

# Moredun's Centenary Science Stories

Volume 1



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# introduction

I am privileged and delighted to introduce the first volume of Moredun's Centenary Stories.

As the name suggests, we planned to celebrate Moredun's Centenary in 2020 with a number of special events which our staff, collaborators, and Moredun Foundation members could all enjoy – including a brief account of some of our successful scientific outputs. COVID-19 has put a delay but not a stop to progress and we look forward to 2021 when we will celebrate 100+1 years!

It has been a very difficult task to choose the topics to include and then what to cover in each story. I asked the scientists currently working on the topics of the stories to give me 3-4 scientific papers which they felt were the "principal milestones" in developing the research and leading to different impacts for farmers, vets, landowners, scientists and all who benefit from our work. The stories, therefore, are far from comprehensive and are designed to tell some of the ups and downs of the scientific process along the way, using language that hopefully is accessible to most readers.

In some stories, I have referred to previous scientists employed at Moredun, while some names are mentioned in the references; in other stories I have mentioned a small number of current staff and students. In all cases, the success of Moredun's science throughout the last 100 years is almost entirely down to the vision, skills, and sense of purpose of all the many staff who have contributed across the Moredun Group. I would also like to acknowledge the financial support from the Scottish Government in particular, via its many acronyms, now RESAS, for their ongoing commitment to longer-term strategic research which is the essence of our work.

In each story I have tried to show why the subject matter is contemporary and where it fits with the current Global Grand Challenges we all face - Emerging infectious and zoonotic diseases, exemplified by the current COVID-19 crisis; endemic diseases adversely affecting welfare and reducing production efficiency, in turn impacting on Climate Change targets; food safety and security; and finding solutions to these problems including diagnostics, vaccines and disease control programmes. I believe these approaches make Moredun more than fit for the future.

There is however always benefit in Looking Forward While Glancing Back. The Moredun Research Institute was originally named the Animal Disease Research Association (ADRA) and my favourite section from Ken Angus's "A History of ADRA" is the quote from the poem written by Richard Barnfield in the 16th century. It is called the Shepherd's lament and reads:

*My flocks feed not,  
My ewes breed not,  
My rams speed not.  
All is amiss:  
What can be the cause of this?*

Over the years Moredun has not just identified the biological agents causing disease but has made significant strides in developing options to prevent the diseases occurring in the first place.

Finally, some time ago I found a document relating to ADRA called "A Short Survey of The Institute's first 25 years", published in the Scottish Farmer in January 1946. The article covered the establishment of the organisation, recent research findings, including the treatment of "milk fever" in dairy cows, and the cause of "pine" in sheep, and of course the economic value of the Institute to Agriculture. I was particularly taken that many of the key issues we face today were also faced by our predecessors. The subtitle of the paper "A Short Survey of The Institute's first 25 years", was "By Persevere". Now, this may be grammatically incorrect but I really liked it, and I hope you may get a feel for some of the dedication to finding long-term solutions to complex infectious diseases of multiple livestock through reading our Centenary Stories.

*Professor Julie Fitzpatrick  
Scientific Director and Chief Executive*



# Malignant Catarrhal Fever

Photo: Felix LamKester



The disease Malignant Catarrhal Fever (MCF), as its dramatic name suggests, is a particularly severe disease of cattle, buffalo and deer, inducing multiple clinical signs and pathological lesions, and usually resulting in death – truly malignant!

The disease MCF had been recognised in cattle in Africa for many decades and associated with herds co-grazing with wildebeest (<sup>1</sup>Plowright et al, 1960). The Maasai tribesmen in East Africa, particularly adversely affected by the high mortality in their livestock, would regularly move their cattle long distances to poorer grazing in order to avoid wildebeest during the calving season when transmission of the disease was known to occur. The virus, eventually identified as causing the disease was *Alcelaphine herpesvirus 1* (AIHV-1), with wildebeest acting as asymptomatic (infected but showing no clinical signs) hosts.

In Scotland in 1980, <sup>2</sup>Buxton and Reid demonstrated that the condition could be transmitted to rabbits via intra-peritoneal injection of tissues from a red deer with MCF which had been in contact with lambing sheep. The rabbits demonstrated clinical and pathological signs that were indistinguishable from rabbits infected with the virus of MCF of wildebeest origin. Thus, the “sheep-associated agent” was initially recognised and successfully passaged in cultures of infected cells capable of inducing MCF in rabbits. This agent, identified eventually as *Ovine herpesvirus 2* (OvHV-2), had sheep as the asymptomatic hosts and was recognised to occur globally where sheep are in contact with susceptible species.

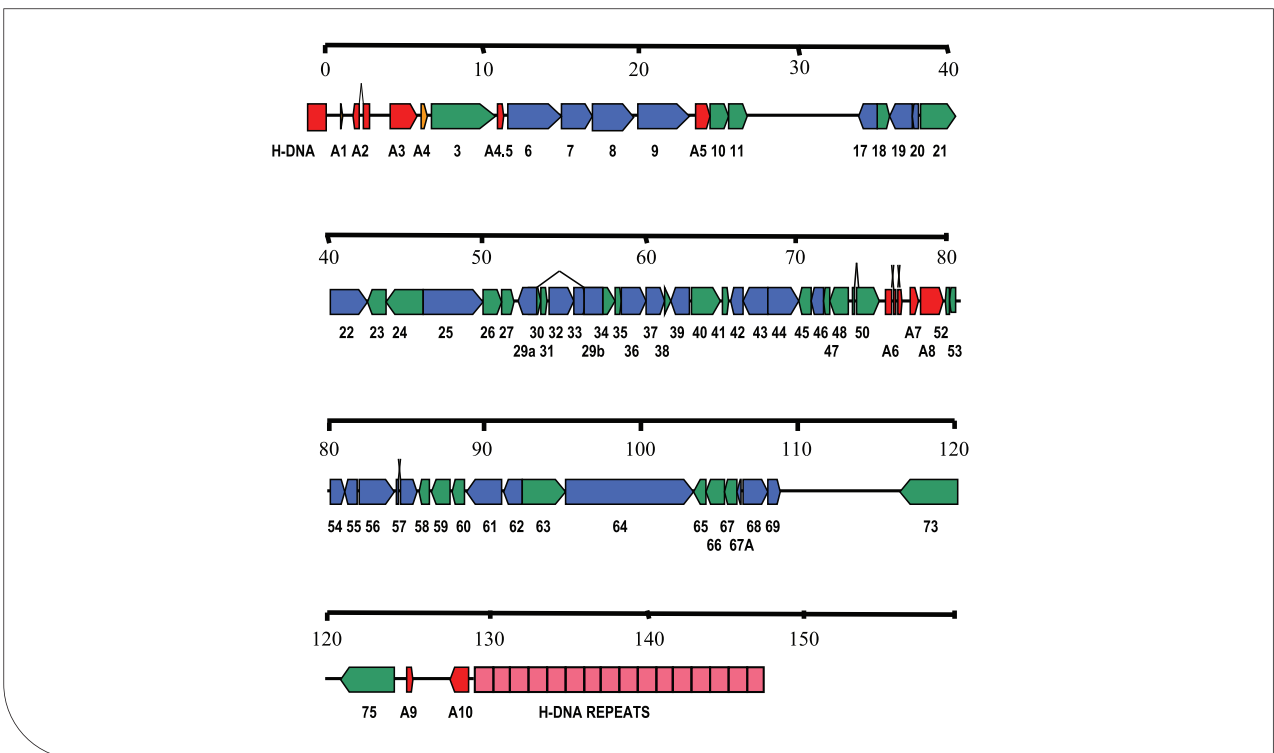
The experimental rabbit model was important in indicating that the course of infections with AIHV-1 and OvHV-2 was similar in this species and paralleled that in the natural MCF-susceptible species in Africa and elsewhere. The Moredun Group also found that a virulent strain of AIHV-1 (named C500) became attenuated, or less able, to cause MCF in rabbits after repeat passage in bovine cells. Subsequent molecular genomic studies identified genetic changes associated with attenuation, suggesting the virulent mechanisms of this virus and opening the door to possible vaccination approaches (<sup>3</sup>Handley et al, 1995).

Cow with classic head and eye signs of MCF.



The attenuated AIHV-1 C500 virus was stabilised over many generations of cell growth prior to being tested as a potential vaccine for MCF. These studies were performed in Moredun’s specialised animal accommodation where it was possible to provide cattle which had not been previously exposed to the virus and where there was a low risk of transmission of virus between cattle. The vaccine was injected intramuscularly into cattle with an adjuvant (a compound which helps stimulate immune responses in host animals), while the challenge virus was administered intra-nasally, mimicking what was considered to be the most likely route of natural infection. Nine of ten vaccinated cattle were protected with high levels of virus neutralising antibody in nasal secretions associated with lack of clinical or pathological signs (<sup>4</sup>Haig et al, 2008).

This promising experimental study led to the exciting prospect of testing the AIHV-1 C500 vaccine in herds with naturally occurring infection initially in Tanzania, led by Dr George Russell of Moredun, Professor David Haig of the University of Nottingham, and Professor Sarah Cleaveland of the University of Glasgow. Results were inconclusive due to few cases of MCF occurring in the unvaccinated, control cattle, indicating the importance of ensuring disease transmission between cattle and wildebeest during the experimental period. Colleagues at the International Livestock Research Institute (ILRI) in Kenya were subsequently ready to collaborate in a randomised vaccine trial on the Institute’s farm near Nairobi, where half the cattle received an injection with vaccine plus adjuvant and the other half received a control injection with adjuvant only. The injections were boosted four weeks later and cattle allowed to mix and graze with wildebeest herds to hopefully allow natural exposure to AIHV-1. Transmission was successful, with the control animals showing clinical signs and many being positive by Polymerase Chain Reaction (PCR, a molecular diagnostic) testing for AIHV-1. A small number of vaccinated cattle showed evidence of MCF, however the vaccine efficacy was shown to be 80.1%, a highly statistically significant result (<sup>5</sup>Cook et al, 2019).



*Genome of AIHV-1 - two regions apparently altered during attenuation were in the centre of the genome (orf50-A7) and to the right of gene A9.*



MCF is a very significant problem across Africa, with clear differences in the cattle populations in different regions. In southern Africa, owners of private wildlife and game parks are under pressure from neighbouring commercial cattle herds which suffer severe economic losses due to MCF and where control of the disease is seen as a national priority. Losses due to MCF also occur in smallholder and medium-sized farming enterprises across southern Africa. In East Africa, MCF affects cattle in all principal farming systems but with the main focus being on pastoralist communities or agro-pastoralists at livestock/wildlife interfaces where wildebeest and cattle are most likely to come into contact. Issues around the ability of pastoralists to access, afford, use and benefit from any future MCF vaccine is a subject which has been discussed at international workshops with Moredun scientists and colleagues from University of Glasgow, Sokoine University of Agriculture, Tanzania; WSU Paul G. Allen School for Global Animal Health, USA, and many others. While MCF in the UK is sporadic, this work is an example of research collaboration among continents and specialists in pathology, virology, epidemiology and development science.

Impact of research endeavour depends on the technologies developed being taken up by manufacturers, distributors and users, both public and private. At the time of writing, a number of commercial companies are progressing plans to scale-up the Moredun MCF vaccine, to register it with appropriate regulatory authorities and to allow greater numbers of cattle to be vaccinated in both East and South Africa. It is only then that it will be possible to judge the benefit of the early work undertaken 50- 70 years ago on the health and welfare of both livestock and their owners in some of Africa's most fragile ecosystems.

I would like to thank Dr George Russell for his help in developing this story.

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## Poultry Red Mite Vaccine Development

This project started in 2006 following a chance conversation between Sir John Campbell and Professor Julie Fitzpatrick, at the Farmer's Club in London about the problems facing the egg-laying industry. Two scientists at Moredun who were already developing a vaccine against a different species of mite, the sheep scab mite, also had interests, and some history, in researching poultry red mites. These scientists, Dr. John Huntley and Dr. Alasdair Nisbet, were invited to visit Sir John's egg production unit in Midlothian to see the scale of the problems facing the industry. These included increased waste resulting from rejection of eggs due to blood spotting, irritation of hens by the bloodsucking mites, and significant staff time on cleaning and disinfection of poultry accommodation. Immediately after the visit, the scientists applied for funding from the British Egg Marketing Board Educational Trust for a PhD studentship to demonstrate that, if hens were vaccinated with extracts of the mite and mites were then fed with the blood from these hens in a controlled laboratory setting, antibodies in the vaccinated hens' blood would kill a proportion of the mites. The PhD student employed on this project, Harry Wright, successfully demonstrated this phenomenon, showing proof of concept that it was indeed possible to vaccinate hens against this parasite (Wright et al., 2009). One of the essential elements of this approach is that the mites need to suck sufficient blood to take in the antibodies which then go on to kill the mites. As the name suggests, poultry red mites are voracious bloodsuckers (being red because of the blood that they consume), and the ideal target for the first ectoparasite vaccine for avian species.





The mite extract which provided this protection was highly complex, being composed of hundreds of different proteins and so the next step in the process was to try to understand which components of the mite extract were really responsible for protection to allow them to be exploited in a commercialisable, synthetic (artificially constructed rather than originating from the natural organism) vaccine. To fund this work, Dr. Nisbet applied to the BBSRC for an Industrial Partnership Award in collaboration with Zoetis and, with substantial additional funding from the Japanese company Akita Co. Ltd, embarked on a 3 year project with Dr. Kathryn Bartley and Dr. Harry Wright to identify the protective components of the mite extract and to produce them as synthetic proteins. Three proteins in particular were identified as the principal protective components of the extract and they retained this protective activity when used as a prototype vaccine in a controlled laboratory setting (Bartley et al., 2015).

The final piece in this puzzle was to establish if the prototype vaccines (both the mite extract identified in Wright et al., 2009 and the cocktail of three synthetic proteins identified in Bartley et al., 2015) protected hens from poultry red mite in a field setting in commercial-style hen houses. A field trial was therefore commenced, funded by the BBSRC, Zoetis, Akita Co. Ltd., The British Egg Marketing Board Trust and The Genomia Fund. The broad spectrum of funders of this research reflects the importance of poultry red mite across the world and the need for an animal-based solution to the severe infestations recognised by industry.

The outcomes of the field trial gave great encouragement for the concept of controlling poultry red mite in commercial premises by vaccination, because the mite extract identified in Wright et al., 2009 led to a 78% reduction in the numbers of mites in the premises (Bartley et al., 2017). However, the cocktail of three synthetic proteins did not provide protection to the hens and this outcome has prompted our team to invest a great deal of time and effort in the last three years to develop the tools that we need to optimise this vaccine. These include identifying vaccine components that will induce strong prolonged immune responses; developing an accurate system for screening multiple vaccine formulations quickly with minimum impact on hens (for which Dr. Francesca Nunn was awarded the International 3Rs Prize in 2019); sequencing the genome (the DNA) of poultry red mite and developing gene-silencing tools (methods of making genes unable to produce proteins) to identify further good vaccine candidates. This work has put Moredun in a very strong position to continue this work to a successful outcome.

Poultry red mite: *Dermanyssus gallinae*.



Poultry red mites living in hen accommodation.

This project also demonstrates the importance of The Moredun Foundation's close link with the farming community and how these interactions identify the major diseases and conditions adversely affecting the health and welfare of food-producing species. Moredun's scientists are able to understand the problems farmers face and are then better placed to develop research pathways for novel products such as vaccines and practical disease control programmes. This approach is becoming an increasing focus globally with the emphasis moving from reliance on chemical therapeutants to "greener" and more sustainable disease prevention in all species. High quality, affordable protein, in the form of eggs, will be an essential element in feeding the estimated growth in the global population.

I would like to thank Dr Alasdair Nisbet for his help in developing this story.

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## Enzootic Abortion in Ewes



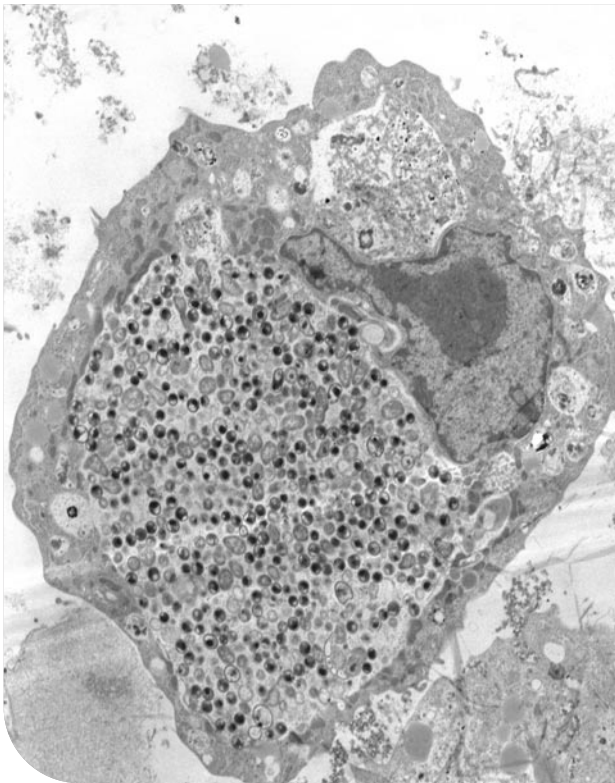
Abortion continues to be a significant issue in sheep production globally with infection due to *Chlamydia abortus* being the most common in many countries. The name "Enzootic Abortion in Ewes" (EAE) reflects that *C. abortus* can infect many ewes and ewe lambs in a flock resulting in abortion storms and weak-born lambs which often die or fail to thrive. It is common veterinary practice to carry out whole flock treatment with long-acting antibiotics during an abortion storm, a treatment option which is increasingly unacceptable due to the importance of anti-microbial resistance of bacteria which may infect the human population. *C. abortus* is a zoonotic pathogen which can cause foetal or maternal death when pregnant women become infected sometimes through handling lambing ewes.

Moredun has a long history of research on EAE with the first identification of the causal agent, originally named *Chlamydia psittaci*, in 1936 by Scottish scientists Prof John Russell Greig and Prof John Trevor Stamp (both former Directors of Moredun Research Institute) and colleagues. This was a landmark achievement in the history of *Chlamydia* research, ultimately leading to the development of the first vaccine (inactivated) against this devastating disease of sheep at Moredun in the 1950s. This vaccine was initially commercialised in 1958 by the Wellcome Foundation Ltd and remained highly successful in controlling the disease in the field until the late 1970s when vaccine breakdowns started to occur, believed at the time to be a consequence of more virulent strains emerging. The vaccine was eventually withdrawn in 1992.

Since then Moredun scientists have been instrumental in increasing understanding of the pathogenesis of *C. abortus*. Key to this was the discovery and role that the cytokine interferon-gamma has in controlling infection (<sup>1</sup>Brown and Entrican, 1976) which has proved extremely important both as a marker of infection and in the assessment of efficacy of trial formulations of new vaccines. The discovery of the three-dimensional structure and functional activity of one of the major surface proteins, MOMP, and the identification of a complex family of membrane proteins, Pmps (<sup>2</sup>Longbottom et al, 1998) resulted in the scientists, David Longbottom and Susan Wyllie, receiving prizes in recognition of their work. These discoveries have contributed significantly to new approaches taken towards the development of next generation vaccines and in the development of reagents and novel diagnostic tests for detecting infected animals, one of which was commercialised in 2015.

*Sheep and goats in pastoralist communities in Tanzania.*





Numerous *C. abortus* organisms located inside cells.

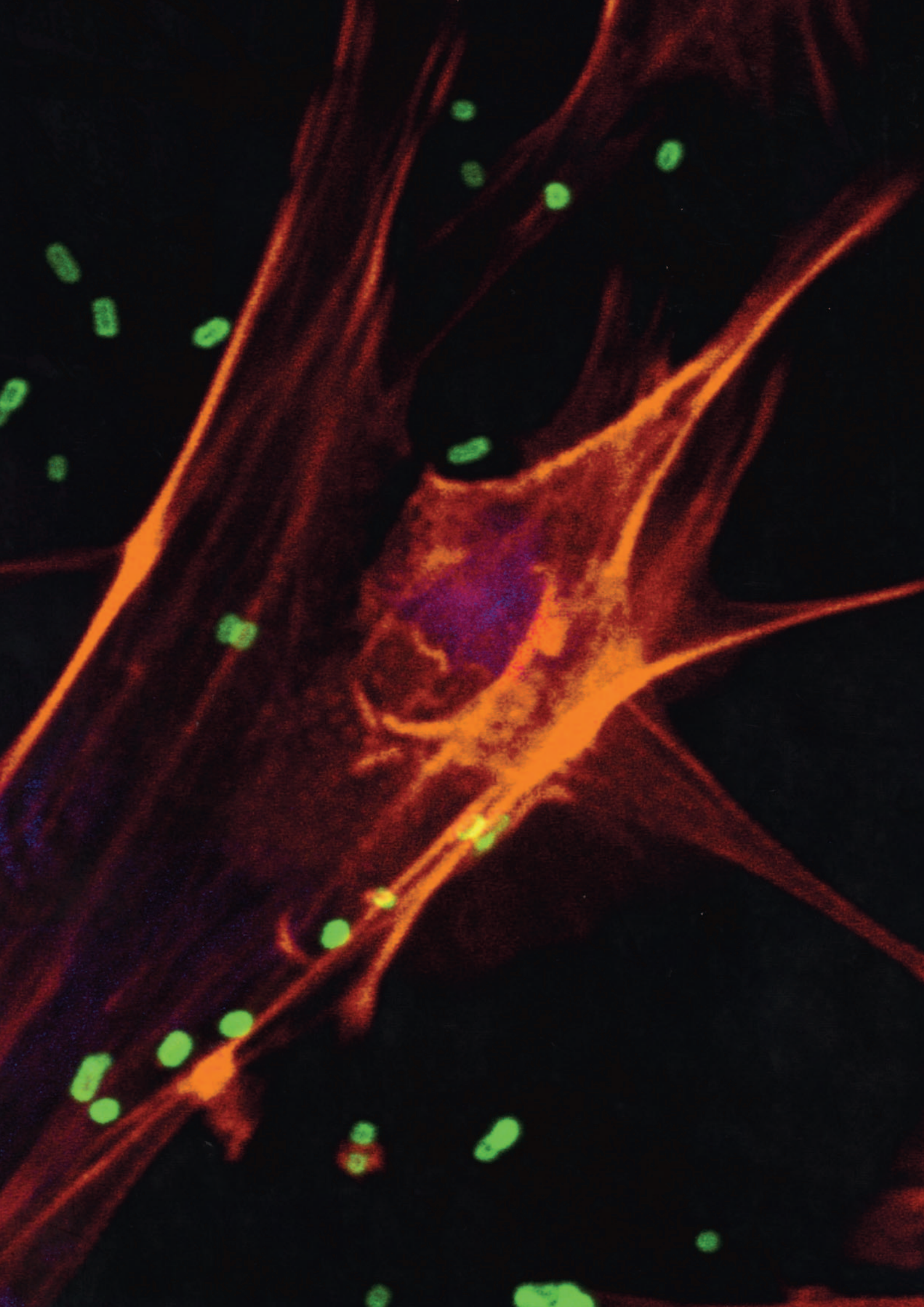
Further work at Moredun discovered that the currently available commercial live attenuated (altered so as viable/replicating bacteria should not remain) vaccine can cause disease in some animals, while most recently it has been shown that this vaccine strain is not actually attenuated (<sup>3</sup>Longbottom et al, 2018) thus accounting for its disease-causing potential and the need to develop a safer vaccine. The development of a model of latency (hidden form of disease) for this pathogen, where infection remains unapparent in the non-pregnant animal and only becomes evident during a subsequent pregnancy, has led to a fundamental change in understanding of the infection and disease process and how protection occurs (<sup>4</sup>Longbottom et al, 2013) which in the intervening years has changed the approach to developing a next generation vaccine. Underpinning development of such a vaccine has been two key publications involving, at that time, the ground breaking sequencing of the first chlamydial pathogen of livestock (<sup>5</sup>Thomson et al, 2005) the first pathogen to be sequenced at Moredun, and more recently, the identification of the proteins present in the outer membrane of the bacterium, all of which are potential targets for vaccine efficacy studies. The scientists are now progressing all of these findings and working closely with the animal health industry in taking this work forward to commercial realisation.

Sheep and goat production is a critical source of animal protein in many countries and continents and reproductive failure is recognised to be a major constraint to optimal efficiency. Vaccine companies globally see the benefit of aiming to produce multi-valent vaccines incorporating a variety of bacterial, viral and protozoal organisms, especially where diagnosis of specific pathogens is logistically difficult. Inclusion of *C. abortus* antigens will be an important component of future vaccines which will play a role in underpinning food security globally.

I would like to thank Dr David Longbottom for his help in developing this story.

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## Foodborne Pathogens

Moredun has focussed its work over the years mainly on the zoonotic foodborne pathogens, those that pass from animals to humans, and sometimes vice versa. Many different types of organisms can be transmitted to humans via food, however, some of the most problematic in terms of human infections are the bacterial species *Campylobacter*, *Salmonella* and Shigatoxin-producing *Escherichia coli* (*E. coli*) (STEC). Moredun's research has spanned from basic scientific knowledge about the mechanisms by which bacteria enter and/or damage animal/host tissues, through identifying the proteins involved in these processes, to tracking the transmission of zoonotic pathogens among host species over time and space. One of the key challenges in addressing foodborne diseases is that the zoonotic pathogens in animals do not usually cause clinical signs in the animals themselves, however, the consequences for humans affected by the same pathogens can be severe.

*Campylobacter jejuni* infections are the most prevalent cause of foodborne disease in the developed world and are responsible for significant economic losses through hospitalisation of patients. The effect of *C. jejuni* in the developing world is even more significant in terms of mortality of young children. Most bacteria causing infections of the gut, or gastrointestinal tract, secrete "virulence factors" which enhance bacterial survival and/or damage the host. Interestingly, *C. jejuni* lacks these virulence factors and it was hypothesised that the bacteria may have its effect through outer membrane vesicles (OMVs), which could potentially deliver damaging bacterial proteins into cells.

Research at Moredun formed part of a study indicating that, indeed, OMVs played an important role in *C. jejuni* infection of cells lining the human gut (demonstrated in cells in the laboratory) and that this was likely to involve fats aiding in transfer of bacterial proteins across the human cell membranes (Elmi et al, 2012). This project was a collaborative venture with the London School of Hygiene and Tropical Medicine who conducted the cell work - an example of strong links between UK organisations.

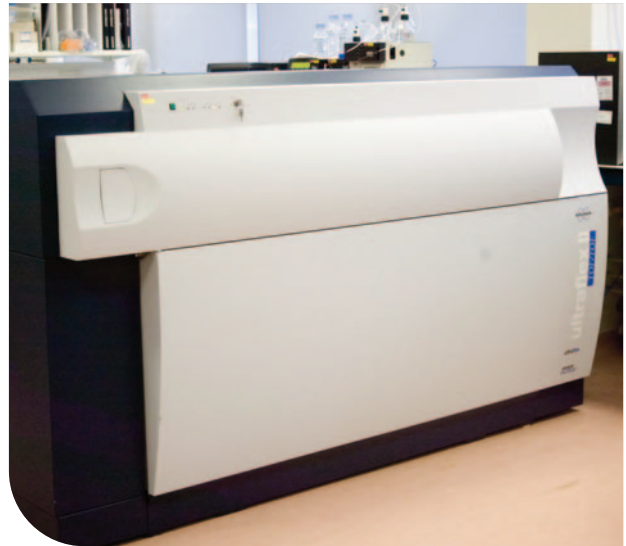


*Campylobacter jejuni* microcolony on gut tissue.

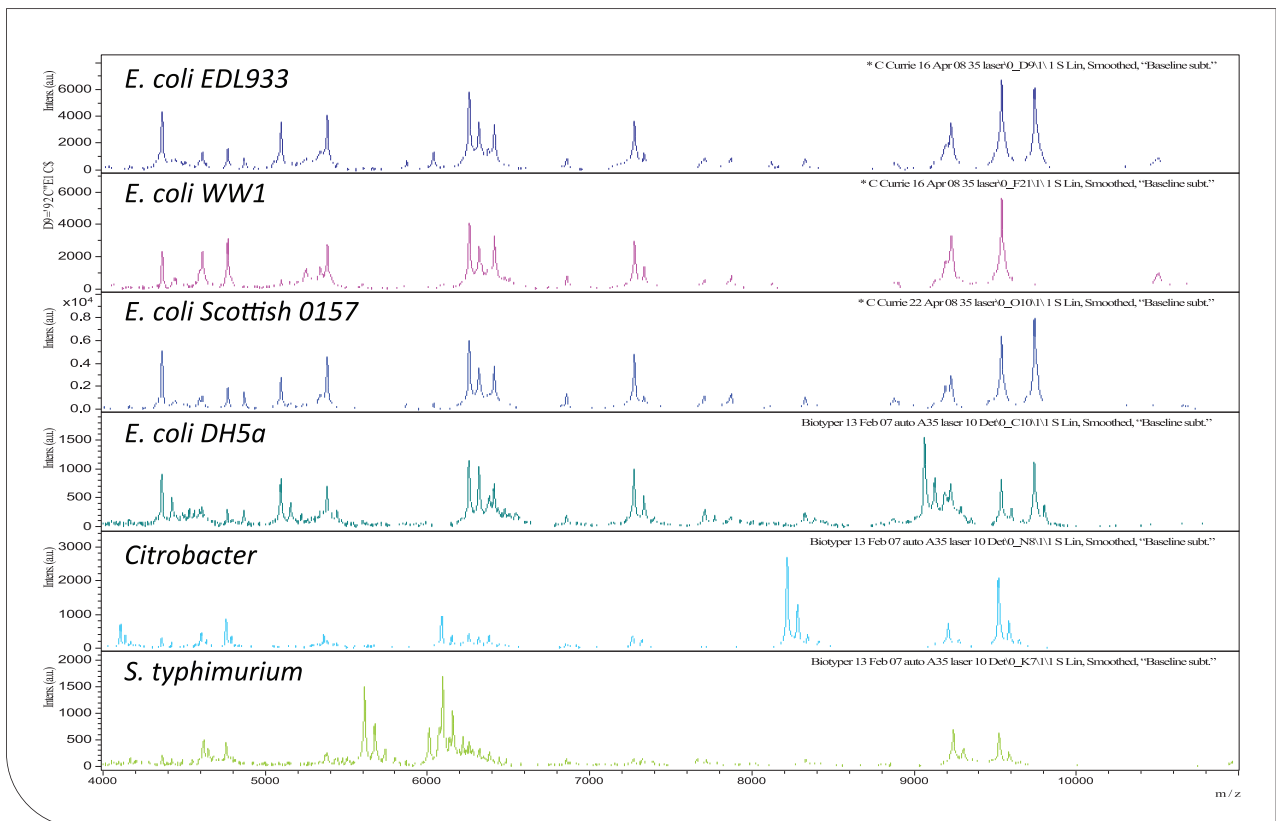
Photo: Professor Paul Everest, University of Glasgow

The “omics” era began some decades ago with innovations in technology which allowed identification of individual genes and their variants or mutations; individual proteins and their component building bricks, amino acids; and metabolites, or chemicals, involved in cell function. Moredun decided to invest in cutting-edge proteomics with the purchase of mass spectrometers and supporting bioinformatics required to translate the outputs. The “Proteomics Unit”, led for many years by Dr Neil Inglis, participated in numerous Moredun projects including identification of proteins for vaccine and diagnostic studies, in addition to fundamental and commercially-focussed work. An excellent example of this approach was the use of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) to differentiate subspecies of STEC bacteria in collaboration with the NHS and specifically with the Scottish *E. coli* O157/STEC Reference Laboratory (SERL). The work included a new approach for MALDI-MS combined with an analytical computational pipeline as a rapid procedure for identifying subtypes of STEC and accurately identifying biomarkers (naturally-occurring biological substances associated with disease) for these strains. The technique was able to differentiate *E. coli* O157:H7, the principal

subtype causing severe and even fatal infections of humans, from other STEC. Overall, nine groups of isolates were distinguished, offering a robust analytical tool useable in reference and diagnostic public health scenarios especially relating to zoonotic disease



Moredun's Ultraflex II MALDI-ToF-ToF mass spectrometer.



MALDI spectra indicating differences between STEC isolates and other bacteria.



Photo: Dr Johanna Baily, University of Stirling

Grey seal cow and pup on the Isle of May colony.

outbreaks (<sup>2</sup>McLean et al, 2018). The analytical computational pipeline was developed by BioSS, one of Moredun's sister institutes in Scotland, using the same dataset as used (or developed) in the biomarker study and published in an international bioinformatics journal (<sup>3</sup>Palarea-Albaladejo et al, 2018). Moredun is currently assessing the application of this technology in the detection of antibiotic resistance in *C. jejuni* and in the study of antibiotic resistance in multiple species of bacteria causing mastitis in cattle and sheep, as the developed pipeline may offer improved timeframes in diagnostic, surveillance and research laboratories.

The routes of transmission of zoonotic pathogens from animals to humans are many and varied. Investigation of "sentinel species" can be useful, including marine mammals where environmental contamination may have taken place. *C. jejuni* was isolated from approximately half of all grey seal pups sampled in the breeding colony on the Isle of May in Scotland. A genomic-based typing method for *Campylobacter* showed that isolates from seals were consistently associated with human isolates (<sup>4</sup>Baily et al, 2015). These results are consistent with either a common infection source for both humans and seals, or direct transmission of human *Campylobacter* to grey seals, raising concerns about the spread of human pathogens to wildlife marine sentinel species in coastal areas. Moredun scientists have also contributed to global collaborations focussing on ecology and biodiversity through studies of infections in seals in remote sites such as Galapagos and Gough Island.

The lead scientist at Moredun on the foodborne research area, Dr Eleanor Watson, sees multiple applications of proteomic and genomic technology in studying foodborne bacteria. Several studies are underway, focussing on *Campylobacter*, which may provide insights into the role of this bacteria in the dissemination of antibiotic resistance genes through the food supply chain and factors which are important in bacterial adaptation within animal reservoirs.

I would like to thank Dr Eleanor Watson for her help in developing this story.

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# Cryptosporidiosis

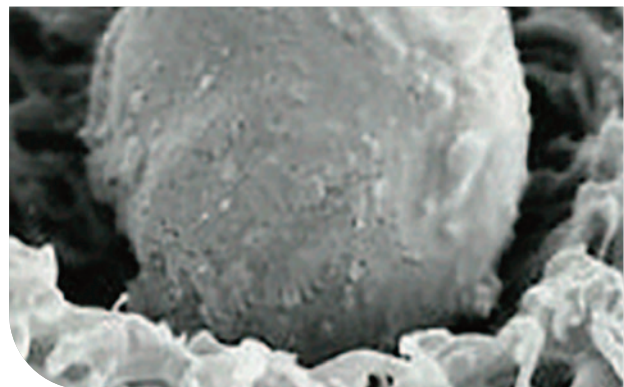
Photo: Andy Wells



*Cryptosporidium parvum* is a parasite which causes diarrhoea in humans and many livestock species. In young or immunocompromised hosts, the infection can be severe and may cause death. Neonatal diarrhoea in lambs and calves poses a real problem for farmers and veterinary surgeons as specific diagnosis can be difficult with a number of pathogens causing similar clinical signs including viral, bacterial and coccidial species. This, in turn, results in regular usage of broad-spectrum anti-microbial drugs on some farms as a "catch-all", a practice which is no longer advisable due to ongoing concerns about anti-microbial resistance.

*C. parvum* is an obligate pathogen which means it only replicates in the host animal and not in the environment, however the infectious stage, the oocyst, persists very well in water, which has been recognised as a common source of infection for humans and animals. Some years ago, Moredun scientists conducted some important, fundamental studies on lambs, raised free of infectious agents, which were given water with varying dilutions of *C. parvum* oocysts. This work indicated that the infective dose for *C. parvum* in lambs was very small indeed - as low as a single oocyst, providing evidence as to why this pathogen was so successful in transmitting to a wide variety of susceptible species (Blewett et al, 1993). This experimental work was critical as it also illustrated the importance of water as a mechanism for the transmission of *C. parvum* and the information was used by water companies in the production of risk assessments for all public water supplies.

More recently, one of Moredun's scientific teams, led by Dr Beth Wells, conducted a study focussing on a water catchment in the Cairngorms National Park; an area which had reported a high level of outbreaks of cryptosporidiosis especially in visitors to the area.



A *Cryptosporidium* oocyst

The research team collected faeces deposited by cattle, sheep and deer within the Glenlivet catchment and water samples from various strategic points along the river providing the public water supply. Using novel sample preparation and specialised molecular genotyping techniques, the cryptosporidia detected could be identified to species level (e.g. *C. parvum* which was the commonest species) and to sub-species level (sub-types), based on genetic variations of the organisms. The results showed that the prevalence, or level, of cryptosporidia was high in the catchment area, that all ages of sheep and especially cattle and deer, were infected and that patterns of infection could be detected among animals and farms (Wells et al, 2015).

The impact of this study has been very significant on both local and national scales and has resulted in considerable dialogue between Moredun and UK water provision companies. On a local scale, improvements in cattle health and welfare on farm has been achieved by engaging with farmers and vets, with the effect of increasing farm efficiency and reducing oocyst output into the environment. Advice included keeping cattle and sheep out of public water supplies through providing water troughs and establishing fencing above the water intake to reduce contamination of water by deer and livestock. This had the effect of reducing the number of infectious oocysts available to wildlife and to humans, through less transmission in to the catchment. Working with gamekeepers and water catchment officers increased knowledge of the disease on farm and helped promote good relations between all concerned leading to improvements in water quality and human health. A further impact study (unpublished) using data from Scottish Water showed that: In the 6 months before the water supply improvements there were 21 raw water positives and 16 final water positives, whereas in the 2 years following improvements there were 2 raw water positives and 1 final water positive for cryptosporidia.



Photo: Andy Wells



*C. parvum* is also an important endemic, or production-limiting, disease of cattle and sheep, however, there have been few studies to date that have measured the long-term effects of cryptosporidiosis. A recent PhD student at Moredun, Hannah Shaw, developed a scoring system for calves which had clinical cryptosporidiosis in early life, through to weaning at 6 months. She then compared the growth rates of the clinically scored calves to calves on the same farm and of the same age which did not have clinical disease. Results showed a significant weight difference of 34 kg between the two groups at six months of age and an economic cost, based on direct costs only, of £130 per affected calf at the point of sale (Shaw et al, 2020). While the true costs of disease also include indirect costs (e.g. extra labour and opportunity costs) this farm trial provides some evidence of the significant economic losses of cryptosporidiosis on a "typical" Scottish beef unit.

I would like to thank Dr Beth Wells for her help in developing this story.

On a national scale, the work highlighted the importance of a "One Health" approach to solve issues arising from zoonotic pathogens (these are organisms which pass from animals to humans or sometimes vice versa), but it also illustrated the benefits of catchment management in water safety. Prior to this, water companies had concentrated on treatments in the final stages of water provision, whereas following the Moredun study, there was a step change to better catchment management and engagement of all concerned. Water companies in dialogue with Moredun included Scottish Water, Welsh Water, Northumbrian Water and Wessex Water. Discussions with the New South Wales, Australian catchment management team gives an indication of the global reach of this scientific approach!

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Photo: Hannah Shaw





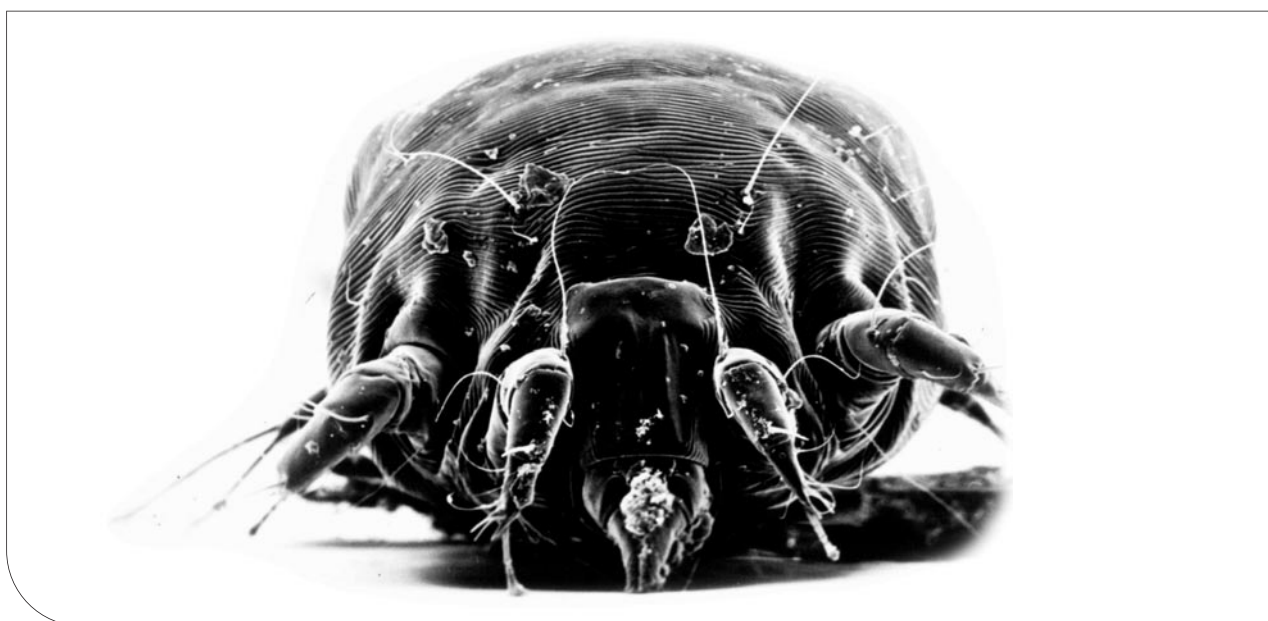
## Sheep Scab

Moredun scientist, Dr John Huntley, played a major role in defining the problem of sheep scab, caused by the mite *Psoroptes ovis*, to global sheep production and contributed to a number of research lines which were subsequently and successfully delivered (<sup>1</sup>van den Broek and Huntley, 2003).

Establishment of a scab mite infestation is the result of a complex interaction between the mite and its host. The hypothesis explored was that the disease might result from allergic substances (protein allergens) produced by the mites, which are then either secreted or excreted onto the skin, causing hypersensitivity and inflammatory reactions in the host. These reactions, which result in the production of a serous exudate on the skin, are conducive to the establishment and maintenance of mite populations then, in turn, result in further skin irritation and subsequent wool loss in sheep. Amongst the many mite allergens involved in triggering this host reaction, one major allergen was identified and termed Pso o 2 due to its similarity to the house dust mite allergen, Der p 2.

One of the major challenges in controlling sheep scab identified by farmers and vets was the need for a diagnostic test, which was not based solely on clinical signs and skin scrapings, both approaches being difficult and time-consuming to perform in the field, especially in

remote farming systems. In 2011, <sup>2</sup>Nunn et al, developed an ELISA test which could be used on blood samples and which would identify early disease and latent (hidden or non-obvious) infestations caused by small numbers of mites. The ELISA test was based on a recombinant (molecularly constructed) version of Pso o 2 and was shown to be an efficient diagnostic test with only very small percentages of false negatives and false positives. Results from the blood test showed that sheep scab cases could be detected within 2 weeks of an infestation and prior to clinical signs being recognised. It was important to then investigate this approach in a commercial setting, but one where movements of sheep could be recorded accurately and flocks tested regularly. A team of Moredun scientists, working with NFUS and the local veterinary practitioner on the Isle of Mull, the late Theresa Wade, showed the utility of the blood test through monitoring of sheep movements on and off the island, demonstrating the potential for reduction of scab in regional areas. The possibility of eradication of sheep scab always being kept in mind!



*Psoroptes ovis* – the sheep scab mite – close up!

Treatment for sheep scab has become more problematic over time, with the use of organophosphate-based sheep dips being associated with potential adverse effects in humans handling sheep and to the environment. For similar reasons other similar products have since been withdrawn from the market. Farmers have generally switched to using the macrocyclic lactone group of injectable drugs (moxidectin, ivermectin, doramectin) for scab control, however, <sup>3</sup>Sturgess-Osborne (2019) showed for the first time that some populations of mites in the UK had become resistant to all three compounds. This growing problem was exacerbated by the routine use of the macrocyclic lactones to treat gastrointestinal parasites in lambs, an example of unintentional interactions in flock health treatments. The attraction of a vaccine approach was clear although the difficulties in generating protection against ecto-parasites (in this case mites living on the surface of the host) had been shown by other scientific groups to be very challenging.

The first protective fractions of *P. ovis* used in a prototype vaccine, demonstrated that vaccination was possible but highlighted that the fractions were complex and difficult to purify and harvest in sufficient quantities for a commercial vaccine (<sup>4</sup>Smith and Pettit, 2004). With modern molecular technologies under rapid development, a recombinant subunit vaccine was prepared containing 7 different antigens. These were tested in experimental pen trials where mites were introduced directly onto the skin of vaccinated and unvaccinated sheep, with encouraging results showing a 63% reduction in the size of skin lesions in vaccinated individuals (<sup>5</sup> Burgess et al, 2016).



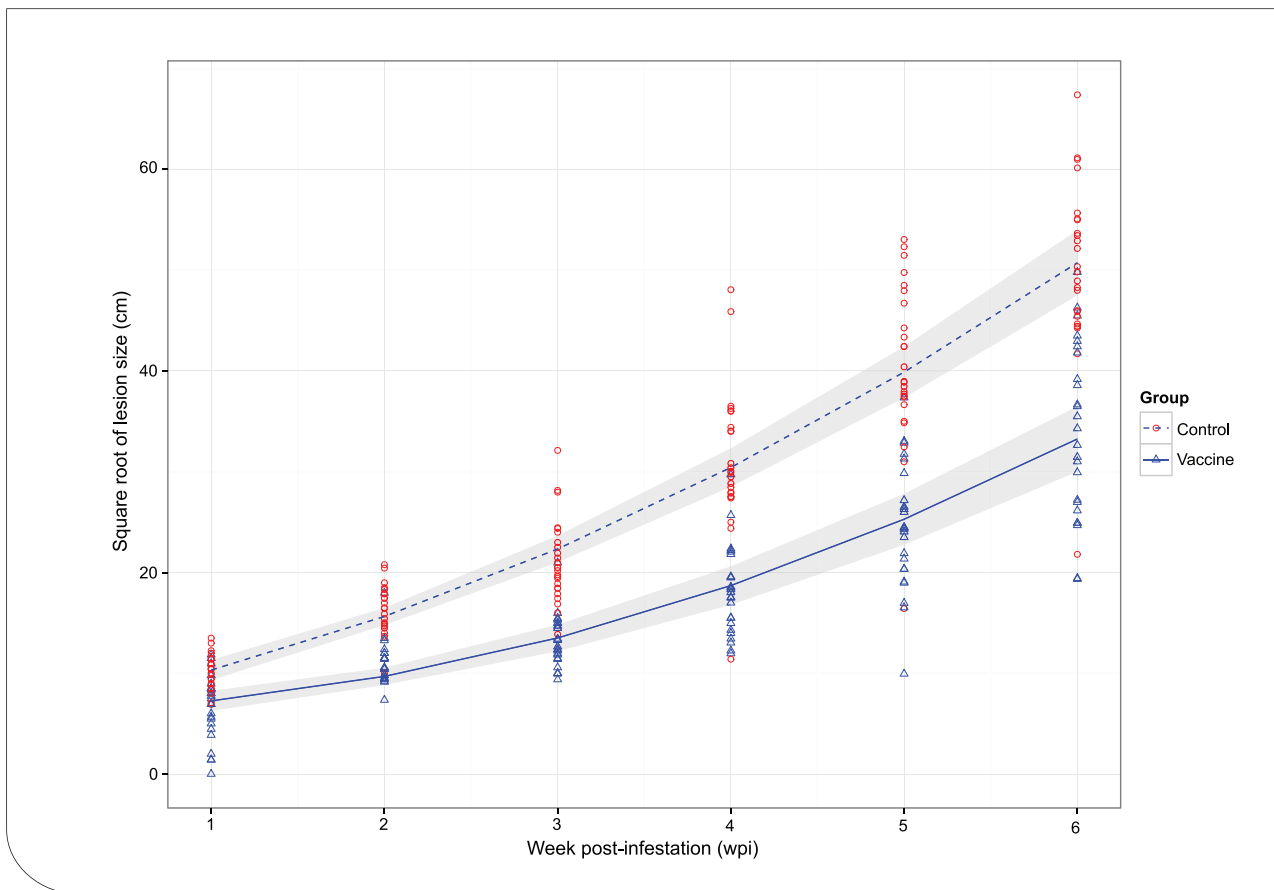
Moredun has always encompassed the ethos of following a pipeline from research to commercialisation where possible and scientists, realising that it is easier and cheaper for vaccines to have fewer antigens, developed a refined prototype vaccine consisting of a cocktail of just 3 antigens. This combination further boosted the efficacy of the vaccine with an 80% reduction in lesion size in vaccinated sheep. The research also improved the method of testing the vaccine experimentally and for more accurately determining vaccine efficacy. This was achieved by establishing mite infestations in a small number of "index" sheep and then introducing them to uninfected sheep in the same pen. The mites transfer very effectively among sheep, as they do on farm, providing a "more natural method of disease transmission".

In the era of Whole Genome Sequencing, <sup>6</sup>Burgess et al (2018) published the first, 63.2-Mb *P. ovis* genome that encodes 12,041 protein-coding genes. This information is the springboard on which future research into *P. ovis* depends, including identifying new diagnostic and vaccine targets, new means of intervention (including drug targets), mapping of resistance genes and future on-farm management of this growing problem.



Sheep showing obvious clinical signs of sheep scab infestation.

Photo: Professor Neil Sargison, University of Edinburgh



The graph shows the size of scab lesions in vaccinated sheep (blue line) and unvaccinated sheep (red line) across a 6-week time course of infestation with *Psoroptes ovis*.

Moredun's leading role in designing disease control programmes for Scotland, where sheep scab is notifiable, and elsewhere in sheep-rearing parts of the UK, Ireland and beyond, is exemplified by the success of Dr Stewart Burgess in attracting Defra/Veterinary Medicine Directorate funds for a study on how best to deal with the issue of antiparasitic resistance in sheep scab mites, involving eight partners from both academia and commercial companies, and EU funds for a project (EASI-GENOMICS) to further refine the *P. ovis* genome and better understand the mechanisms of mite resistance.

I am sorry to say that at the time of writing, we heard the very sad news of the death of John Huntley on the 4th October 2020. He is very much missed at Moredun as a scientist, friend and colleague.

I would like to thank Dr Stewart Burgess for his help in developing this story.

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## Moredun's role in the farming community: Past, present and future



### Thoughts from Ian Duncan Millar, livestock farmer and Chair of The Moredun Foundation

My interest in Moredun and its work was initially aroused through my wish to ensure the best I could for and from my sheep and cattle here at Tirinie. This led me naturally into disease management, which initially was around Enzootic Abortion of Ewes (EAE) on our own farms, but also on our neighbours in the wider Highland Perthshire community. That wider health group then enabled a more open discussion about sheep scab and increasingly, other diseases such as OPA came into the conversation.

Tirinie lies next to the confluence of the Rivers Tay & Lyon with around 130 acres of easy light cropping ground and the rest of the 500 acres we farm (owned and rented) is grassland or rough grazing, meaning our business revolves around traditional livestock. We run 500 ewes & 35 cows, augmented by young stock from other family units further upstream, all geared towards efficient grass and forage finishing of all young stock.

It dawned on me quite early on that managing our stock for the best health, or at least initially, to minimise losses through death and ill health, was actually very cost effective, as well as emotionally less upsetting... So when the opportunity arose to join the board of MRI it seemed a natural progression, and to spend more than eight years as chair of The Moredun Foundation has been a pleasure and honour that was never in the career plan all these years ago!



In 1920 the intention of Moredun's founding fathers was to stop so many of their sheep dying, and from that early beginning our scientists have made so many wonderful discoveries that farmed animals now live long, healthy and productive lives compared to 100 years ago. The drive to improve animal health is as strong today as it was then, only the tools and the language have changed. Moredun now focusses on Diagnostics, Vaccines and Disease Management, which are all connected, and all directed to improve the health of the stock concerned. Interestingly that fits as well now, or perhaps even better than it did 100 years ago, as it addresses the key concerns of the 21<sup>st</sup> century. Better health means better lives and welfare for the stock concerned, a better and more efficient biological system that is farming, optimisation of our impact on the environment in which we operate, a reduced carbon footprint, and a more sustainable industry that uses less antimicrobial drugs, thus contributing to addressing antimicrobial resistance in both farm and human populations. Importantly these same goals also lead to the opportunity for farmers themselves to benefit through greater profit.



As the century has progressed the range of pathogens being studied has increased, along with the species who benefit, and in more recent times the ability of our skilled scientists, has been harnessed to the benefit of human medicine, both through zoonotic disease and through knowledge and understanding that is transferable.



“No man is an island” is a quote from John Donne in the 16<sup>th</sup> century, but was never truer than today. Moredun has a huge opportunity to contribute to human kind through a better understanding of disease pathogens and their interaction with their hosts and the environment. This understanding is absolutely central to the future of “One Health”, and of the “Green Recovery” that is needed for the benefit of future generations that inhabit our planet. And as for my wee corner: If I use the understanding and discoveries from Moredun, I can ensure my stock are fit and healthy. I will have less waste, and use less medicines that might impact other species, and will be able to leave a few corners around the farm where other creatures can co-exist, while still making a meaningful contribution towards food production and maintaining a viable business.



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