ROYAL SOCIETY/ACADEMY OF MEDICAL SCIENCES STUDY ON PANDEMIC INFLUENZA: CALL FOR EVIDENCE

BBSRC RESPONSE

BBSRC is the main UK funder of non-medical biosciences supporting basic, strategic and applied research, training and public engagement activities. The Council supports research on animal disease, including avian influenza, through responsive mode and through some core funding to BBSRC-sponsored institutes, principally, the Institute for Animal Health (IAH) and the Roslin Institute. BBSRC also funds fundamental work on virology, and BBSRC support for research on influenza is shown at **Annex 1**. For comparison, figures for support of research on Foot and Mouth Disease are also given. Control of infectious disease is a priority research area for the Animal Sciences Committee (see http://www.bbsrc.ac.uk/science/areas/as/priorities/inf.html); this priority encourages studies aimed at the effective control of infectious diseases important to UK livestock (including fish and poultry).

Basic and strategic research is of key importance in underpinning the scientific understanding of avian and pandemic influenza. Although BBSRC does not fund research on human disease, the threat of pandemic influenza in man seems very likely to be related to the level of infection sustained in poultry, and control in the avian host is critical. Thus, scientific approaches that are directed towards reducing the disease in the avian host should form a major part of any strategy to prevent pandemic influenza in humans.

BBSRC has recently launched a £4.5M Initiative 'Combatting Avian Influenza' to address research opportunities within the BBSRC remit. This should provide some of the underpinning scientific understanding that should inform policy issues. Specific areas of benefit to addressing avian influenza, highlighted in the call for proposals, are:

- Avian immunology, and innate and acquired immunity
- Chicken genomics and genetic variation
- Virology of influenza infection
- Host-pathogen interactions
- Epidemiology in avian populations
- Vaccine development and delivery

Full details of the initiative, which has a closing date for applications of 26 April 2006, are covered at **Annex 2**. This initiative is anticipated to attract collaborative efforts between the IAH and other research groups, and the facilities for handling infected poultry at the IAH are currently being upgraded to meet anticipated research demand. We will be in close liaison with MRC, who have a parallel call for proposals to work on pandemic influenza. With Government Departments and other funders, BBSRC has participated in the drafting of a MoU between the UK and Chinese Governments on pandemic influenza research and it is anticipated that it will be signed during Lord Sainsbury's visited to China in April. Opportunity for collaborative research with China is emphasised in the initiative call.

Following the outbreak of Foot & Mouth Disease in the UK, BBSRC launched a £10M initiative on the ‘Control of Viral Diseases in Livestock’. Details are at **Annex 3**. An important aspect of this initiative was the additional funding for epidemiological research which was felt to be an essential

component of any emergency planning response. A total of 29 grants were awarded. Of these, 3 were directly relevant to avian influenza and the details are included in Annex 3.

Colleagues at the Institute for Animal Health have contributed the following comments on the research requirements to underpin avian disease management and prevention.

Notes from the Institute for Animal Health

The threat of pandemic influenza in man seems very likely to be related to the level of infection sustained in poultry. Waterfowl are the natural host for AI viruses and it is believed that spread into poultry represents episodic events that may be sustained in the poultry population for varying periods of time. We submit that it is prolonged human contact with infected waterfowl or poultry that increases the risk of pandemic influenza in man - so control in the avian host is critical. Control by depopulation is an obvious option but control through alternative means is the focus at the Institute for Animal Health.

We propose scientific approaches that are directed towards a reduction of AI in the avian host by:

1. Fundamental work to uncover the details of interactions between the host and AI virus
2. Identification of differences in susceptibility of poultry to influenza virus infection so that it may be possible to breed chickens with a reduced potential for spread of the virus
3. Generation of novel vaccines and their testing under controlled conditions to enable control of infection during outbreaks of infection
4. Screening for differences in the responses of poultry to traditional and new vaccines to promote the breeding of birds with optimal protection following vaccination

1. FUNDAMENTAL WORK TO UNCOVER THE DETAILS OF HOST INTERACTIONS WITH AVIAN INFLUENZA TO GIVE FUTURE PROSPECTS FOR CONTROL

The viruses circulating in poultry and waterfowl in South East Asia are lethal for poultry and it appears that some are also lethal for waterfowl. It is unusual for such HPAI viruses to be isolated from wildfowl and these strains are therefore a cause of special concern. In infected poultry, HPAI viruses are not restricted to replication in the respiratory and alimentary tracts of birds but cause a systemic infection which results in rapid death.

How applicable this model is to human infection is not known, but it is clearly a very serious risk factor relating to the severity of the disease. The risks can be considered enormous should the virus acquire the ability to spread to humans more efficiently and then spread from human-to-human without any loss of pathogenicity consequent upon mutation. An additional risk might be that the virus does not spread to humans but that the pig acts as a source of infection. Even if there is no immediate pandemic, a chronic threat remains for AI or AI viruses in pigs to transmit to humans with lethal consequences.

A greater understanding of the interactions between hosts and AI is of fundamental importance and will underpin much our ability to control the spread of this disease. The scientific community needs answers to questions like:

- What factors limit replication and transmission of AI strains within and between different avian and mammalian species?
- Why are some strains pathogenic in geese and ducks but other strains are not?

AI strains of high pathogenicity evolve from strains of low pathogenicity and the bulk of evidence indicates this occurs following transmission to poultry. The evolutionary drive for high pathogenicity is not known and clearly the factors responsible for both transmission and pathogenicity are intrinsically important areas for future investigation.

The factors that affect transmission within and between different avian species have not been as thoroughly investigated as those that affect transmission between avian species and mammalian species. However, similar factors are likely and could include interaction with receptors for virus binding to, and release, from infected cells; the ability of the virus to overcome non-specific inhibition, such as mucins or other glycoconjugates; the ability of the virus to overcome innate immune responses, such as the interferon response or the response of cells though TLR-signalling and the nature of the acquired immune response of the host to infection.

Experiments need to be done to look for avian-species specific signatures in the viruses using quantitative virological techniques that are combined with detailed sequence analyses of within-host variation of the viruses which show a preferential ability to replicate in different avian species.

The host factors that control pathogenicity are also unknown. There are usually differences between the pathogenicity of HPAI viruses in galliform and anseriform species. The current H5N1 virus shows a remarkable ability to cause 'fowl plague' in a wide range of species. It is not known whether this difference is simply the result of the efficiency of virus infection in the hosts or whether there are immune barriers to infection that have been breached by the H5N1 virus. These factors warrant investigation both to unravel aspects of virus replication from host response and a full examination of the host response to infection.

International projects to sequence the genomes of ducks and other hosts will provide information about host defence and susceptibility genes. These sequences will be used to develop the tools to examine host responses in terms of changes in gene expression and protein differences. The sequences will also be used to screen for genetic variation in host genes and search for associations both with susceptibility to infection and with the ability to shed and spread AI infections.

Increased knowledge of the genes and proteins of the host that limit infection may also identify new targets for drugs or vaccine design. Antiviral drugs might therefore be targeted against virus proteins that counter the host antiviral response.

2. IDENTIFICATION OF DIFFERENCES IN SUSCEPTIBILITY OF POULTRY TO INFLUENZA VIRUS INFECTION

The management of poultry breeding and production makes the poultry industry exceptionally well suited to exploiting genetic variants for a useful trait and establishing a trait such as disease resistance within poultry population. From the field there is little evidence for resistance to AI virus infection since, once established, highly pathogenic strains of AI virus (HPAI viruses) spread and kill poultry indiscriminately. However this does not imply that all poultry strains are equally susceptible to infection and able to transmit infection. A successful strategy may be to select poultry that (a) need a higher infectious dose of virus or (b) produce less virus when infected. Both strategies effectively reduce the potential of the virus to spread infection and could reduce the risk of pandemic influenza.

3. GENERATION OF NOVEL VACCINES AND THEIR TESTING UNDER CONTROLLED CONDITIONS

Vaccination is the major method of control for many viruses in the poultry industry, which has considerable experience in developing and administering many live and killed viral vaccines. Traditional and new vaccines against AI viruses have mixed success and can cause problems if they result in protection from disease signs and yet fail to block virus transmission.

Substantial advances in our understanding of the immune response of poultry have been made over recent years with the discovery of genes and proteins that control the immune response. Through our better knowledge of the molecules that contribute to the immune response we hope to improve the efficacy of vaccines against AI viruses that are effective in the avian (poultry) host. Particular discoveries that bear on novel vaccines are the description of proteins that act as molecular adjuvants (such as cytokines and Toll-like receptors) to induce the correct immune responses.

It is not just the genes and proteins that are important to vaccines but vaccines need to deliver the right response to the right place at the right time, and there is increasing knowledge about these parameters. Identification of the most appropriate viral proteins, the best vectors, the more appropriate adjuvants, and the best immunisation procedures are crucial for improving the efficacy of vaccination. Another desirable feature of such vaccines would be that they protect against many serotypes, that is to say they are sustainable. One approach to achieve would be to take advantage of the T-cell response of AI peptides on MHC molecules.

It is essential that infection of poultry flocks with viruses is determined swiftly so that timely slaughter and vaccination can be carried out. Masking the infection by vaccination without eliminating shedding of active virus potentially leads to a situation with the virus out of control - as in South East Asia. New vaccines must be designed with the intent that to be effective in the long term they will totally eliminate or reduce shedding to minimal levels. The need for new vaccines is inescapable since there are doubts about the efficacy of current vaccines when used outside of the laboratory environment.

4. SCREENING OF THE RESPONSES OF POULTRY TO TRADITIONAL AND NEW VACCINES

Host genetics may also play a role in the selection of poultry that respond well to vaccination. There is much evidence that different chickens respond differently to various vaccines. In some cases this may be a general trait towards many vaccines and in other cases the response may be specific for a particular pathogen or vaccine. It is already known that some current vaccines efficiently immunise some chickens but not others. The selection by poultry breeding of those genes which allow the best response to the traditional and any novel vaccines is a desirable goal.

Antiviral innate resistance whether through the interferon system, with its antiviral response, or through other mechanical barriers, is also worthy of investigation and could be considered as part of a suite of traits that could be investigated.

ANNEX 1

BBSRC spend on research on Avian Influenza and Influenza 2002/03 - 2005/06 and Foot and Mouth Disease 2001/02 - 2005/06

Estimated spend on avian influenza compared with all influenza research from 2002/03 onwards

	Estimated spend (Avian flu)					Estimated spend (All flu research, incl avian)			
	2002/03	**2003/04**	**2004/05**	**2005/06 (forecast)**		**2002/03**	**2003/04**	**2004/05**	**2005/06 (forecast)**
CSG projects*	£92,389	£57,643	£191,809	£196,592		£187,389	£146,643	£289,809	£263,592
Grants	£0	£13,594	£165,459	£320,585		£245,253	£585,918	£658,295	£661,676
Studentships	£0	£0	£0	£0		£33,000	£24,500	£32,400	£39,250
Total	**£92,389**	**£71,237**	**£357,268**	**£517,177**		**£465,642**	**£757,061**	**£980,504**	**£964,518**

Foot and Mouth Disease Spend

	Estimated Research Spend:				
	2001/02	**2002/03**	**2003/04**	**2004/05**	**2005/06 (forecast)**
CSG projects*	£675,629	£774,172	£937,838	£912,009	£1,382,550
Grants	£156,275	£148,562	£198,848	£501,553	£760,349
Studentships	£12,380	£13,200	£14,000	£14,400	£7,850
TOTAL	**£844,284**	**£935,934**	**£1,150,686**	**£1,427,962**	**£2,150,749**

*CSG projects are those funded from the Core Strategic Grants to BBSRC Institutes

ANNEX 2

Combating avian influenza

Background

In the light of current public and scientific interest in avian and pandemic influenza, and following a recent meeting of interested research groups and funding bodies, it is clear that many of the potential problems posed by the H5N1 form of avian influenza could be most appropriately addressed by studying the virus in the natural hosts of what is currently still primarily an animal disease.

BBSRC is well placed to sponsor research on both the pathogen and its hosts - and the interactions between them - and supports facilities suitable for *in vivo* studies at the Institute for Animal Health and the Roslin Institute. Accordingly, the Council is making available an additional £4.5M of responsive-mode funding specifically for projects to improve scientific understanding of avian influenza, with the aim of enabling more effective control of the disease.

Objectives

The aim of the initiative is to enhance understanding of the virology, pathology, host-pathogen interactions and epidemiology of avian influenza in its animal hosts, with a view to generating the underpinning scientific knowledge that will enable the development of more effective methods for its control.

Scientific Scope

Relevant areas of research, appropriate to BBSRC's remit, include:

- Avian immunology, and innate and acquired immunity
- Chicken genomics and genetic variation
- Virology of influenza infection
- Host-pathogen interactions
- Epidemiology in avian populations
- Vaccine development and delivery

Applications for collaborative projects are strongly encouraged, particularly where they would make use of BBSRC facilities at the Institute for Animal Health or elsewhere.

The initiative will be implemented in parallel with the Medical Research Council's call for proposals for influenza research that was announced recently.

Work on human immunology falls within the remits of both BBSRC and MRC, but will not be a focus for BBSRC funding under this initiative. Studies of animal models of human diseases are outside the remit of BBSRC.

Applicants who are uncertain about which Council to direct a proposal are advised to discuss their proposed project with the Designated Contact or the MRC (influenza@headoffice.mrc.ac.uk).

As part of this call for proposals, BBSRC is keen to establish scientific links with avian influenza researchers in China. Further information may be obtained from Tim Willis.

Application Procedure

The closing date for the initiative is 26 April, and applications will be considered by BBSRC's relevant responsive-mode committees at their meetings in September, alongside other applications.

Applications should be submitted in the usual way on the BBSRC Je-S form, with the 'BBSRC Combating Avian Influenza Initiative' selected as 'Mode' within the 'Project Details' section.

The initiative will be subject to the Council's normal responsive-mode eligibility criteria, but applications from BBSRC-sponsored institutes will not count against their responsive funding caps.

Although many of the projects awarded will be highly focused, BBSRC encourages applications up to 5 years' duration and/or of a larger collaborative nature where the science warrants this.

The additional funding for this initiative will be available for allocation only as part of the grants round immediately following the 26 April closing date. Future responsive-mode proposals for research on avian influenza will be considered in the usual way, in full competition with all other applications.

ANNEX 3

COMBATING VIRAL DISEASES OF LIVESTOCK INITIATIVE

(http://www.bbsrc.ac.uk/science/initiatives/animal_disease.html#1 for full details)

Background

The 2002 spending review settlement allocated funds to BBSRC for studies of the fundamental biology of the interactions of a range of infectious organisms with their hosts, especially viral persistence, epidemiology of animal infectious diseases and related investigations in support of improved diagnostics, and vaccines. This work will be co-ordinated with that of other funders, particularly Defra and SEERAD.

Aims of the Initiative

Recent events have highlighted the global threats to UK farming from viruses. International trade in animals and animal products and speed of movement of animals and people mean that we can no longer afford to be complacent about viral diseases of livestock; we live on a global farm. In addition global warming may be a significant factor in exotic virus disease spread.

There is no single solution to the problem of viral infections of livestock, but key to future disease control measures is a better understanding of the patho-physiology of the host-pathogen interactions. If we understand how pathogens persist, are transmitted and cause disease we will be in a position to; develop better methods of disease detection; improve husbandry methods to reduce disease transmission; develop novel and more effective vaccination, other therapeutic and control strategies; and identify genes associated with disease resistance/susceptibility to permit the breeding of naturally resistant stock.

Objectives

Although a number of separate objectives are listed below, it is not intended that these should be seen as disconnected elements. An over arching aim for this initiative will be to bring about greater collaboration and integration between all those groups that are able to contribute to a deeper understanding of the patho-physiology and epidemiology of animal viral disease. Therefore in addition to those already working in the animal viral disease area, we also wish to encourage groups from other areas such as human virology who can contribute relevant expertise.

A. Scientific Objectives

A1. To increase our understanding of the molecular, immunological and physiological mechanisms of pathogenesis of important viral pathogens of livestock (including farmed fish and poultry). The aim of this work should be to increase understanding of both the effects of the pathogen on the host and the host on the pathogen, particularly in the areas of persistence, mucosal infections, virulence determinants, and susceptibility/resistance in the host. New, post-genomic technologies offer great opportunities for understanding at the host-virus level.

A2. To increase our understanding of the epidemiology of viral diseases of livestock, including experimental, statistical and mathematical modelling approaches. Studies could, for example, target: modes of disease transmission, progression of disease/infectivity, invasion/persistence criteria, the role of host resistance/ susceptibility in epidemiology, and the emergence of virulence (see notes below concerning funding mechanisms – applicants addressing this objective may only apply as part of a grouping through Fund 1).

A3. To exploit fundamental knowledge obtained in model system and in vitro research in order to better understand viral disease in target species. Model-hopping approaches could be particularly appropriate here.

A4. To develop a suite of functional genomic, proteomic, virological and immunological resources to permit molecular analysis in both viral pathogens and their target species, and underpin the development of appropriate vaccines.

A5. To facilitate the utilisation of the newly acquired molecular pathological information in the advancement of novel husbandry, diagnostic, therapeutic and vaccination strategies to combat viral disease of livestock.

Consequently the following will be taken into consideration when assessing applications:

- Collaborations that fully utilise the expertise and facilities that already exist in institutes and universities
- The likelihood that the proposed project will increase the cadre of young scientists capable of undertaking integrative studies of target species disease.

CVDL INITIATIVE: GRANTS RELEVANT TO AVIAN INFLUENZA

There are 3 projects:

1. **Grant Reference:** BBSB00093/ BBSB00522
   Current Institution of Principal Investigator/Co-Applicant: Institute For Animal Health (IAH)/ University of Cambridge, Penn State University College of Medicine
   Institution of Grant: Institute for Animal Health (IAH)/ University of Cambridge
   Leader: McCauley J/ Gog J R, Holmes E C
   Title: Dynamics, selection and pathogenicity in avian influenza: from individual to population
   Total Value of Grant: £558,777

2. **Grant Reference:** BBSB00239/ BBSB00301
   Current Institution of Principal Investigator/Co-Applicant: University of Cambridge/ The Roslin Institute (RI)
   Institution of Grant: University of Cambridge/ Roslin Institute (RI)
   Leader: Tiley L/ Sang H
   Title: Suppression of avian influenza virus replication using inhibitory RNAs
   Total Value of Grant: £322,313

3. **Grant Reference:** BBSB001152
   Current Institution of Principal Investigator/Co-Applicant: St Mary's Hospital Medical School
   Institution of Grant: Imperial College London
   Leader: Skinner MA
   Title: Defining the avian antiviral type I interferon system
   Total Value of Grant: £182,555

   Grant Reference: BBSB00344
   Current Institution of Principal Investigator/Co-Applicant: St George's University of London
   Institution of Grant: St George's University of London
   Leader: Goodbourn S
   Title: Defining the avian antiviral type I interferon system
   Total Value of Grant: £222,351