Institution: London School of Hygiene & Tropical Medicine (LSHTM)

Unit of Assessment: UoA1 – Clinical Medicine

Title of case study: Exploitation of virus-like particles for vaccinology and the development of safe efficacious Bluetongue virus vaccine

1. Summary of the impact

Recent outbreaks across Europe of Bluetongue, a viral disease particularly affecting sheep, have driven research at LSHTM by Professor Polly Roy and her team, resulting in the Bluetongue virus (BTV) becoming one of the best understood viruses at the structural and molecular levels. The research has ultimately enabled the creation of several promising new vaccines. In addition the Roy group has contributed towards exploiting virus-like particles (VLPs) as a method to produce safe vaccines against human and animal viral pathogen. The most advanced example is a BTV vaccine for livestock, which is manufactured by Boehringer Ingelheim (BI).

2. Underpinning research

Bluetongue has been recognised as a viral disease of livestock for more than 100 years, first in Africa and then in the USA, Australia, Asia and more recently Europe. Spread by *Culicoides* midges, it affects all ruminants, but sheep are most susceptible with up to 70% of cases resulting in death. Since 1998, there have been several BTV outbreaks in Europe, which have killed more than 2m animals, causing serious economic losses to the agricultural sector.

Polly Roy, who joined LSHTM as Professor of Virology in 2001, is known as a leading authority on BTV and her salient contribution has been the first complete molecular understanding of this widespread viral pathogen. Her work also represents a model system for a number of similar viruses that are pathogenic to humans and animals (e.g. Rift Valley fever virus). At LSHTM, Roy's team carried out a series of studies using multidisciplinary approaches to provide a detailed understanding of every aspect of the BTV replication cycle – from virus entry via genome replication to virus assembly, cell-to-cell transmission and the engagement of the virus particle with the host cell and egress of the viral particle. This complete understanding has not only paved the way for improved diagnostics and vaccines for BTV, but has generic applications for viral vaccine design and development.

Roy was the first to demonstrate that simultaneous high level expression of multiple proteins in the same eukaryotic cell leads to the assembly of multilayered VLPs, mimicking authentic virus particles lacking viral genome.[3.1–3.4] Further, as a proof of concept, the team subsequently demonstrated that these empty VLPs were highly protective in animals against virulent viral challenge, as well as being completely safe.[3.1–3.4]

Following several outbreaks of BTV in Europe from 1998, Roy embarked on a vigorous research programme at LSHTM with a view to designing improved VLP-based vaccines. As a first milestone, she pioneered highly versatile improved multiprotein expression vectors (funded by BBSRC and patented in 2008, Patent number P522720PCT) for the development of promising vaccines.[3.1]

In the second milestone, Roy generated VLPs for European Bluetongue serotypes (five consecutive EU awards from 2000–2013) and tested their protective efficacies in different European breeds of sheep (France, Spain, Greece). In each case, she was able to demonstrate full protection against virulent viral challenges,[3.2–3.4] a strong translational outcome to the underpinning basic research.

In the third milestone, Roy transferred her knowledge to a vaccine manufacturing company, BI, which conducted successful clinical trials in 2012/2013 (details in Section 4).

A concurrent important research area has been the development of the first reverse genetics (RG) system (patented in 2008, PCT/GB08/03945) for BTV, which allows the synthesis of infectious virus solely from synthetic genes following their introduction into cells. This new technology allows

directed virus genetic manipulation, facilitating the creation of novel genetically attenuated BTV strains as safe efficacious vaccines. The research demonstrated their protective qualities in sheep and cattle and using similar approaches could be broadly applied to other animal and human viral pathogens.[3.5–3.6]

3. References to the research

3.1 Noad, R, Stewart, M, Boyce, M, Celma, C, Willison, K and Roy, P (2009) Multigene expression of protein complexes by iterative modification of genomic Bacmid DNA, *BMC Molecular Biology*, 10(paper 87), doi: 10.1186/1471-2199-10-87.

3.2 Stewart, M, Bhatia, Y, Athmaran, TN, Noad, R, Gastaldi, C, Dubois, E, Russo, P, Thiéry, R, Sailleau, C, Bréard, E, Zientara, S and Roy, P (2009) Validation of a novel approach for the rapid production of immunogenic virus-like particles for Bluetongue virus, *Vaccine*, 28(17): 3047–3054, doi: 10.1016/j.vaccine.2009.10.072.

3.3 Stewart, M, Dubois, E, Sailleau, C, Viarouge, C, Bréard, E, Desprat, A, Thiéry, R, Zientara, S, and Roy, P (2013) Bluetongue virus serotype 8 virus-like particles protect sheep against virulent virus infection as a single or multi-serotype cocktail immunogen, *Vaccine*, 31(3): 553–558, doi: 10.1016/j.vaccine.2012.11.016.

3.4 Stewart, M, Dovas, CI, Chatzinasiou, E, Athmaran, TN, Papanastassopoulou, M, Papadopoules, O and Roy, P (2012) Protective efficacy of Bluetongue virus-like and subvirus-like particles in sheep: presence of the serotype-specific VP2, independent of its geographic lineage, is essential for protection, *Vaccine*, 30(12): 2131–2139, doi: 10.1016/j.vaccine.2012.01.042.

3.5 Matsuo, E, Celma, CCP, Boyce, M, Viarouge, C, Sailleau, C, Dubois, E, Bréard, E, Thiéry, R, Zientara, S and Roy, P (2011) Generation of replication-defective virus-based vaccines that confer full protection in sheep against virulent BTV challenge, *Journal of Virology*, 85(19): 10213–10221, doi: 10.1128/JVI.05412-11.

3.6 Celma, CCP, Boyce, M, Van Rijn, PA, Eschbaumer, M, Wernike, K, Hoffmann, B, Beer, M, Haegeman, A, De Clercq, K and Roy, P (2013) Rapid generation of replication-deficient monovalent and multivalent vaccines for Bluetongue virus: protection against virulent virus challenge in cattle and sheep, *Journal of Virology*, 87(17): 9856–9864, doi: 10.1128/JVI.01514-13.

Key grants

3.1 Roy, Improving Baculovirus Expression of Multi-protein Complexes in Insect Cells, BBSRC, 6/2005–5/2008, £295,769.

3.2–3.4 Roy, EC, FP6 STREP, Improved Vaccines for Bluetongue Disease (BTVAC) #044211, European Commission, 1/2007–9/2010, €840,000.

3.5 Roy, Recovery of Bluetongue Virus from Nucleic Acid: Configuration, Optimisation and Application, BBSRC, 12/2007–6/2011, £563,947; Roy, Bluetongue Virus Reverse Genetics: The Way Forward for Bluetongue Vaccines, BBSRC 7/2008–12/2011, £572,143.

3.6 Roy, Development of Multivalent Vaccines for BTV, EHDV and AHS, ORBIVAC, European Commission FP7 Program, # 245266, 2/2010–1/2014, €2,999,729.00.

4. Details of the impact

Roy's groundbreaking research on Bluetongue and related orbiviruses has laid the foundations for fundamentally new vaccines. Existing vaccines for some BTV serotypes are available as relatively crude preparations which carry serious risks; for example, a live attenuated BTV vaccine which has been in use in Africa for many years can cause severe clinical signs of disease in European sheep breeds. Affected sheep can even infect feeding midges, with the possibility of transmission to non-vaccinated areas. As a result, the use of these vaccines in Europe is not recommended.

A direct result of Roy's research was the provision of material for a VLP vaccine for BTV which would carry no such risks. In 2011, Roy formed a commercial partnership with BI, a pharmaceutical company in Germany, with a view to testing whether a VLP Bluetongue vaccine based on her patented technology could be produced on a large scale. After the positive outcome of these initial tests, BI performed several clinical trials with two different vaccine candidates, both in sheep and cattle (2012–2013). The fact that the manufacture of VLP-based vaccines does not require containment level 3 and hence needs fewer precautions during production is a significant commercial advantage for the production of the vaccine and for the company. The results obtained from these trials were 'excellent', according to BI, and the company has declared itself 'very confident' that it will be able to market this 'highly innovative vaccine against Bluetongue disease' within the next few years.[5.1] BI plans to manufacture VLPs of several European strains initially, each developed by Roy's team at LSHTM.

Through their work on Bluetongue, Roy and colleagues pioneered and delivered proof of concept for the VLP technology for vaccine development. This has been generally available to all scientists[5.2] and has contributed to a number of other VLP protein-based vaccines, including influenza, SARS, ebola, FMDV and others. VLPs have been used for the development of an influenza vaccine, with the US company Novavax announcing in March 2011 that it had been awarded a contract by the US Department of Health and Human Services for the advanced clinical and manufacturing development of VLP vaccines for the prevention of seasonal and pandemic influenza. The first phase 1 clinical trial of its VLP vaccine candidate for the avian-origin A(H7N9) virus was announced in July 2013.[5.3]

As part of her work on Bluetongue, to address the poor performance of current attenuated vaccines, in 2011 Roy began overseeing the development and trial of a polyvalent vaccine effective against most serotypes based on the unique reverse genetics system pioneered in her research. This work has been carried out in collaboration with EU veterinarian partners (BTV reference laboratories in France, the Netherlands and Germany). The vaccine has provided full protection in challenge studies carried out in 2011 and 2013 (Matsuo et al.; Celma et al.)[5.4] and has the capability to uniquely offer most of Europe's estimated over 150m sheep and cattle population cross-serotype protection. Several commercial partners have already shown an interest in producing and marketing the vaccine including DELTAMUNE, Pretoria, South Africa.[5.4]

During and after the UK Bluetongue outbreak in 2008, Roy contributed to the understanding of the virus and its effects among the general public by giving a number of press interviews.[5.5.] Her work has been publicised in media directed at farmers.[5.6] She has also featured in the scientific and official press. Professor Douglas Kell, BBSRC Chief Executive, said, 'This is an exciting development and offers great potential for future vaccine development. This approach could allow us to make safer and more effective vaccines against a range of viral diseases";[5.7] and a BIS press release noted. 'A research team led by Professor Polly Roy ... has recently reconstructed a Bluetongue virus in a test tube … This is an important step in vaccine creation.'[5.8]

5. Sources to corroborate the impact

5.1 Head of Preclinical and Clinical R&D, Boehringer Ingelheim Veterinary Research Center GmbH & Co KG: letter to Roy providing details of LSHTM/BI cooperation and outcomes to date.

5.2 Noad, R and Roy, P (2003) Virus-like particles as immunogens, *Trends in Microbiology*, 11(9): 438–444, doi: 10.1016/S0966-842X(03)00208-7.

5.3 Novavax (2013) Novavax initiates first phase 1 clinical trial of its A(H7M9) influenza vaccine candidate, press release 8 July 2013, http://www.novavax.com/download/releases/2013-07-08%20H7N9%20FSI%20Final.pdf (accessed 6 November 2013).

5.4 Chief Executive Officer and Scientific Officer Of Deltamune (Pty) Ltd, a South African based Company: letter to Roy stating their interest in Bluetongue virus DISC vaccine.

5.5http://www.lshtm.ac.uk/research/publicationsandimpact/casestudies/pollyroy_bluetongue_vaccine.html

5.6 Mackenzie, G (2011) Bluetongue marker vaccine one step closer, *Farmers Weekly*, 2 August, http://www.fwi.co.uk/articles/02/08/2011/128148/bluetongue-marker-vaccine-one-step-closer.htm (accessed 25 November 2013).

5.7 Biotechnology and Biological Sciences Research Council (2011) Scientists take a step towards developing better vaccines for bluetongue, *ScienceDaily*, 1 August, http://www.sciencedaily.com/releases/2011/08/110801160217.htm (accessed 6 November 2013).

5.8 Department for Business Innovation & Skills (2012) Government to invest £20 million in synthetic biology, press release 9 November, http://news.bis.gov.uk/Press-Releases/Government-to-invest-20-million-in-synthetic-biology-682fa.aspx (accessed 6 November 2013).