A validated experimental model of *Mycoplasma bovis* pneumonia in calves

David Reddick and Cliff Ramage, Moredun Scientific.

**Acute bovine respiratory disease (ABRD) continues to be a significant economic and welfare problem in calves. Severe cases can lead to mortality but more common outcomes include decreased growth rates, particularly where there is permanent lung damage.**

The disease syndrome can be caused by a number of different viruses and bacteria. The main viruses are: respiratory syncytial virus (RSV), parainfluenza type 3 (PI3) virus, infectious bovine rhinotracheitis (IBR) virus and less obviously bovine viral diarrhoea (BVD) virus. *Mycoplasma bovis* is one of several bacterial species which can be involved, the others being *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*.

To meet the requirement of animal health companies looking to test the efficacy of novel vaccines and therapeutics Moredun Scientific offers a validated experimental model of *M. bovis* infection in calves for use in client studies. The model has been used successfully in both vaccine and pharmaceutical trials.

**Model Overview**

(Therapeutic efficacy studies)

Calves are screened (by ELISA) to ensure that *M. bovis* antibody levels are below a defined cut off prior to transportation to the study site. At between 5 and 8 weeks of age the animals are challenged with an *M. bovis* isolate over three days. For four days post final challenge, the animals are clinically observed and enrolled on the study once clinical signs of respiratory disease are observed. Post enrollment clinical observations are carried out once daily for 14 days at which point the animals are euthanased and the lungs assessed for the presence of *M. bovis* specific lesions.

**Challenge Model**

The strain of *M. bovis* most commonly used in the model was isolated from a calf with clinical respiratory disease in the UK. This isolate has been used in a number of different client studies with considerable success. The growth of this and other *M. bovis* isolates has been fully validated using defined growth media and conditions and the production of challenge material to a defined level is reproducible within tightly defined limits.

**Clinical Signs**

A critical aspect of *M. bovis* studies is to ensure a consistently high challenge success rate. The Moredun model, which utilises endobronchial deposition of a low volume/high titre challenge on three consecutive days, has been shown to be effective with up to 70% of challenge animals showing clear clinical signs of respiratory disease within a four day period post challenge.

The model has been validated to produce clear signs of clinical disease including increased rectal temperature, increased respiratory effort/rate, abnormal demeanour and in some cases nasal discharge. These clinical signs allow enrollment of animals on therapeutic trials based on clearly defined and validated criteria. The standard design for enrollment requires animals to have an increased rectal temperature (≥39.5°C) and either abnormal demeanour or respiration.

**Lung Pathology**

The main determinant of the efficacy of the challenge for the majority of studies which use the Moredun model is the percentage of total lungs with lesions. The mean % lung damage in the control groups is generally 15 to 20% and significant differences between treated and control groups are routinely observed. Figure 1 shows an example of lung lesion scores following challenge in a therapeutic study.

**Bacteriology**

There are a number of options available to confirm the presence of the challenge isolate in the lungs of study animals. The most effective method is recovery of the bacteria from lung lavage fluid samples collected at necropsy with samples titrated onto *Mycoplasma* specific agar for accurate colony counts.
Disease Models
In addition to the M. bovis model Moredun Scientific offer validated experimental models of respiratory disease for a range of bovine pathogens which are available for client studies. The range includes models for bacterial and viral pathogens.

We have significant experience in conducting studies with Mannheimia haemolytica with both vaccines and therapeutics and have several recent publications in partnership with clients which refer to the use of the model:

Research
Pasturella multocida causes major welfare problems and economic losses in a range of hosts and in many areas of the world. It is responsible for both respiratory and systemic disease in cattle and buffalo, but a detailed understanding of, and the ability to control, the underlying molecular mechanisms during host-pathogen interaction is lacking.

The organism is re-emerging as a primary contributor to acute bovine respiratory disease, accounting for up to 15% of pneumonic pasteurellosis in the UK. There is no current test that differentiates between dangerous and less harmful forms of the bacterium, nor any European vaccine and the industry needs new and effective control measures.

The current focus areas of the Pasteurella Research Group at the Moredun Research Institute are the molecular characterisation of bovine isolates of P. multocida during carriage and disease, what happens during infection and the involvement of biofilms in disease. The aim is to develop new diagnostic and control methods for diseases caused by this bacterium.

Diagnostic tests
The Virus Surveillance Unit at Moredun provides specialist virological support to Scottish disease control centres. The unit has used the platform technology of real time polymerase chain reaction (PCR) to develop a new generation of highly sensitive, quantitative tests to detect and identify, bovine respiratory viruses. A multiplex test is now available for the detection of the viruses involved in bovine respiratory disease complex (calf pneumonia) in a single sample. The test detects bovine herpesvirus type 1, bovine respiratory syncytial virus and bovine parainfluenza virus 3.

For further information about how Moredun Scientific can support your bovine respiratory disease projects please visit our website or contact David Reddick: reddick@moredun-scientific.com

Moredun Research News
Parasitic Worm control consortium
Scientists from the Moredun Research Institute are involved in an exciting new EU project to develop new and sustainable strategies to control parasitic worms in sheep and cattle.

The ‘GLOWORM’ project (an abbreviation for “GLObal changes in parasitic WORMs”) will run for 3 years and will harness the expertise of 14 research groups throughout Europe, with the help of a 3 million euro grant from the EU 7th Research Framework Program.

Infection with parasitic worms represents a significant economic and welfare burden to the European livestock industry. The increasing prevalence of anthelmintic (wormer) resistance means that current control programmes, based on routine anthelmintic treatment, are costly and unsustainable in the longer-term. Recent changes in the epidemiology, seasonality and geographic distribution of parasitic worms have been attributed to climate change, however, other changes in the environment (e.g. land-use) and in livestock farming (e.g. intensification and changing management practices) will also have an impact.

The main objectives for the project are:
• to design improved diagnostic tests to improve surveillance of parasitic worm infections levels across Europe.
• to provide EU-wide modelling of infection risk in space and time with the aim of both supplying farmers with improved, up-to-date, information and designing new control strategies.
• to test new and innovative control strategies under field situations.

Ruminant Immunology Project
Researchers at Moredun have secured nearly £1 million from the BBSRC and the Scottish Government to learn more about the immune systems of livestock to aid the development of sheep and cattle vaccines. Scientists from Moredun and the nearby Roslin Institute, with support from an industrial partner, AbD Serotec, will undertake detailed investigations of the immune responses of sheep and cattle, vitally underpinning the development of future vaccines. The team will be led by Moredun’s Professor Gary Entrican.
Liver fluke is caused by *Fasciola hepatica*, a highly pathogenic flatworm parasite of ruminants. There is ongoing research at Moredun focussed on improving the diagnosis of active fluke infection in the host animal, determining the efficacy of flukicidal treatment as a measure of emerging resistance, monitoring fluke infection levels on farm as a predictor of disease risk and identifying novel vaccine targets on the surface of the fluke gut.

Moredun Scientific offer the following tests which can be used to study the efficacy of flukicidal drugs:

- **Faecal egg counting service** – indicates the presence of adult liver fluke in the faeces of infected animals.
- **Coproantigen ELISA test** – detects the presence of immature and mature fluke through measurement of fluke coproantigen secreted from both immature and mature fluke in the faeces of infected animals.

The tests can be carried out on samples from individual animals or on composite samples (combined samples from 10 animals) which provides information on the presence of fluke within a flock or herd.

Animal Health Research Club

Moredun Scientific is a partner in a newly formed Animal Health Research Club (ARC) which unites farmers, breeders and pharmaceutical companies in the UK in the fight against animal diseases. The club will invest around £9.5 M of public and private money in research projects which aim to improve animal health and welfare through better understanding, management and control of pests and pathogens. The club is being led by the Biotechnology and Biological Sciences Research Council (BBSRC), with additional funding from 12 company members and the Scottish Government.

The partners hope that by drawing on the complementary expertise of academia and industry the club will spark new ideas for keeping animals healthy and free from disease. The company members of the ARC will provide important input to the strategic direction of the Club’s research. This ensures that the Club is broadly directing its funding to areas where the commercial sector sees scientific bottlenecks.

For further information visit: www. www.bbsrc.ac.uk

Biosafety testing

The biosafety testing business of Moredun Scientific continues to develop and we have recently recruited additional staff to meet the demand from clients. Our focus is on *in vivo* and microbiology tests for the presence of adventitious agents in cell lines and cell banks used in the development and manufacture of human and veterinary biopharmaceuticals. Tests are conducted to GLP/GMP standards to meet international regulatory requirements.

For further information contact please contact John Murray: jmurray@moredin-scientific.com

Approval as French R&D Subcontractor

Moredun Scientific has received notification from the French Ministry of Research that it has successfully renewed its status as an approved subcontractor for research and development projects. This approval enables our clients who are private companies subject to corporate tax in France to claim R&D tax credits for work subcontracted to Moredun Scientific. The process required us to present a recent development project that demonstrated our competence as a subcontractor.

Recent publications

The following publications highlight recently published studies conducted in partnership with our clients:

- The efficacy of a combined oral formulation of derquantel-albendactin against anthelmintic resistant gastro-intestinal nematodes of sheep in the UK. Geurden et al, Veterinary Parasitology April 2012.
- Duration of efficacy of tulathromycin (Draxxin®) for the treatment of experimentally induced *Mycoplasma bovis* respiratory infection in calves. Moyaert et al World Biautrics Congress 2012.
- Colostrum from cattle immunized with a vaccine based on iron regulated proteins of *Mannheimia haemolytica* confers partial protection. Makoschey B et al Vaccine 2012, 30 pp969-973.
- The efficacy of monepantel against naturally acquired inhibited and developing fourth-stage larvae of *Teladorsagia circumcincta* in sheep in the United Kingdom Ramage et al, Veterinary Parasitology November 2011.

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Disease Models
Our disease models and areas of expertise are shown below:

Respiratory Diseases
- Bovine Virus Diarrhoea Virus (BVDV)
- Bovine Herpesvirus-Type 1 (BHV-1)
- Parainfluenza Virus Type 3 (PI3)
- Mannheimia (Pasteurella) haemolytica (bovine, ovine)
- Pasteurella multocida (bovine)
- Mycoplasma bovis
- Actinobacillus pleuropneumoniae (porcine)
- Mycoplasma hyopneumoniae (porcine) – under development

Mammary Gland Diseases
- Staphylococcus aureus mastitis (bovine)
- Streptococcus uberis mastitis (bovine)
- Escherichia coli (bovine)

Reproductive Diseases
- Bovine Virus Diarrhoea Virus (BVDV)
- Neospora caninum (bovine)
- Chlamydia abortus (ovine)
- Toxoplasma gondii (ovine)

Diseases of the Gut
- Escherichia coli (bovine, ovine) – porcine under development
- Rotavirus G6 & G10 (bovine, porcine)
- Coronavirus (bovine)
- Salmonella spp. (porcine)

Parasitic Diseases
- Endo and ecto Parasitic Disease (Bovine, ovine, equine, avian natural and experimental infections)

Systemic Diseases
- Pasteurella trehalosi (ovine)
- Streptococcus suis (porcine)

Contact us
Moredun Scientific
Pentlands Science Park
Bush Loan
Penicuik
EH26 0PZ
Scotland, UK
Tel: +44 (0) 131 445 6206
Fax: +44 (0) 131 445 6205
Email: info@moredun-scientific.com
www.moredun-scientific.com

Rigorous quality systems
Our independent Quality Assurance department has expertise in Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), VICH-Good Clinical Practice (GCP), ISO 9001:2000, ISO 17025 and ISO 14001. All studies are conducted to meet regulatory guidelines.

State of the art animal facilities
GLP accredited animal accommodation ranging from conventional farm accommodation, to specific pathogen free (SPF), Containment Level 3 or gnotobiotic units.

Laboratory facilities
We can support all aspects of safety and efficacy studies.