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The Prohealth Project: A review of some of the findings of relevance to the poultry industry

Abstract

The EU-funded Prohealth project aims to improve the health of conventionally farmed poultry and pigs by a wide-ranging programme of research into the causation of so-called "production diseases". The project consortium includes a broad range of skills and interests, including experts on research on consumer attitudes. Here we review the general background to the project and then consider results in the area of consumer perception, biosecurity assessment and establishment of normal gut flora in young birds. The project web-site includes newsletters and references to other project areas also of interest to poultry producers.

Keywords

egg, egg consumption, microbiology, production disease, microbiome, biosecurity, farm hygiene, hatching egg sanitation

Introduction

In 2012 the EU Commission announced its intention to fund research under Framework Programme 7 on **Sustainable animal production: an integrated and multi-factorial Approach.** The aim of this was to contribute to our understanding of the multi-factorial dimension (infectious agents, genetics, nutrition, and management factors) of diseases of poultry and pigs "linked to the intensification of production, so-called 'production diseases'", and so to help us to arrive at effective control strategies and reduce the impact on animal health and welfare. I was asked to help a consortium of 22 partners led by the University of Newcastle and one of our first tasks was to define what a 'production disease' is (the term had previously been applied mainly to metabolic diseases of ruminants). The definition we proposed was

"Diseases which tend to persist in animal production systems and, typically, become more prevalent or severe, in proportion to the potential productivity of the system". Our bid was successful and the Prohealth project started in December 2013 and will run until November 2018. Our consortium includes a broad range of partners including academic institutions, breeding companies, and small and medium enterprises relevant to the project objectives. They are active in 10 different EU member states and one associated country and have, collectively, a good geographic spread and involvement in poultry and pig production.

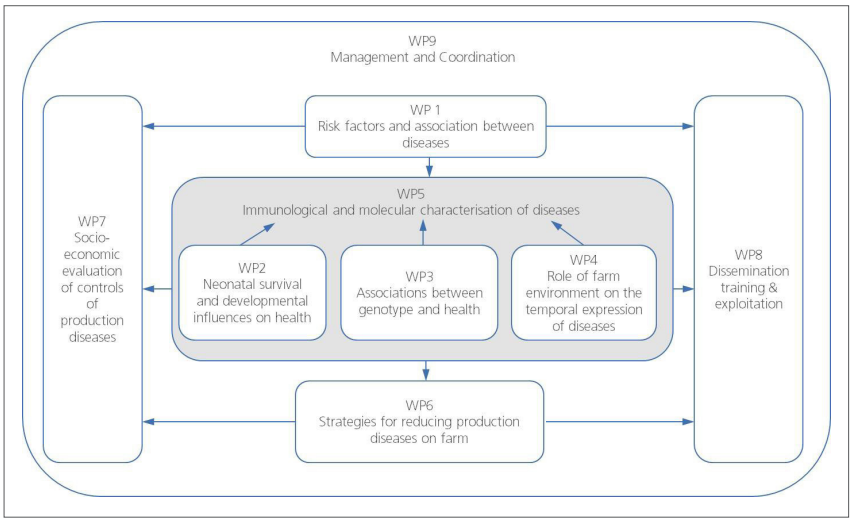

Figure 1 Project Overview

The project has a broad ranging and ambitious set of objectives to:

- identify the risk factors for production diseases and establish associations between diseases;
- explore the role of genetic and environmental factors on neonatal survival and in exerting longer-term developmental influences on health;
- evaluate the effects of genetic selection for productive traits on susceptibility and identify strategies to mitigate these;
- determine the role of variation in farm environment on the temporal expression of production diseases;
- characterize the microbio-immunological changes and identify pathological changes at the molecular level which take place during production diseases in order to develop diagnostic tools;
- synthesize strategies to reduce the impact of production diseases on a farm and assess the efficacy of improvement strategies in reducing disease prevalence or severity; and
- identify economically viable and socially acceptable ways to control pathologies, with emphasis on animal welfare implications.

Much more detail is available on the project web-site http://www.fp7-prohealth.eu .

The work has been organised in a series of work packages to target a range of issues in the areas outlined below. Much has been achieved on the basic characterisation of production diseases and their risk factors in poultry. Work is currently ongoing on some selected interventions based on these findings and this will be reported in due course. Here we will briefly review some of the completed work which is likely to be relevant to poultry production now and in the future.

Social Science and Economics

Clark et al (2017) have published the findings of their detailed meta-analysis of consumer attitudes to farm animal welfare and identified a gap in relation to the control of production diseases in livestock. They did find quite a lot of variability in attitudes to modern farming but they also identified willingness to pay a small premium for products associated with improved disease control and welfare. They found that consumers mostly view modern production systems negatively and tend to voice concerns about naturalness and humane treatment. Many they also tended to be focussed on human health concerns. Most consumers had little knowledge about production diseases and how they are controlled, though they did tend to refer to antibiotic use. The same research group have gone on to carry out a large survey of consumer attitudes in 5 European countries. A range of possible interventions were presented and, for layers, the order of preference found was as shown in **Table 1**. Considering that the consumer claims to have little technical knowledge about the topic they appear to have chosen quite sensibly those topics which might be grouped as 'good management' as their preferred approach. Given the identified low premium that they are willing to pay, some of these approaches may not always be economically viable. However, broadly speaking, this work supports the approach the layer industry has been taking for many years, of focussing on controlling any egg-associated human health risks and offering products produced in different systems. This work also helps us focus on specific disease control mechanisms which have consumer acceptance and emphasises that 'do nothing' is the least favoured approach.

Biosecurity

Although biosecurity was not included as a specific intervention in table 1, it is a significant component of the number 1 choice, "Enhanced hygiene and disease prevention measures". It is, of course, high on our list of priorities currently because the unusually high numbers of avian influenza outbreaks in both wild birds and poultry over the past few years. Colleagues at the University of Ghent have developed detailed questionnaires to help poultry producers assess their biosecurity practice and also applied these independently on farms of different types. It only takes about 20 minutes to carry out the survey and the system immediately gives a report with suggestions for areas of improvement. Separate biosecurity surveys are freely available for broilers and layers :

Layers: https://www.survey.ugent.be/lime/index.php/519199/lang-en

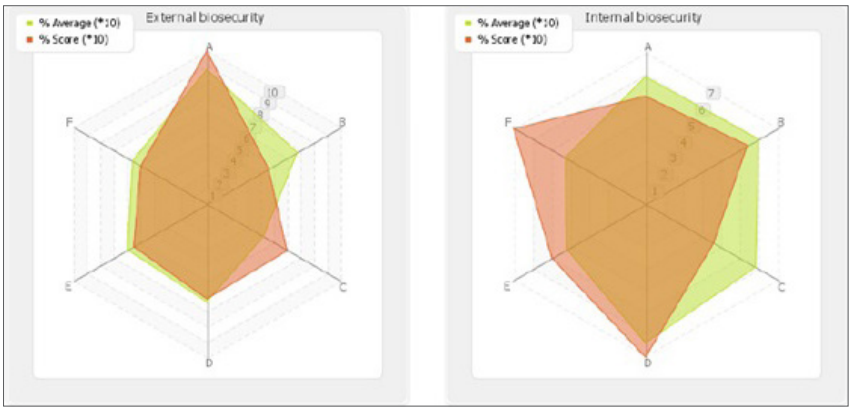

Figure 2 Biocheck Graphic output

Broilers: https://www.survey.ugent.be/lime/index.php/981521/lang-en

The graphic output is intended to allow each user to see how he is doing for both 'external' biosecurity (between the farm and the rest of the environment) and 'internal' (within farm). Perfection would be 100% in all categories (and would be shown as a red hexagon with the maximum score all way around). The red hexagon represents the score of the respondent and the green the average of all respondents. The developers point out the benefit of repeating the survey after having done improvements to see the effect on scores. A total of 124 layer farms were visited for assessment of biosecurity in 3 countries (T. van Limbergen, pers.comm.). These included enriched cages, deep litter and perchery systems. Internal scores were, on average 71% whereas external scores were 62%. The best scores were those relating to egg management (88%) whereas the poorest related to the entry of staff and visitors (41%). This points to some relatively simple and inexpensive opportunities for improvement, by focussing on procedures and systems for the entry of staff and visitors.

Table 1. Consumer preferences with respect to disease control interventions in laying chickens

Order	Intervention
1	Enhanced hygiene and disease prevention measures
2	Housing that allows birds greater freedom to move
3	Providing materials and an environment where animals can perform natural behaviours
4	Reducing the number of animals in a given area
5	Improvements in housing design
6	Enhanced maintenance of the quality of the bedding
7	Housing that protects the animals from adverse natural conditions
8	Providing a price premium that encourages enhanced animal health
9	Enhanced control of air movement in the houses
10	Adjustments to feed or diet composition
11	Changes in the amount and time of light provision
12	Adjustments to the quantity of feed available
13	The use of vaccination
14	Using antibiotics and medicines to treat sick animals
15	Use of feed supplements e.g. probiotics
16	The preventative use of veterinary drugs including antibiotics
17	Doing nothing

Farm Hygiene Assessment

A key aspect of within-farm biosecurity is effective cleaning and disinfection between successive flocks in the same farm and building. With increasing regulatory focus on the approval of biocides and protection of the health of workers it is important to carefully monitor these processes. Visual assessment is an important component of this but does need microbiological assessment for confirmation.

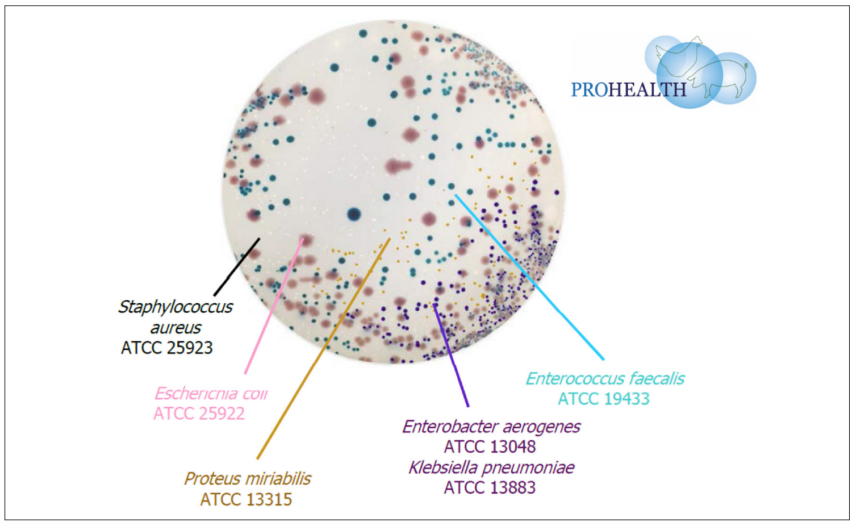

Figure 3. Colony colour and morphology on UTIC agar – similar formulations are available from different suppliers.

Many assurance schemes require specific measures such as absence of Salmonella sp, or Total Viable count on hygiene swabs. Absence of Salmonella is very important but tells little about the efficacy of disinfection against other flora, whether pathogenic or beneficial. We developed a simple protocol to semi-quantitatively assess the microbiology of environmental and post-placement samples. Post-placement samples were, mainly, boot swabs but also included dusts, chick-papers etc. These were in varying proportions among the types of birds examined in accordance with the types of samples normally submitted for other purposes. Briefly, the method involves a peptone pre-enrichment (so that we are focusing on microbes which favour body temperatures, and also reduce variability associated with intervals from sampling to testing). The broths are then diluted and swabbed and streaked on a chromogenic medium developed for human urinary tract infections (UTIC), and separate media to detect lactobaccili, pseudomonads, and staphylococci. Scores are logarithmic and take into account dilution where appropriate. The novelty of the technique lies in the use of a single chromogenic agar allowing identification of major classes of bacteria **(Figure 3)**, and the use of an enrichment to provide more consistent results and favour pathogens.

Over 2500 poultry-origin samples were examined by this method over a 15 month period. The mean scores obtained on post cleaning and disinfecting samples from pullet and layer farms are shown in **table 2**. The rough spectrum of "patho-potential" runs from low on the left to high on the right, and the areas are sorted by the combined E.coli + Staphylococci score. The mean scores are generally satisfactory, and Lactobacilli and enterococci are commonly present. Higher scores of pathogens in this series were more common from water tanks and drinkers, walls and floors.

This relatively low-tech approach may be helpful in addressing the challenge of controlling production-related disease and maintaining productivity with reduced use of anti-microbials.

We went on to compare pre-placement hygiene and post-placement (boot swab) samples evaluated with the same technique in over 800 samples from broiler

Table 2. Scores for post-cleaning and disinfection environmental swabs

Source	Tested	Lactobacilli	Enterococci	Coliforms	Proteus	Pseudomonas	Staphylococci	E.coli
Water Tank	66	2.33	1.73	0.88	0.76	0.32	2.14	1.29
Walls	93	1.90	1.39	0.97	0.16	0.56	1.74	1.42
Drinkers	93	1.98	1.24	0.98	0.18	0.42	1.67	1.44
Floor	93	2.11	1.16	0.90	0.16	0.47	1.58	1.53
Air inlets	109	2.28	1.43	0.82	0.34	0.55	1.98	1.12
Feeder	57	2.07	1.21	0.70	0.12	0.21	1.74	1.21
Fan(s)	83	2.12	1.04	1.04	0.11	0.54	1.48	1.36
Feed Bins	48	1.94	1.06	0.50	0.06	0.04	1.60	0.92
Service area	48	1.92	0.92	0.96	0.17	0.17	1.60	0.90

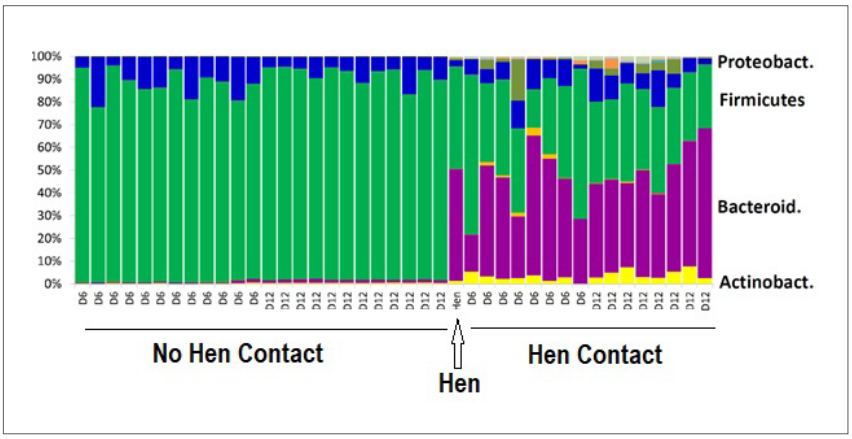

Figure 4. Caecal microbiome assessment of individual layer type chicks at 6 and 12 days of age, with and without direct exposure to an adult hen (with the pattern of the hen shown in the middle of the chart).

chickens and to evaluate associations with health and processing reject parameters (McMullin et al 2017). It was possible to identify clear differences in microbial profiles between samples and sample types. Residual bacterial contamination of farm environments showed clear positive and negative associations with particular flock traits relevant to production disease. There were also significant associations between specific genera in boot swabs samples and relevant flock traits. The analysis supports the need for intervention strategies which focus broadly on the relationship between pathogen and beneficial organisms along the lines of the "Weed/Seed/Feed"-type strategies which are being used increasingly in the poultry industry.

Hatching Egg Sanitation

Colleagues in Denmark and Cyprus have recently published the results of their studies on egg sanitation (Olsen et al. 2017). This work is particularly interesting because there is wish to develop alternatives to formaldehyde in egg fumigation. It demonstrated that repeated treatments can substantially reduce microbial loading of egg surfaces and went on to study the detailed microbiome of egg surfaces. The authors rightly point out that conventional microbiology will only identify culturable bacteria and that 16S RNA sequencing will allow identification of all bacterial species colonizing the eggshell of hatching eggs, but it will not distinguish between live or dead bacteria.

There may be a role for the use of the enhanced hygiene monitoring protocol in routine checks of egg sanitation to help focus on viable organisms of key pathogenic classes, supplemented by molecular techniques where more detail is required.

Establishment of normal intestinal flora

Our colleagues in the Institute of Veterinary Research at Brno provided support to various parts of this project, especially in relation to the composition of intestinal and caecal flora using sequencing of 16s RNA products to classify bacteria regardless of culturability. A nice example of this (Rychlik, 2016) was a series of experiments in which one group of layer chicks were reared from day of age with an adult hen, the other without. Exposure to the hen resulted in a much more diverse and adult-like flora as early as 6 days of age **(Figure 4)**. They have repeated this study on a number of occasions with similar results giving them confidence in the repeatability of this phenomenon. Separate work has demonstrated that establishment of an adult-like flora can take 4-16 weeks in conventionally reared chickens.

Rearing commercial pullets along with adult chickens would be likely to have unintended adverse consequences, by, for example, increasing spread of pathogens and heat-stressing the hens. However this work emphasises the potential benefit of using defined or undefined adult flora administration in very early life to help replicate the flora maturation which would occur naturally through exposure to adults. This approach has been widely applied to reduce risk of colonisation with Salmonella sp for over 30 years (Wierup et al, 1987). Recently in-hatchery administration of such products has begun to be widely applied as an alternative to "starter" medication with antimicrobials.

The same group (Varmuzova et al 2015) have gone on to use a Salmonella Enteritidis challenge model to demonstrate a very obvious activation of inflammatory markers **(Figure 5)** at day 4 after infection, which was declining by day 14 post infection. The inclusion of 2 plant extracts in the feed markedly reduced this response. However detailed examination of the microbiota showed that the combination of SE challenge and plant extract supplementation resulted in the greatest deviation of flora from the normal pattern. So far this experiment has only conducted once so these findings should be interpreted with caution. While Salmonella Enteritidis is becoming a rare occurrence in most European countries, other pathogens may well interact with the immune system, the microbiota and use of nutritional supplements to destabilise gut flora.

Gene markers and disease

The team at the University of Nottingham veterinary school, has been conducting wide-ranging research on gene-activation associated with disease in poultry, including, in particular coccidiosis and clostridial infection. They are particularly interested in a case of leg weakness in 10 week-old brown-egg layer strain pullets with a histopathological diagnosis of lymphoplasmacytic neuritis. They analysed lymphocyte expression and found that the CD72 gene was up-regulated in these birds. The preliminary findings suggests that while CD8-type cell numbers may be increased, they may not function properly. It is possible that this work will help improve our understanding of so-called B-type Marek's lesions and/or idiopathic peripheral neuropathy.

Other activities

Here we have reviewed only a small proportion of the work carried out under the Prohealth project relevant to poultry. Both the topics discussed and much other useful work carried out in a range of areas will be of interest to the industry. A scientific conference is planned to take place on November 27th/28th 2018 in Ghent, Belgium. Various publications are planned and the web site(http://www.fp7-prohealth.eu) is a good way of following these developments.

Finally, readers may find the recently-launched "Online Poultry Journal" of interest. This initiative by the consortium provides a consolidated news feed from different sources on topics relevant to poultry and pig health.

This 'Online Poultry Journal' may be accessed at:
http://www.fp7-prohealth.eu/knowledge-platform/online-poultry-journal/

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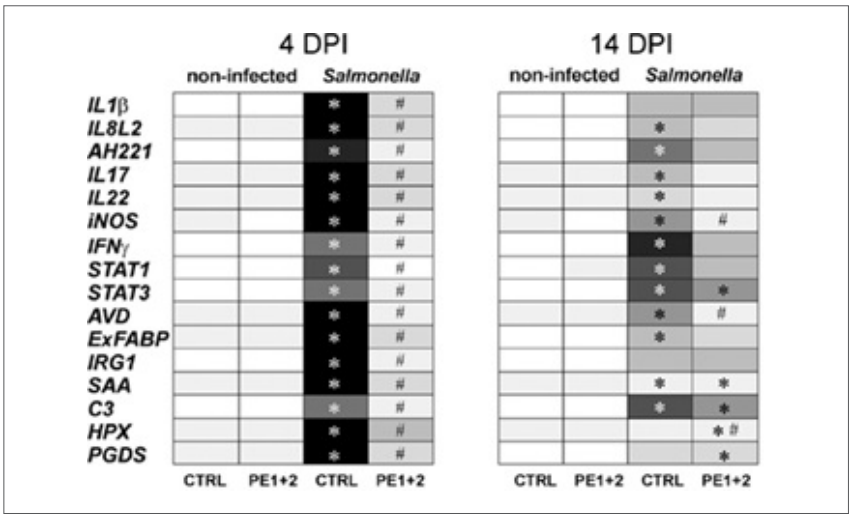

Figure 5. Expression of cytokines and acute phase genes in chickens after S. Enteritidis infection. CTRL = Chickens fed control diet; PE1+2 = Chickens fed control diet supplemented with plant extracts. Darker shading indicates higher expression level.