmann, S.	95
Hogenkamp, A.	86
Holt, P. S.	37
House, J.	75
Huang, X.	53

## J

Jackwood, M. W.	13
Jaffredo, T.	36
Jansen, C.	38, 39, 40
Jenkins, K.	54
Jenkins, M. C.	16
Jensen, S.	55
Jiang, A.	95
Jiang, H. J.	45
Jiao, X.	41, 42
Jones, R. C.	27
Jørgensen, P. H.	15
Jungierius, A. P.	82
Jungersen, G.	25, 60
Juul-Madsen, H. R.	14, 15, 43, 44, 47, 60, 61

## K

Kabell, S.	7
Kaiser, P.	22, 27, 28, 56, 88, 90, 95
Kannan, L.	70
Kapczynski, D. R.	45
Karpala, A. J.	4, 54
Kaspers, B.	32, 33, 34, 35, 78
Kastelic, S.	21
Kaufman, J.	2
Keeler, C. L. Jr.	46
Kelly, H.	64, 65
Kim, H.	95



Kim, K-W.	95
Kim, S.	16, 17, 95
Kimpton, W. G.	5
Kjærup, R. M.	44, 47, 60, 61
Kobinger, G. P.	62, 63
Kocsis, K.	6, 7
Kolesárová, M.	48, 49
Kopulos, R.	12
Korte, J.	34
Krohmann, C.	78

## L

Lai, H. T. L.	50
Lalmanach, A-C.	68
Lambrecht, B.	52, 72
Lammers, A.	51, 67, 84
Langenberger, D.	95
Lardinois, A.	52
Lauková, A.	48
Lay, J. O.	70
Lee, M-K.	95
Lee, T.	95
Lemiere, S.	27
Levkut, M.	48, 49
Li, J.	53
Lian, Z.	22
Lillehoj, H.	65
Liu, X.	53
Liyanage, R.	70
Long, J. A.	95
Lowenthal, J. W.	4, 5, 54
Lowther, S.	4
Lundén, A.	89

## M

Magnusson, S. E.	89
Magor, K. E.	3, 26, 55, 83
Mahgoub, H.	56
Maillard, J-C.	69
Mallick, A. I.	79
Mane, S.	95
Mansour Haeryfar, S. M.	80
Marcais, G.	95
Marty, H.	68
Marz, M.	95

Maughan, M. N.	46
McElroy, A. P.	17, 95
Megens, H-J.	95
Mesa, C.	55
Minkó, K.	36
Miska, K. B.	16
Modise, T.	95
Molhoek, E. M.	85
Molnár, D.	20, 57, 58
Moon, D.	26, 55

## N

Nagy, É.	79
Nagy, N.	6, 7, 8, 20, 24, 36, 57, 58, 92
Namikawa, T.	91
Narat, M.	21, 59
Nefedov, M.	95
Neulen, M-L.	30
Nieuwland, M. G. B.	67
Norup, L. R.	14, 15, 43, 44, 47, 60, 61
Notredame, C.	95

## O

Ogunremi, D.	62, 63, 64, 65
Oláh, I.	6, 7, 8, 20, 54, 57, 58, 92
Opatova, P.	87
Osnas, E. E.	76
Oven, I.	59

## P

Palya, V.	6, 7
Pan, Z.	41, 42
Pantin-Jackwood, M.	66
Parks-Dely, J.	55
Parmentier, H. K.	50, 51, 67, 82
Parvizi, P.	79
Pasick, J.	62, 63
Paton, I. R.	95
Payne, J.	5
Payne, W. S.	95
Pedersen, A. R.	15
Peeters, B.	73, 74
Penski, N.	78
Permin, A.	61



Pertea, G.	95
Pharr, G. T.	24
Pleidrup, J.	44, 47, 60, 61
Post, J.	38, 73, 74
Prickett, D.	95
Promerová, M.	1
Puiu, D.	95

## Q

Qioa, D.	95
Quéré, P.	68, 69

## R

Raineri, E.	95
Ramirez-Yanez, G.	75
Rath, N. C.	70
Rautenschlein, S.	71
Rauw, F.	72
Read, L. R.	79, 80
Rebel, A.	38
Rebel, J. M. J.	19, 73, 74, 77
Rebeski, D.	52
Reed, K. M.	95
Reemers, S.	38, 40, 86
Revajová, V.	48, 49
Rodríguez-Lecompte, J. C.	75
Rosenberger, J. K.	46
Ross, K.	26
Rothwell, L.	22, 27, 90
Ruiz-Hernandez, R.	2, 81, 93
Russell, P.	22

## S

Sadeyen, J-R.	22
Saggese, M.	13
Salzberg, S. L.	95
Schat, K. A.	76
Schatz, M. C.	95
Schenk-Weibhauser, K.	33
Scheuring, C.	95
Schmidt, C.	46, 95
Schmieder, S.	34
Schokker, D.	77
Schou, T. W.	61
Schroeder, S.	95

Schumacher, M.	33
Schusser, B.	78
Schwarz, A.	71
Seeliger, C.	32
Ševčíková, Z.	48
Sharif, S.	75, 79, 80
Shen, H.	43
Shi, F.	23
Shivaprasad, H. L.	13
Sibille, P.	69
Sijts, A.	40
Singh, S.	12
Slaminková, Z.	49
Smith, A.	2, 89
Smith, E. J.	95
Smith, J.	95
Smits, M. A.	77
Sonstegard, T. S.	95
Spišáková, V.	48
Stadler, P. F.	95
Staeheli, P.	78
Staines, K.	2, 81, 93, 94
Steensels, M.	52
Strompfová, V.	48
Su, H.	53
Sumners, L. H.	16, 17
Suo, X.	53

## T

Tafer, H.	95
Tang, D-C.	12
Tefsen, B.	18
Thebo, P.	89
Tkalcic, S.	13
Toàn, T. X.	69
Tomasekm, O.	87
Toro, H.	12, 84
Tu, Z. J.	95

## V

Vahlenkamp, T.	78
van Arendonk, J. A. M.	82
van de Haar, P.	39
van den Berg, T.	52, 72
van der Poel, J. J.	82



van Die, I.	18
van Dijk, A.	85
van Drunen Little-van den Hurk, S.	62
van Eden, W.	39
van Eijk, M.	86
van Ginkel, F. W.	84
van Haarlem, D.	39
Van Tassell, C. P.	95
van Zoelen, D.	74
van Zutphen, L. J. W.	51
Vandervlen, H.	83
Vaughn, L. E.	37
Veldhuizen, E. J. A.	85, 86
Vervelde, L.	18, 19, 38, 39, 40, 73, 86
Viertlboeck, B. C.	29, 30
Vilella, A. J.	95
Vinkler, M.	1, 87

## W

Ward, A. C.	31
Wasilenko, J.	66
Waters, V.	88
Wattrang, E.	14, 89
Webster, R. G.	3, 83

Werling, D.	88
Williams, K.	95
Woo, P.	28
Wu, Z.	22, 90

## X

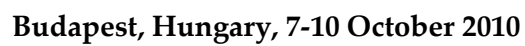
Xia, J.	26
---------	----

## Y

Yamamoto, Y.	91
Yates, L.	12
Yin, G.	53
Yorke, J. A.	95
Young, J.	2, 33

## Z

Zhang, H-B.	95
Zhang, L.	95
Zhang, X.	95
Zhang, Y.	95
Zhao, W.	41
Zimin, A. V.	95
Zou, J.	53









## Main activities of PROPHYL Ltd. and Biovo Ltd.

- ▶ Vaccine and SPF egg production
- ▶ CRO animal test facility for GLP and GCP testing of vaccines and pharmaceuticals, supporting research and development processes of multinational companies
- ▶ Production of biological materials (blood, sera, egg yolk antibodies, etc.) under SPF and commercial circumstances
- ▶ Distribution of Veterinary vaccines and medicines

---

If you need further information please do not hesitate to contact us!

E-mail: [jbenyeda@prophyl.hu](mailto:jbenyeda@prophyl.hu)



## HUNGARIAN EQUINE REHABILITATION AND HEALTH SERVICE

I founded the **Hungarian Equine Rehabilitation and Health Service** in 1999 with the aim of establishing such an animal health service in the Transdanubian region that is able to diagnose and treat the surgical, internal medical and reproduction diseases of the large animals both in the field and in clinical conditions.

It was an important consideration to provide a high-standard service for the Equistrain Academy of the Diagnostic Imaging and Radiation Oncology, University Kaposvár despite its distance from Large Animal Clinic of the Faculty of Veterinary Science, Szent István University. In the interest of it's continuous development, a well- equipped equine service was resulted. This Service can provide medical attendance both in the field and in the clinic working together with several veterinarians.

The Hungarian Equine Rehabilitation and Health Service lays emphasises to help the veterinarians working on the field with their high quality equipments and also takes part in different research and training programmes. It submitted successful tenders both inland (Széchenyi Plan) and in the EU (GVOP, GOP programmes).

Our staff take an important role in national and international animal and human healthcare researches, have scientific (PhD) and clinical (Equine Specialist) qualifications and take part continuously in training programmes. We have been regular presenters at the annual Congress of Association of Hungarian Equine Practitioners for several years in each autumn. We performed more than 35.000 treatments and 950 operations between 1999-2009.

Our fundamental philosophy is that with a cooperation based on respect and sincere helping intention (Hungarian Equine Rehabilitation and Health Service-Colleagues-Owners) we are able to provide the most effective help both the sick animal and its worried owner, too.

### Contact

7400 Kaposvár, Guba Sándor út 40.  
Fax: +36-82-410 542  
Mobil: +36-30 300 4914  
[www.pannonlogogyasz.hu](http://www.pannonlogogyasz.hu)

**Dr. Ákos Tibor Hevesi Ph.D, MRCVS,**  
**Equine Specialist**  
**Leading veterinary**



Protect your eggs. Protect your profits.

## Nobilis<sup>®</sup> the *trusted* name in poultry vaccines

Infectious diseases can have a dramatic negative effect on flock performance and profitability. For decades Intervet has been a trusted partner in poultry production, delivering safe, effective and innovative disease solutions.

The Nobilis range of live and inactivated vaccines protects against a wide range of viral, bacterial and parasitic diseases.

Vaccines such as Nobilis E. coli, Nobilis Salenvac T and Nobilis IB 4/91 have revolutionised poultry health worldwide, making the industry less vulnerable to risk whilst safeguarding profitability.

Intervet's continued commitment to research guarantees future vaccine development, which will protect your assets against the challenges of current and future disease threats.