




SAFEHOUSE and RESCAPE



Assessing and controlling **the risk of eggs** becoming contaminated by ***Salmonella*** after the move of laying hens to '**enriched cages**' and **alternative housing systems**



RESCAPE and SAFEHOUSE are two Specific Targeted Research Projects (STREP) funded under the 6th Research Framework Programme of the European Union. Commission of the European Communities Research Directorate - General - (Food)





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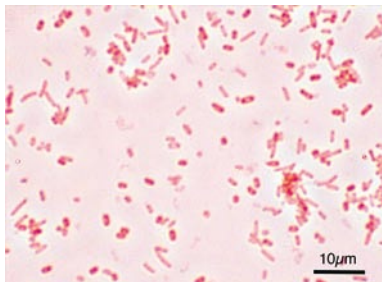


SAFEHOUSE AND RESCAPE: AN INTRODUCTION

By 2012, battery cages for laying hens will have been phased out (council directive 1999/74/EC). As a result, the EU egg industry has recently started introducing “enriched” cages, barn or free range systems to ensure the welfare of laying hens and thus comply with consumer demands.



These modifications will, however, affect productivity, working times and costs, as well as the environment of buildings (dust). It raises fears as to a resurgence of food-borne infections because of possible contact between eggs and litter, which is particularly conducive to bacterial proliferation. Eggs are of high nutritional value. They are used frequently without cooking and are therefore one of the major sources of food borne infection of bacterial origin. The urgent need to collect qualitative and quantitative information on contamination of eggs in alternative production systems was underlined by the Scientific Panel on Animal Health and Welfare of the European Food Safety Agency (EFSA Journal, 2005, 197, 1-23). It is particularly relevant in the context of Regulation (EC) No 2160/2003 that aims to detect and control *Salmonella* and other zoonotic agents at all relevant stages of production.



In this context, the European Commission has been funding (€4.66 million) since October 2006, two European projects, The SAFEHOUSE and RESCAPE Projects. These two “Specific Targeted Research Projects” are organizing the activities of 22 public and private sector laboratories in 11 countries over a period of three years and respond to the strong demands firstly for information on the impact of these new production systems on the hygienic quality of eggs, and secondly for development of new approaches to encourage better microbial safety in these alternative production systems.



The complementary nature of these two European projects should encourage the emergence of economically viable methods which, for poultry farmers and the poultry industry as a whole, will facilitate the transition towards alternative systems or “enriched” cages. Enhancing the health safety of eggs should bolster consumer confidence in this product.



SAFEHOUSE AND RESCAPE: FACTS AND FIGURES

- The SAFEHOUSE and RESCAPE Projects are two specific targeted research projects supported by €4.66 million under the Sixth Research Framework Programme of the European Union (Priority 5, Food quality and Safety).
- Both projects started on the 1st October 2006 and will take 3 years to complete.
- The SAFEHOUSE and RESCAPE Projects involve 22 public and private sector laboratories in 11 European countries.

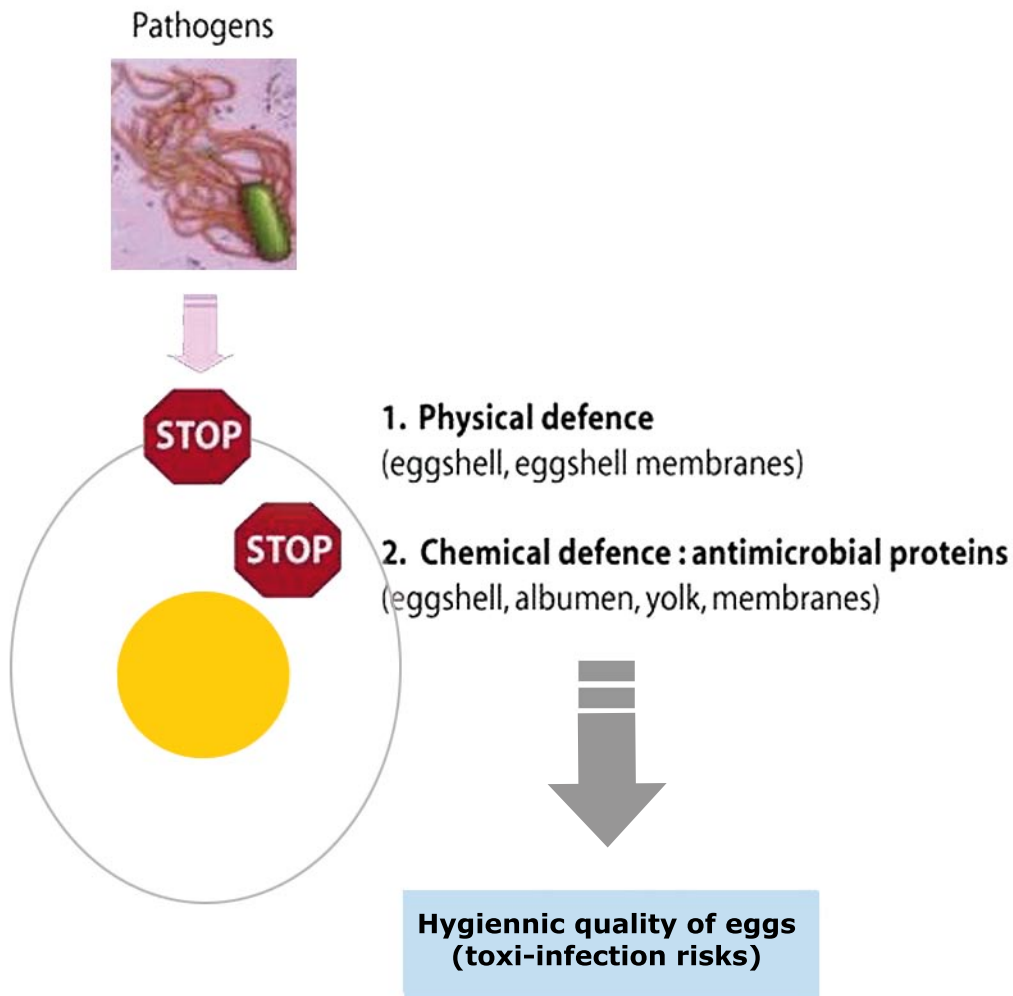


Figure 1: The egg has its own natural defenses against invading pathogens



SAFEHOUSE 2006-2009

Summary



The aim is to predict the potential risk to the consumer of the move to more welfare-friendly laying hen housing systems. This will be achieved by developing qualitative and quantitative risk assessment models using EFSA monitoring data, in combination with new data on the prevalence of *Salmonella* in egg-laying flocks kept in different housing systems. In addition to this, standardized infection models with *Salmonella* will be used in experiments to directly assess the effect of the housing system on colonization of the gut and reproductive tissues and on the spread of a *Salmonella* infection within a group of hens, and finally on egg contamination. Using highly specialized molecular methods the specific factors responsible for colonization capacity of *Salmonella* Enteritidis will also be identified. Furthermore, underlying mechanisms of differences in prevalence of *Salmonella* in the different housing systems will be investigated by analyzing the role of the level of stress in hens, of the intestinal and reproductive microbiota, and the hens' immune competence. Finally the role of parasite contamination, in particular red mites, and of the housing system design will be explored with industrial partners in order to propose combined strategies to control *Salmonella* infections in non-conventional housing systems.

Key objectives

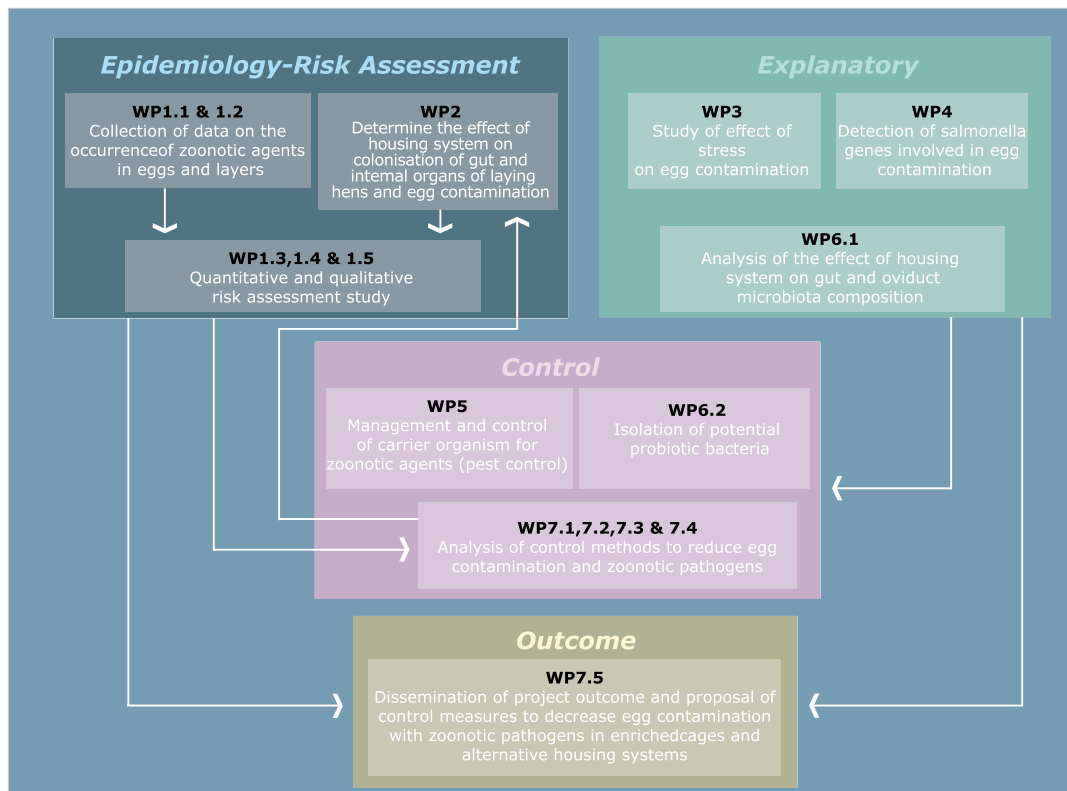
- To improve the evaluation of the risk to the consumer of contamination of eggs with *Salmonella* and other infectious agents, caused by the move to more welfare-friendly egg-production systems for laying hens.
- To propose methods of reducing any potential, additional food safety risks generated by alternative systems for laying hens, taking into account the need to maintain eggs of high quality.





SAFEHOUSE 2006-2009

Safehouse structure



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Epidemiological analysis and risk assessment modeling to evaluate the prevalence of zoonotic pathogens in laying hens housed in different housing systems in the field

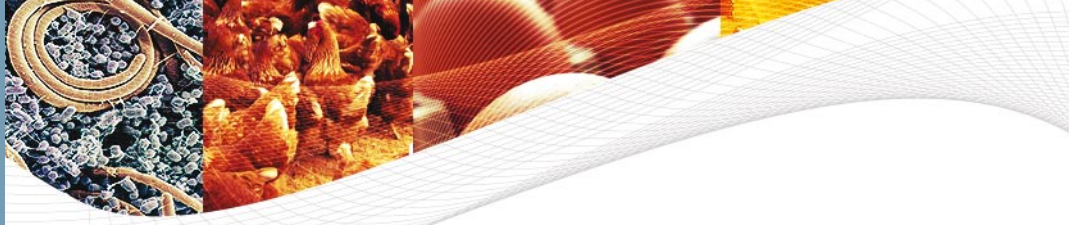


In this study quantitative data will be collected and analyzed to evaluate the effect of the housing system on egg contamination by zoonotic agents in the field, in order to predict the potential risk to the consumer of the move to more welfare-friendly laying hen housing systems. This will involve carrying out risk analysis studies using EFSA monitoring data, in combination with new data derived from cross-sectional and longitudinal studies in flocks housed in different housing systems. To generate valuable data on the potential changes in egg contamination rates when laying hens are moved to more animal friendly housing systems, data will be collected using a multidisciplinary approach.

Besides using historical data (from monitoring programs in Denmark and Switzerland), a longitudinal as well as a cross-sectional field trial will be conducted to gather new quantitative data on the prevalence of *Salmonella* and other zoonotic agents in egg-laying flocks kept in different housing systems.

Samples will be taken in 5 flocks of each housing type (conventional, enriched cage system, aviary system) in Germany and Denmark in the longitudinal study, while for the cross-sectional study 50 flocks will be sampled in Belgium, Germany and Switzerland. In Greece and Turkey at least 5 flocks of each housing type will be sampled in the cross-sectional study. In Italy, 50 flocks housed in battery cages, 50 flocks of floor-raised

laying hens and 20 organic flocks will be sampled. The risk analysis studies will focus on *Salmonella*, but will also cover other potential zoonotic agents, antibiotic resistance gene transmission and residues. Qualitative and quantitative risk assessment models will be developed, to provide us with estimations of the risk of human illness due to the consumption of eggs, related to the housing system.



SAFEHOUSE 2006-2009

Study of the effect of the housing system on colonization of layers and egg contamination by *Salmonella* using experimental infection experiments under controlled conditions of hens kept under different housing condition

Standardized experimental infection models with *Salmonella* will be used to directly assess the effect of the housing system on colonization of the gut and internal organs, including reproductive tissues, on spread of a *Salmonella* infection within a group of laying hens, and on egg contamination. Although field observations are an important and necessary tool to analyze the effect of the housing system on *Salmonella* infections, field conditions harbor a wide variety of other factors that can potentially influence the infection outcome (use of control measures, management of the company, flock density and many others). The sole effect of housing conditions can be better evaluated when experiments are performed in controlled conditions using reproducible and standardized infection protocols.



Study of the effect of neurotransmitters and corticosteroids on immunocompetence in laying hens and egg contamination by *Salmonella* using experimental infection experiments under controlled conditions



Data are accumulating on the profound changes in host responses that occur when animals are exposed to damaging conditions, both psychological and/or physiological. Common stresses include poor housing and a poor social environment. Studies are also beginning to demonstrate the often profound effects that compounds central to the animal host's reaction to stress can have on pathogen behavior, particularly on virulence gene expression and invasiveness. In this study the effects of the housing environment on stress responses of laying hens will be assessed, and the outcome of stress induction on the pathogenesis of infections with zoonotic agents, including *Salmonella* will be evaluated. Specifically, the underlying mechanisms of possible differences in prevalence of *Salmonella* and other zoonotic agents in different housing systems will be investigated by analyzing the stress responses (levels of adrenaline/noradrenalin and corticosterone in hen blood and faeces), the composition of the microbiota and immune competence, both in experimental setups and in the field (and WP7). The effects of the administration of these compounds on the course of *Salmonella* infection and especially on egg contamination rates will also be examined.



SAFEHOUSE 2006-2009

Understand how *Salmonella* infects eggs and the mechanisms it uses to become resistant to antibiotics

Salmonella Enteritidis has a predilection for oviduct colonization and egg contamination and is the most frequently isolated serotype from eggs. Using highly specialized molecular methods it will be tried to identify *Salmonella* Enteritidis specific factors necessary for reproductive tract infection of layers. Comparative genomic indexing will be used to compare genomes of egg contaminating with non egg contaminating serotypes. Gene expression studies inside oviduct cells will also be explored. Validation of gene functions will be done using infection trials with knock/out mutants in the identified genes.



Evaluate the efficacy of arthropod-specific fungi in combination with other methods, for control of arthropod carriers, particularly ectoparasitic mites



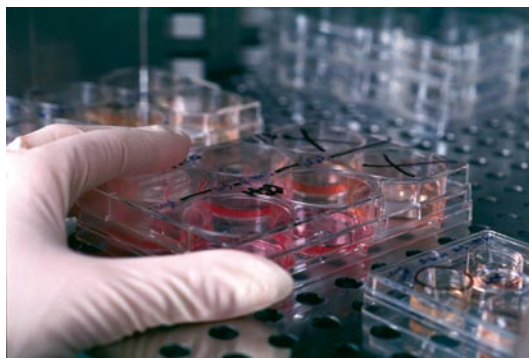
To control *Salmonella* and other zoonotic agents in the alternative housing systems, the project will propose simple and cost-effective measures such as steering gut flora composition towards a protective flora by changing feed composition. Also vector control, in particular red mites, and optimisation of the housing system design are key issues. Arthropods may act as carriers and in some cases reservoirs of zoonotic bacteria. With the present lack of effective control methods, in particular against the red poultry red mite *Dermanyssus gallinae*, and the fact that enriched cage and alternative housing systems means more favorable conditions for arthropod pests, increasing problems can be expected with the transition to alternative housing systems. Effective pest management strategies need urgent development, therefore in Work Package 5 non-chemical control methods will be developed using diatomaceous earth and arthropod-specific fungi.



SAFEHOUSE 2006-2009

Investigation of the composition of the intestinal and oviduct microflora of hens kept under different housing conditions of hens kept under different housing conditions

The natural microbiota in the intestinal tract constitutes a very important barrier against pathogens, including *Salmonella*. It is well known that the microbiota is influenced by internal as well as external factors, such as diet, stress, antibiotics and antimicrobial growth promoters. The impact of housing systems is less well investigated, but since housing will most likely have an influence on stress levels and many other factors, a certain influence is to be expected. In this study, the composition of the microbiota in the intestinal tract and the oviduct will be investigated using molecular techniques and culture methods. By describing differences in gut and oviduct flora composition when laying hens are moved to more welfare friendly housing systems, this study will attempt to aid in explaining differences in *Salmonella* status of animals, housed in different housing systems, based on gut microbiota composition.



Analysis and dissemination of methods to control food safety risks in enriched cages and alternative housing systems



Studies will be conducted on *Salmonella* vaccine development and safety in alternative housing systems, and optimisation of housing system design will be explored together with industrial partners. Combined strategies will be proposed to control *Salmonella* infections in no-conventional housing systems and this information will be disseminated to the poultry industry.



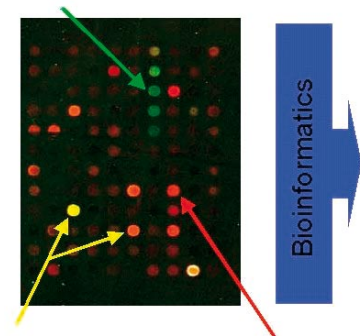
Summary



RESCAPE aims to develop new methods to reduce the risk of eggs becoming contaminated by potentially harmful bacteria. One option to enhance the safety of eggs is to develop new, non-invasive techniques to measure the internal and external quality of eggs and thus improve sorting in packaging centers. In this context, the RESCAPE project will look at ways of improving the sensitivity of the acoustic test device so that it can detect cracked eggs and eggs containing micro-cracks. An optical transmission spectra method to analyze the internal quality of eggs will also be further developed, and an image analysis system for detecting dirty eggs optimized. All 3 measurements systems will then be assembled into a common platform and tested under commercial conditions.



The RESCAPE project will also evaluate the feasibility of using novel techniques such as microwave technology, hot air pasteurization, gas plasma sterilization and modified atmosphere packaging to decontaminate the shell surface of eggs without altering the egg quality



Eggs are unique because of their natural ability to limit bacterial growth. The RESCAPE project will therefore also consider the environmental and genetic variability in the components of the eggs' natural defense system against the entry and growth of bacteria. The potential usefulness of molecular genetic markers (Single Nucleotide Polymorphism) to select and breed for their ability to produce eggs with improved antimicrobial defenses will also be investigated as a longer term approach to improving egg safety.

Key objectives

- To enhance the microbiological safety of eggs by looking at ways of improving the technology for egg grading to enhance the detection of eggs with the greatest risk to the consumer and to develop new innovative decontamination treatments for eggs.
- To investigate ways of reinforcing the natural anti-microbial and mechanical defense mechanisms of eggs using molecular genetic markers to select and breed hens which produce eggs with improved antimicrobial properties.

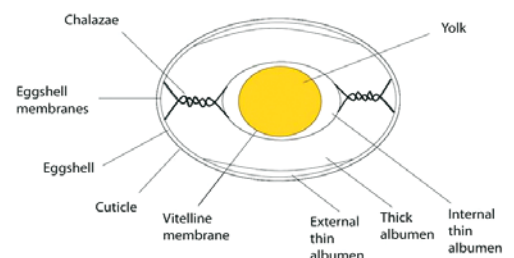
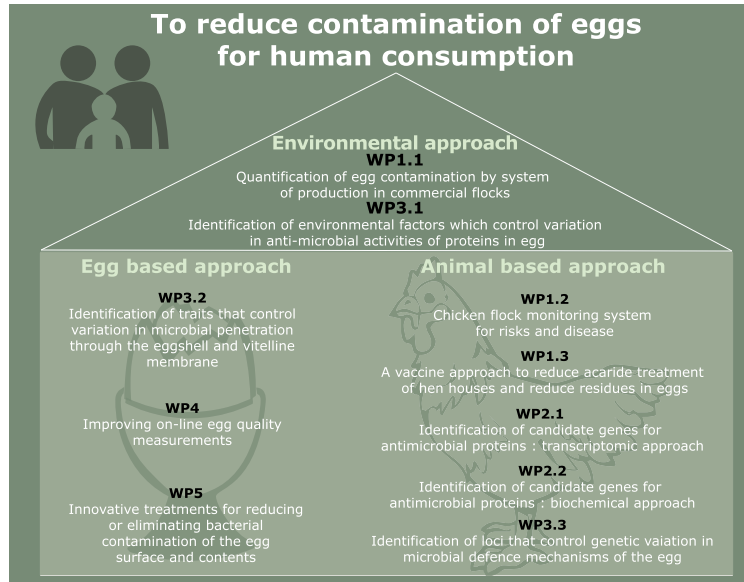


Figure 2: The components of the egg

Rescape structure

RESCAPE will implement a multidisciplinary strategy to reduce the risk of eggs unfit for human consumption to stay in the human nutrition chain. This will be realized by reduction in egg contamination using 3 complementary approaches viz. an environmental approach (farm design and management), an animal based approach (hens and breeding), and an egg based approach (egg decontamination and sorting).



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Reducing egg contamination in commercial flocks



The aim of this study is to provide additional quantitative epidemiological data from cage and non-cage systems in France which will reinforce the epidemiological work of the SAFEHOUSE Project and to develop a trial vaccine against poultry red mite. A cross sectional epidemiological study will be conducted in France in thirty farms, with litter (free range standard, floor raised aviary) and without litter (conventional and enriched cages). This study will also look at the impact of air quality on human and animal health. In addition, a longitudinal study will be carried out in three production systems: conventional and enriched cages and a free range system throughout the laying period. Existing networks in France will be used to monitor microbial contamination in different poultry farm systems from production level to egg storage.

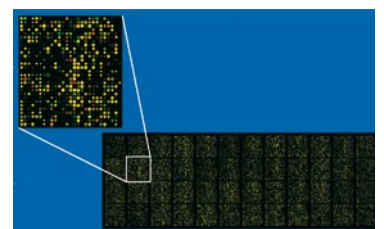
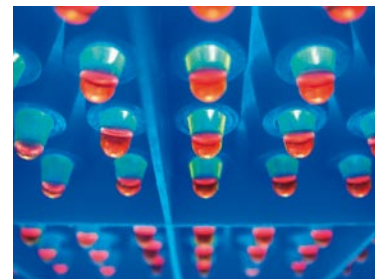
RESCAPEs contribution to reduce potential residue contamination in eggs will be the development of a trial vaccine against poultry red mite to prevent chemicals use. The first step will be to quantify the hen's immunological reaction to red mite under natural or induced infestation, then to characterize the most immuno-dominant antigens and finally to compare vaccination impact in a number of commercial farms.

Screening candidate genes involved in antimicrobial egg defences

The aim of this study is to assess antimicrobial activity of egg white fractions and purified proteins and to make use of genomics and bioinformatics to identify candidate genes which could be used in breeding programs aimed at improving the antimicrobial defence of eggs (WP3).

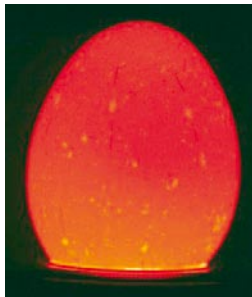
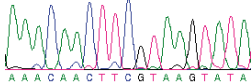
Eggs are unique because of their aptitude to limit bacterial growth and remain edible even after weeks of storage in contrast to other animal proteins. In this study transcriptomic and proteomic approaches will be used to identify proteins with putative antimicrobial activities. Bioinformatics tools combined with data from the literature will help define potential interesting functions and therefore candidate proteins.

In a parallel study, a library of chicken cDNAs will be cloned into bacteria to screen proteins that have the capacity to kill the host pathogen. The expression of the candidate proteins including proteases, antiproteases and antimicrobial peptides will be further investigated by real-time PCR using specific primers. Some of these proteins will then be purified to characterise their functional properties allowing the selection of novel candidate genes to be evaluated for marker assisted selection programs as described in WP3.



RESCAPE 2006-2009

Identification of phenotypic and genotypic variation controlling the antimicrobial defence of the egg

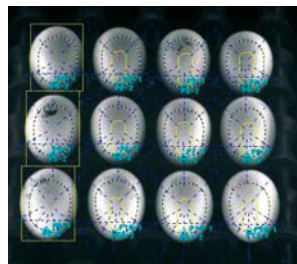
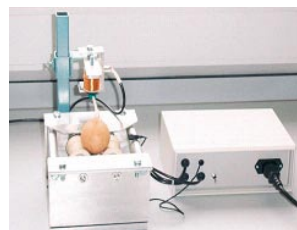


The environmental and genetic variability in the eggs' defence against the entry and growth of bacteria including those posing a risk to consumers will be investigated in this study. Genes identified to have anti microbial properties in WP2 will be evaluated for their contribution to the genetic variance in the eggs natural defence. This will be tested by association analysis using markers in the candidate genes within a structured commercial population (2000 offspring) where we have precise knowledge of pedigree. This will provide genetic markers that can be implemented in breeding programmes to reduce risk from zoonotic disease.

Environmental factors which can alter the antimicrobial properties of the egg either at the level of the hen or on the egg during storage and transportation will also be examined. These include the role of micro-cracks and the vitelline membrane on bacterial penetration and how the effect of temperature on the hen and on egg storage alters this antimicrobial activity.

Improving egg quality measurement techniques

This study will develop new ways for detecting 'risky eggs' during grading. The sensitivity and performance of the Acoustic Crack Detector developed by KU Leuven, (Belgium) will be enhanced so that it is able to detect eggs containing microcracks. Secondly, the possibility of directly or indirectly detecting the presence of bacteria within the egg contents by changes in the eggs freshness measurements will be assessed using an optical transmission spectra measurement technique. Thirdly, the measurement time for a novel method of detecting dirt on eggs will be reduced and a method for detecting damage to the cuticle will also be developed for both white and brown shelled eggs. Finally, these new technologies will be combined into one measurement platform prototype which will then be tested in two commercial packing stations in France and in Belgium respectively.





RESCAPE 2006-2009

Innovative egg treatments for reducing bacterial contamination of the egg surface and its contents.



This study will assess the efficiency of alternative and innovative decontamination techniques to egg washing. The first technique proposes to decontaminate table eggs by using microwaves: the heat generation and its transfer to the egg, the effect of the time and power of the microwave treatment on the foamability, turbidity, and rheological properties of albumen and yolk as well as its lethality against reference bacteria will each be explored.

Similar parameters will be used to test hot air pasteurisation at different combinations of air speed/temperature/time/direction by means of Computational Fluid Dynamics (CFD) analysis. The third approach aims to decontaminate shell eggs using gas plasma sterilisation. Laboratory scale cold plasma sterilisation equipment will be developed and different process parameters (treatment time, power, kind of discharge apparatus etc.) will be tested for their efficiency on pathogens most frequently associated with eggs. Finally two methods to protect the egg will be evaluated. A modified atmosphere packaging system (N_2 , CO_2 and O_2 and non-conventional, N_2O and Ar gas mixtures) will be tested at various conditions of egg storage (temperature, duration...) and levels of surface contamination of the eggs and the effect of a chitosan coating will be tested on the survival of bacteria and for reducing bacterial eggshell penetration.

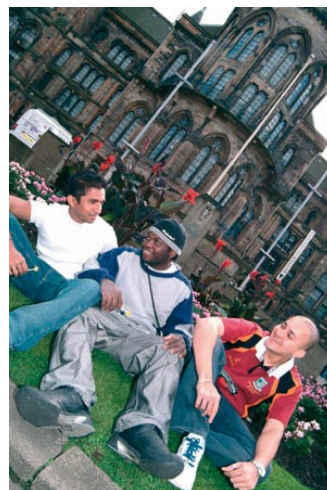


SAFEHOUSE AND RESCAPE

Raising public awareness and dissemination of results arising from Safehouse and Rescape

The coordinators of the SAFEHOUSE and RESCAPE projects are taking a thorough approach to ensure that all stakeholders including professional organisations, and companies who are directly associated with the egg production chain are regularly updated about the scientific objectives, progress, results and practical applications of both of these projects.

In addition to this informative booklet a joint annual newsletter will be produced and published on each of the SAFEHOUSE and RESCAPE websites. In addition, joint articles will appear in the popular poultry press, and scientific papers will be presented at International conferences leading to publication in peer reviewed journals.



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SAFEHOUSE

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