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Standard Report

Document Status: With Council
BBSRC Reference: BB/D007542/1

Scheme: Standard grant
Type: PI on a single grant

Award Holding Organisation

Organisation	University of Liverpool	Research Organisation Reference:	6250 VPR10031
Division or Department	Veterinary Pathology		

Title of Research Project

Effect of immunosuppression associated with point-of-lay on Salmonella infection and immunity in laying hens

Project Details

Start Date	03/07/2006	Duration of Grant (months)	45
End Date	02/04/2010	Total Grant Value	£ 199157.7
Report Due Date	22/06/2010		

Investigators

Role	Name	Organisation	Division or Department
Principal Investigator	Dr P Wigley	University of Liverpool	Veterinary Pathology

Objectives

The main objectives of the research in order of priority [up to 4000 chars] at proposal time

1.Determination of the nature of immunosuppression in point-of-lay hens.
2. To determine whether immunosupression at point-of-lay increases susceptibiity to salmonellosis in the chicken
3. To ascertain whether vaccination is effective measure against Salmonella challenge at point-of-lay in chickens
4. To initiate development of novel vaccine strategies and use of adjuvants/non-specfic immune stimulation that may overcome point-of-lay immunosuppression

The main objectives of the research in order of priority [up to 4000 chars] at report time

Currently this information is not available electronically. Please refer to original application.

Publication Summary

	Refereed Journal	Conference Proceedings	Books	Popular Journal	Other
Total	1	1	1	0	0
Total with Industrial Co-Authors	0	0	0	0	0
Total with International Co-Authors	0	0	0	0	0

Publications

Type	Title	Author(s)	Reference			
			Name	Year	Vol.	Page
Refereed Journal	Immunobiology of systemic salmonelosis	Dr Lucy Chappell Professor Pete Kaiser Professor Paul Barrow Dr Michael Jones Dr Claire Johnson	Veterinary Immunology and Immunopathology	2009	128	53
Conference Proceedings	Cellular and functional changes in the reproductive tract assocaited with onset-of-lay in chickens	Dr Claire Johnson Dr Paul Wigley	Veterinary Immunology and Immunopathology	2009	128	243
Book	The avian reproductive immune system	Dr Paul Wigley Professor Karel Schat Professor Paul Barrow	Avian Immunology	2008		289

Results and Outputs

Resources generated.
Provide details of any new products or processes that were developed, e.g. reagents, cell lines, transgenic lines, software, analytical tools or methodology.

Development of potential vaccine adjuvant for killed poultry vaccines

Animal research addressing the 3Rs):
i) welfare
Describe any outcomes from the research that are relevant to animal welfare that are not covered in the full report.

Use of enriched environments for poultry experiments do not impair infection models

Animal research addressing the 3Rs:
ii) reduction and replacement
Describe methods that were developed to reduce and replace animal experiments: the development of new alternative methods to animal models including in vitro methods, computational methods, non-animal methods and non-invasive in vivo methods.

Use of cell culture in inital experiments. Experiments refined and improved through improved experimental poultry facility-renovated in response to BBSRC funding and improved welfare and enrichment of poultry rooms

Other results and outputs.

In addition to the detailed publications, two other manuscripts are in preparation for submission in the next three months

Results Exploitation and Knowledge Transfer

Data/sequences lodged in public access databases. Give details of database(s).

Not applicable

Licenses and/or patents. Give full details.

None

Spin-out company. Give details of incorporation date, turnover, staff numbers, etc.

None

Industrial collaborations. Include grants from, or formal collaborations with, industry or elsewhere, or exchanges of staff, materials or results.

Development of links and projects with industry including major poultry producers (Vion & Aviagen). nutrition companies (Dansico). Formal collaboration with Provexis on BBSRC LINK grant 'The potential role of soluble plantain fibre and its components in preventing colonisation and invasion of the intestinal mucosa by S.Typhimurium-BB/G01969X/1'.

Science in Society

Describe activities undertaken to enhance public engagement with the biosciences by the PI, component grant holder, PhD student or employee funded during the course of the grant. Activities can cover broader areas than your grant and could include media interactions and media training, public meetings and lectures, exhibitions, open days, school events, etc. Presentations at scientific conferences or articles in refereed journals do not count as public engagement.

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1. Seminar given to 5th and 6th forms West Kirby Grammar School, Wirral on infectious diseases and the use of animals in research April 2006 (PI)
2. Interactive session on 'Animals and Disease' given to primary school children (Years 2 and 3) St Silas CoE Primary, Toxteth, Liverpool and Woodfall Primary, Neston, Cheshire. This used Vet School resources (bones etc.) to understand animals and their diseases pitched at 6-8 year olds April and May 2008 (PI)
3. Participated in 'Easter School' at School of Veterinary Sciences. A three day taster programme to offer potential students from diverse and non-standard 'A' level backgrounds the opportunity to experience a University Veterinary School and its research in biosciences. April 2009 (PI)
4. Organised a stand at the 'Big Bang' science fair in Manchester representing the National Centre for Zoonosis research. This inolvolved a game for the under-12s matching the 'cuddly zoonotic pathogen' (e.g. Salmonella, Ebola Virus) to an important 'cuddly' animal reservoir (e.g. chicken, bat).March 2010
5. The researcher and PI were able to support two summer studentships during the course of the grant. The first project was a Nuffield 6th form studentship working on 'Salmonella in greenfinches'. The student Miss Hannah Bradon won 'Astra Zeneca Young Innovator of the Year 2007' at the BA Crest Science fair on the basis of her work which also lead to a publication in BMC Veterinary Research (Hughes et al BMV Veterinary Research 4:4 2008). The second project was an undergraduate project funded by the Society for Applied Microbiology that investigated the role of Salmonella Type III secretion systems on survival in protozoa. This work involved colllaboration with BBSRC IAH Compton and lead to a publication in Applied and Environmental Microbiology (Bleasdale et al Applied Environ Microbiol 75 1793 2009). Both pieces of work were featured on the front page of the University website.

Summary

The research described in lay terms, that could be publicised to a general audience [up to 4000 chars]

Original Summary

Salmonella enterica is one of main causes of bacterial food poisoning in the world. One type of Salmonella called Salmonella Enteritidis has the ability to infect the eggs of chickens that are infected with Salmonella bacteria. If infected eggs are eaten raw or poorly cooked, there is a chance that the person may become ill. In most cases this results in diarrheoa and a short term illness but in young children, the elderly or people with immune systems that do not work well, Salmonella infection can be very serious. In some case it may result in death. In recent years vaccination has been used as part of the control of Salmonella in chickens. However just before hens begin laying eggs (point-of-lay), their immune system becomes suppressed or less effective. This may mean that even vaccinated chickens are more likely to catch a Salmonella infection at this point. This research will find out which parts of the immune system do not work well at the point-of lay. We will then test Salmonella vaccines and measure how well they stimulate the immune system at point-of-lay and whether these vaccinated birds are more likely to get Salmonella at this time.The research will allow us to improve how and when we vaccinate chickens against Salmonella so they are protected when they start to lay eggs. It will also help us make better vaccines that protect chickens better and for longer than current vaccines.

Revised Summary

Salmonella enterica is one of main causes of bacterial food poisoning in the world. One type of Salmonella called Salmonella Enteritidis has the ability to infect the eggs of chickens that are infected with Salmonella bacteria. If infected eggs are eaten raw or poorly cooked, there is a chance that the person may become ill. In most cases this results in diarrheoa and a short term illness but in young children, the elderly or people with immune systems that do not work well, Salmonella infection can be very serious. In some case it may result in death. In recent years vaccination has been used as part of the control of Salmonella in chickens. However just before hens begin laying eggs (point-of-lay), their immune system becomes suppressed or less effective. This may mean that even vaccinated chickens are more likely to catch a Salmonella infection at this point. In this research we have shown that this change is mainly due to a reduction in the numbers of a type of cell in the immune system known as a T helper or CD4+ lymphocyte that are important in controlling the type of immune response important in Salmonella infection of the chicken. We have also shown for the first time how

the cells of the immune system within the reproductive and egg laying parts of the hen are organised and that there are considerable local changes to the immune system during the start of the egg laying period.
We have shown that hens are also infected more easily or are more susceptible to infection when the reduction in T lymphocytes is greatest. However the use of vaccines, and in particular those current used in the UK, help provide protection to the hens that greatly reduces the chance of Salmonella infecting eggs.
We have also shown that by stimulating the immune system together with vaccination helps provide increased protection. This is called an adjuvant-named after the Latin word 'adjuvare' meaning help because it 'helps' a vaccine be more effective. We have tried a new adjuvant that contains a type of DNA molecule found only in bacteria called a CpG motif. CpG motifs make the immune system think that there is 'danger' from infection and 'switch on the immune system'. By including CpG motifs and a messenger from the chicken immune system called interferon, we were able to make simple vaccines protect better against infection.
We also tried to use CpG motifs to help the hen clear Salmonella infections. Although this worked on cultured cells from the immune system called macrophage where Salmonella hides during infection, it did not work in infected animals. We did however show that a certian form of Salmonella which is very rare in the UK called Salmonella Gallinarum, but kills many chickens throughout the world was not cleared from cells by CpG stimulation. This was not expected and has helped us better understand how this type of Salmonella causes disease in chickens.
Most importantly we have shown that the chicken immune system works less well when they first start to lay eggs and the reasons why this happens. However it is good news that the vaccines we use in the UK to prevent egg infection by Salmonellla clearly work well enough to prevent egg infection.

Technical Summary

at proposal time

The project will investigate the mechanisms that underlie the observed suppression of adaptive, and in particular cell mediated immunity at the onset of sexual maturity of laying hens and the effect these changes have on immunity to Salmonella enterica serovar Enteritidis. Salmonella Enteritidis may be transmitted vertically through eggs and thereby poses a risk to public health through the consumption of poorly cooked contaminated eggs. Although vaccination is employed in the UK in the control of Salmonella in laying hens, the immune mechanisms that underpin protection are not known. It is also unclear whether there is a gap in immunity at point-of-lay in vaccinated animals that may compromise protection to salmonellosis. The first aim of the project is to characterise changes in the immune system in developing hens. Flow cytometry will be used to determine the levels of T and B lymphocytes in the spleens of hens. Further characterisation of CD4 and CD8 positive, along with T cell receptor type (TCR1, 2 or 3)will also be performed. Immunocytochemical staining will be performed on tissues, including the oviduct and ovaries which are the main sites of Salmonella infection. Whilst these assays will indicate changes in cell numbers and population distributions, analysis of T cell function will be determined by proliferation to stimulation with the mitogens ConA and PHA. Immunoglobulin levels will be determined by ELISA.
In a second series of experiments, we will characterise the immune response to Salmonella infection and to vaccination with currently available live and killed vaccines. Cellular changes will be determined as outlined above. T cell proliferation to Salmonella antigens will be determined and specific antibody responses to Salmonella (IgM, IgG and IgA) determined by ELISA. Expression of key cytokines, IL-2, Il-4, IL-10, IL-12 and interferon-gamma will be performed on tissues by qRT-PCR. The use of magnetic cell sorting will be done to obtain specific ce

Staff

Role Name	Name / Post Identifier	Grade / Scale	Start Date	End Date	Gender	Qualifications gained on project
Researcher	Dr Claire Johnson	RA1A	03/07/2006	15/02/2008	Female	BSc PhD
Researcher	Dr Michael Johnston	RA1A	03/11/2008	03/05/2009	Male	BSc PhD
Researcher	Ms Catherine Hartley	RA1A	01/08/2009	02/03/2010	Female	BSc, MSc, MIBIOL

Staff Destinations

Name	Organisation details	Employment type
Dr Claire Johnson	University of Wales, Swansea Medical School United Kingdom	FurtherTraining
Dr Michael Johnston	Trinity College Dublin Ireland	HigherEducationResearch
Ms Catherine Hartley	University of Liverpool United Kingdom	HigherEducationResearch