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Moredun's Centenary Science Stories

Volume 2

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introduction

Here is the second volume of Moredun's Centenary Science Stories which I am pleased to send to our Moredun Foundation members and supporters during the second main COVID-19 lockdown in Scotland in the early months of 2021.

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 to progress and we are now in 2021 when we will be holding some special events to celebrate 100+1 years! Moredun staff are proud that our mission remains today, as it has always been "Promoting animal health and welfare through research and education".

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.

Moredun has consistently focused its research on infectious diseases of livestock mainly as these have been identified by Foundation members, livestock farmers and vets, as the most challenging areas requiring solutions. Increasingly, Moredun scientists are exploring the interactions among infectious diseases of livestock with wildlife and the environment. As Scotland continues to explore "Land Use" across the country, Moredun's work is contributing to sustainable livestock farming - producing high quality, safe food from our land while protecting our unique ecosystems and biodiversity. Another direction of travel for the Moredun Group is a widening of the animal species being studied. Originally Moredun worked on diseases of the iconic Scottish livestock species, mainly sheep and beef cattle. Over the years, dairy cattle, pigs and poultry, have been added to the list, especially where experience of pathogen types has allowed transferable approaches to research. Most recently, Moredun has established expertise in aquaculture both in terms of innovative research, predominantly on vaccines, as well as commercial contracts studying novel products for EU registration. Finally, Moredun's most recent contributions are to the human population through provision of testing for SARS-Cov-2, the cause of COVID-19, through biosafety testing of the commercial vaccines against COVID-19 and through innovative work on the virus in animal models - more of this in future stories.

Moredun focuses on outputs, outcomes and impact from its work on vaccines, diagnostic tests and disease control programmes due to the combination of specialized infrastructure and facilities which allow work on pathogens dangerous to humans and animals to take place, and its skilled scientific and support staff who are able to rise to new challenges. I hope you will see evidence of this in reading some more of Moredun's Centenary stories.

As I finish this introduction, I would like to acknowledge the huge ongoing efforts of staff to continue their valuable work, with some based on site at the Pentlands Science Park, while others are working from home.

Professor Julie Fitzpatrick
Scientific Director and Chief Executive

Liver and Rumen Fluke

Liver fluke is a growing problem across Scotland and the rest of the UK. Caused by the flatworm parasite, *Fasciola hepatica*, the distribution of the disease in sheep and cattle has moved from mainly being a west-coast problem to being a country-wide one.

The reasons for this are many and varied: increasing wetter and warmer periods for development of the mud snail intermediate host, changing farming patterns including less drainage of wet ground thus increasing the snails' favourite habitat, and increased drug resistance in fluke which then travel inside their animal hosts to different parts of the country through animal movement.

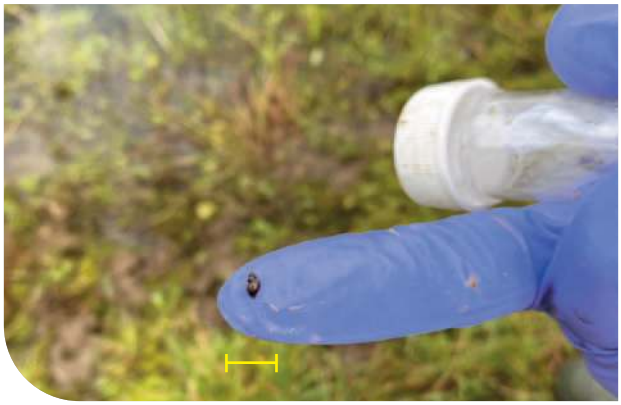
Adult liver fluke.

Photo: Sinclair Stammers, https://micromacro.co.uk/

Above: A good day for snails!? Moredun scientists sampling on the Caerlaverock Estate, Solway Firth.

The scientific father, or even grandfather, of fluke research, at least in the UK, is Dr C.B. Ollerenshaw, who featured in the film "Liver fluke in Great Britain", a public information film for farmers produced in 1965. A main plank of this communication was Ollerenshaw's Index for Liver Fluke Forecasting, based on the prevailing weather patterns, and to this day it forms the basis of the UK mainland's predictions of liver fluke disease. Dr Ollerenshaw has met up in recent years with Dr Philip Skuce, Moredun's resident fluke expert, where the two discussed the progress of research on this important and still common parasite. Moredun's recent innovation in Knowledge Exchange has included the creation of animated films, designed to inform the farmer, vet and other interested parties, about the pathogens, host animals and methods of treatment and control. "Fight the Fluke" is a good example and is available at https://vimeo.com/268388860/d0250dd844

Moredun's fluke research has mainly focused on the area of diagnostics. The most frequently used method currently relies on faecal egg counts (FECs), which have their limitations, as eggs are only produced by adult fluke (at least 10-12 weeks old), however, most of the clinical and pathological effects of fluke are caused by the immature fluke burrowing into the liver tissue of infected animals. Experimental studies by researchers in Spain had shown that coproantigens of fluke showed promise as an early marker of disease (coproantigens are parasite-specific proteins secreted by fluke as they migrate and feed, and which appear in faeces, representing a simple, non-invasive sampling substrate). A commercial coproantigen ELISA test came onto the market in 2012 (BioX Diagnostics, Belgium) and Moredun scientists were amongst the first to evaluate it in the field. Experimental challenge studies conducted at Moredun had shown the test to be capable of detecting fluke several weeks before eggs appeared in the faeces, however, field studies conducted by the Moredun fluke group in 'fluke central', SW Scotland, showed that faecal samples were positive by coproantigen diagnostics and FEC at approximately the same time ([1]Gordon et al, 2013). This result, and similar observations by others, led to the development of a more sensitive version of the coproantigen ELISA and this is now increasingly used as a more rapid and convenient field test for fluke, and also as the default choice for testing the efficacy of drug treatments for fluke (flukicides).



Liver fluke intermediate host mud snail, Galba truncatula. Scale bar = 10mm

There is a very limited array of flukicides which can be used to treat fluke, with the most effective for all stages of liver fluke being triclabendazole (TCBZ). The utility of this product has resulted in it being used routinely on farms across the world, resulting in a high level of resistance developing in fluke across most farming systems investigated to date. [2]Gordon et al (2012) conducted a dose-and-slaughter trial in sheep, using the infective stage of fluke derived from sheep, which had active infection in spite of numerous treatments with TCBZ. This provided the first confirmation of TCBZ-resistant liver fluke in the UK, through propagation of fluke surviving TCBZ treatment. The high economic cost of identifying new molecules exhibiting flukicidal activity for ruminant parasites limits this treatment approach, making a vaccine alternative for fluke control highly attractive.

Photo: Pat Bain, Rowett Institute for Nutrition and Health, University of Aberdeen.

Natterjack toad.

Moredun scientists have had considerable success in employing "hidden" gut antigens as vaccine targets for blood-feeding parasites, such as *Haemonchus contortus*. A similar approach was taken with liver fluke, also a blood-feeder, where gut antigens, predominantly cysteine, aspartyl and other proteases were purified from parasite extracts at Moredun ([3]McAllister et al., 2012). The proteins were purified successfully, however, when injected into sheep over a series of trials, they produced no demonstrable protective effect against fluke, indicating the varied and complex immunity associated with fluke infestations. Vaccination against fluke is still one of the hardest nuts to crack of veterinary science worldwide and the subject of considerable continued research effort.

Rumen Fluke: Increasingly, rumen fluke species, have been identified in the stomachs of sheep and cattle, especially in Scotland and Ireland, where the weather is wet (usually) and warm (sometimes). Moredun were first to recognise that the rumen fluke species involved was, in fact, *Calicophoron daubneyi*, an invader from continental Europe and not *Paramphistomum cervi* from wildlife, as the textbooks would have the reader believe ([4]Gordon et al, 2013). Moredun scientists also subsequently found that *C. daubneyi* utilises the same mud snail intermediate host as liver fluke and is often found as co-infections in livestock on the same farms. The pathological and clinical relevance of rumen fluke is still disputed, certainly by farmers, but its increased level or prevalence leaves many unanswered questions. Moredun has recently established an experimental challenge model of rumen fluke in sheep, so that the interactions between parasite and host species may be investigated in more detail.

Moredun's research on liver fluke and rumen fluke has extended to the interactions among grazing ruminant livestock, wildlife species and the environment. This was stimulated mainly by agri-environment schemes which encourage practices such as reduced drainage of farmland to support rare species, including natterjack toads and key wetland birds.

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Photo: Philip Skuce

Adult rumen fluke on the surface of a cow's stomach.

https://www.sruc.ac.uk/downloads/file/4067/skuce_et_al_-_liver_fluke_risk_and_agri-environment_schemes

Addressing issues of sustainable farming along with protecting biodiversity have been possible through multi-disciplinary research conducted by Moredun on Foundation members' farms and in collaboration with agencies including NatureScot, the Wildfowl and Wetland Trust, the Amphibian and Reptilian Conservation Trust, the Game and Wildlife Conservation Trust and the Royal Society for the Protection of Birds. Since 2016, Moredun has been working with local land managers to investigate conservation grazing practices at the Caerlaverock Estate on the Solway Firth. This is specifically in relation to liver fluke risk to livestock grazing on the saltmarsh (merse).

There is a perception amongst farmers that grazing the merse and associated marginal areas may increase the fluke risk to their stock. These areas provide habitat for natterjack toads, which are an endangered and protected species in Scotland. Conservation grazing by livestock helps maintain short grass and open areas favourable for natterjack toads to hunt and breed, therefore, it was encouraging that very low levels of liver fluke and rumen fluke eggs were found in cattle going onto the merse, with no evidence of the intermediate mud snails on the saltmarsh itself. While this seems to be a win-win for conservation farming, the potential consequences of changes in land-use will be an increasingly important focus for researchers in future years.

I would like to thank Dr Philip Skuce for his help in developing this story.

Vaccine studies for *Teladorsagia circumcincta* and *Ostertagia ostertagi*

Parasitic gastroenteritis (PGE) of ruminants caused by a variety of worm species has long been recognised as a major constraint to optimal ruminant production worldwide. While huge gains have been made scientifically in the prevention of viral and some bacterial diseases, worms, as large multi-cellular organisms which reproduce sexually, have evolved to evade host defence mechanisms and to maintain their presence in different livestock populations. Interestingly, livestock diseases caused by gut worms have been identified as one of the priority foci for research and development by many international organisations in both developed and developing countries. The prospect of vaccines against gut worms as a main plank of disease control is attractive as the level of resistance to drugs used to treat worms increases worldwide.

One of the commonest species causing PGE in temperate climates is *Teladorsagia circumcincta*, the brown stomach worm of sheep which has a lifecycle described in the figure below.

Another important stomach worm, *Ostertagia ostertagi*, affects cattle health and production in similar regions of the world to *T. circumcincta* and both species have comparable life cycles.

While preventive management measures can be adopted to address various stages of the parasite lifecycle, the juvenile (late larval) and adult worms present in the abomasum (or fourth stomach), where they develop, mate and produce eggs, are relevant targets for vaccination. These parasites damage the lining of the abomasum reducing absorption of food nutrients causing production loss and eventually diarrhoea in cattle and sheep.

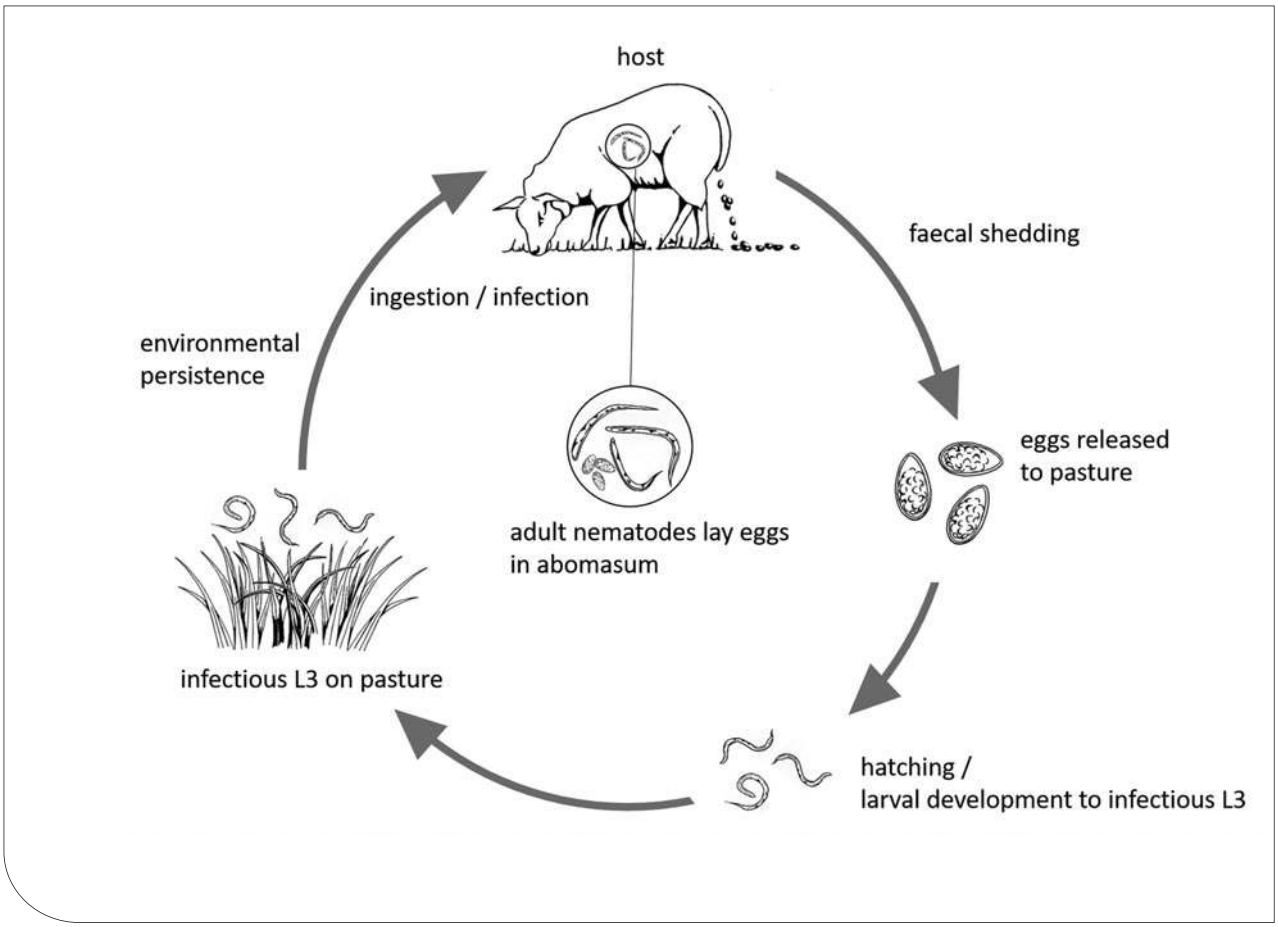
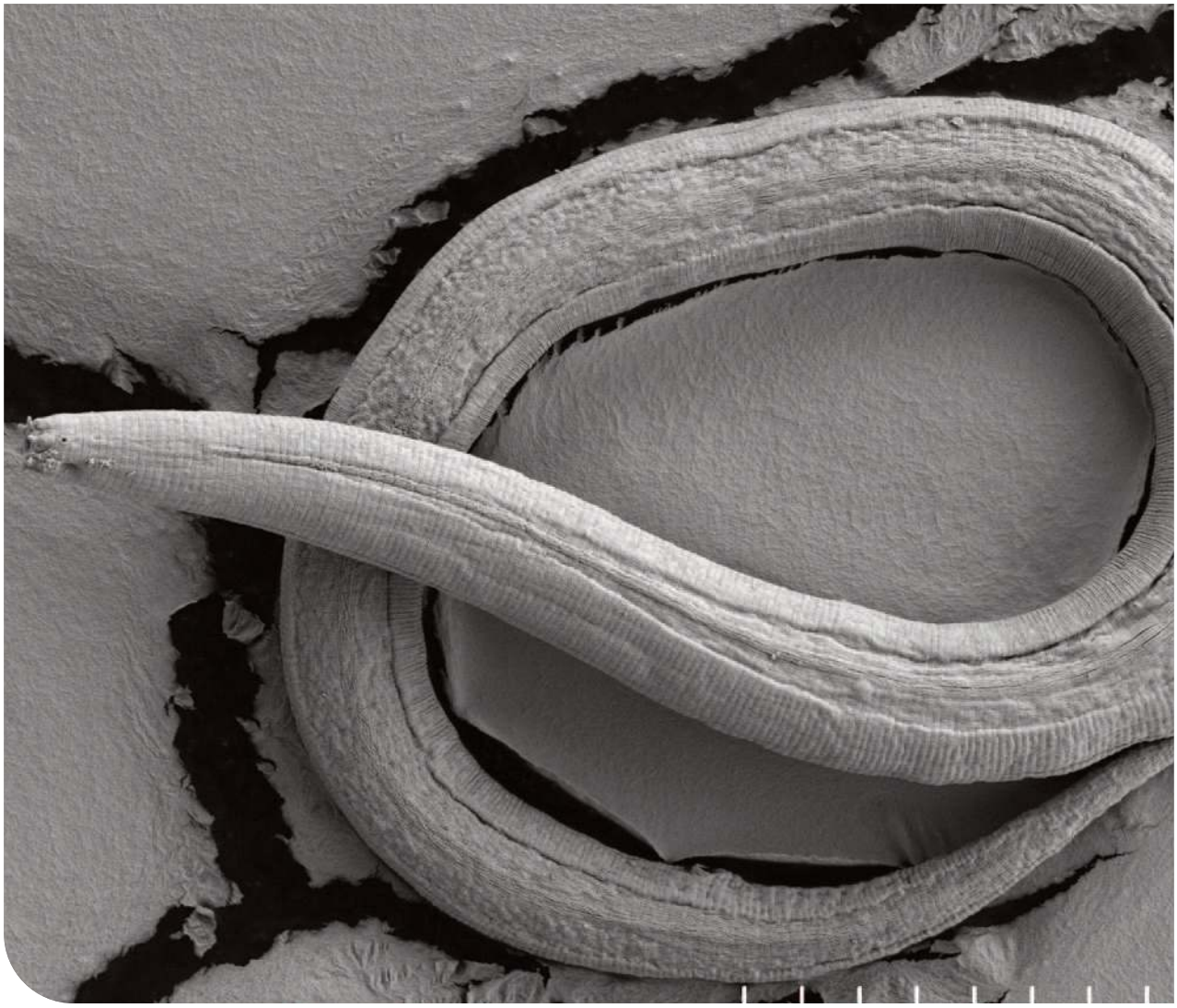


Diagram: Scott Hamilton



A high powered scanning electron micrograph of a juvenile (larval) Teladorsagia circumcincta.

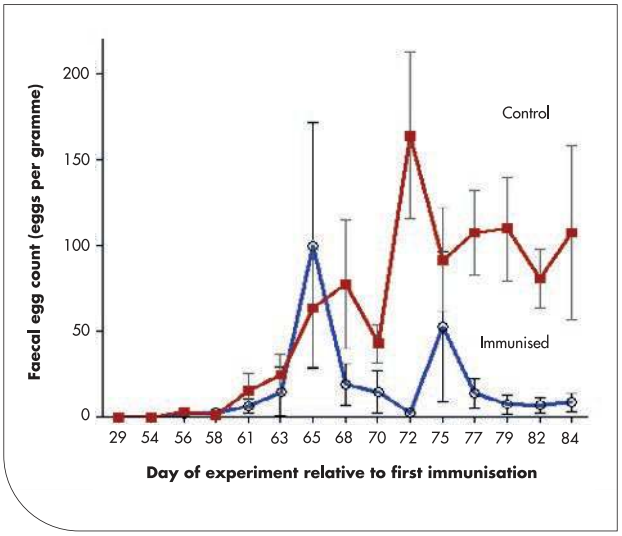
Early studies indicated the importance of the immune response in the abomasum of sheep in protection against these parasites. Lymphatic fluid draining the abomasum of sheep which were immune to *Teladorsagia circumcincta*, was shown to contain both immune cells (lymphoblasts) and antibody (IgA). When this fluid was injected into the bloodstream of susceptible recipient sheep, it stunted worm growth and/or reduced the worm burden ([1]Smith et al., 1986). This showed that the resistance acquired by sheep after prolonged exposure to *T. circumcincta* could have an immunological basis, suggesting that vaccination might be a feasible approach to control.

Different stages of T. circumcincta have adapted to survive and persist in very different environments, both on pasture and within the host animal as shown in the lifecycle above. The fourth larval stage, the L4, is found either dwelling inside the abomasum or in its lining. In order to identify the molecules which are important for larval survival in different host "niches", analysis of the transcriptomes (all the messenger RNA molecules expressed from the genes of an organism) and proteomes (proteins expressed by an organism) of the larvae was undertaken by Moredun scientists. Results showed that many of the genes were expressed at higher levels in the mucosal-dwelling larvae when compared with those living in the lumen, with one, named Tck6, capable of affecting T cell immunity ([2]McNeilly et al., 2017). This study provided evidence that niche- as well as stage-specific analyses of parasite transcriptomes are important in identifying parasite molecules of potential importance for survival within the host.

Attempts to produce recombinant (artificially constructed rather than being derived from the native organism) subunit vaccines against parasites in many animal species, including humans, has proven very difficult for scientific groups worldwide. Moredun scientists selected a number of potential vaccine candidate antigens from *T. circumcincta* for experimental vaccine trials on sheep. The antigens chosen included four which had been identified as targets of mucosal (IgA) antibody against L4, the larval stage most closely associated with the abomasal tissues of infected sheep; one antigen from the canine hookworm which had been shown to induce protection in dogs and which *T. circumcincta* has an equivalent with a similar structure, and three antigens produced by the larval stage which have molecules with potential to suppress the immune response of sheep. This approach attempted to induce immunity to antigens which would protect the sheep against the parasite, while reducing responses that might maintain the infection. The vaccine "cocktail" plus an adjuvant (a component which enhances immunity generally) was administered three times, following which the sheep were given a continuous challenge with infective larvae to mimic the situation in sheep grazing infected pasture. The outcome of these trials was measured by faecal egg counts (FEC) and by counting the number of worms in the stomach at post-mortem. The trials provided the best evidence recorded to date for any *T. circumcincta* recombinant vaccine experiment with up to 70% fewer FEC in vaccinates than in the control sheep, and 75% fewer adult worms in vaccinates than in control sheep ([3]Nisbet et al., 2013). The scale of these reductions is considered to be around the level of protection required to enable breaking the parasite life-cycle in the field.

Progress has since been made in reducing the number of recombinant antigens included in the vaccine cocktail. This is to decrease the complexity of manufacturing the vaccine and to reduce costs. These "reductionist" studies take many months to perform and involve very significant work in collecting both worms and larvae post-mortem in order to assess vaccine efficacy – the adult worms are small and the L4 larvae even smaller, thus the exercise is like looking for a needle in a haystack but messier!

Research to develop roundworm vaccines has been very well supported by Scottish Government funding over the years. The international excellence of Moredun scientists in this area was recognised through the award of two, large (9m Euro) grants from the EU, called "Paravac" and "Paragone". These projects involved collaborations with multiple partners across Europe and further afield.



The graph shows the faecal egg counts from sheep immunised with the T. circumcincta vaccine cocktail (shown in blue), compared to non-immunised control sheep (shown in red).

There is the enticing prospect that certain recombinant vaccine antigens identified for *T. circumcincta* might also protect against *O. ostertagi*, or other species of roundworms which affect ruminants. Moredun's future work in this area will therefore focus on exploiting our current vaccines to maximum effect against multiple species while also evolving these vaccines to make sure that they have the highest levels of efficacy. The "holy grail" of the ideal combination vaccine effective against multiple worm species remains evasive however significant progress is being made.

I would like to thank Al Nisbet and Tom McNeilly for help in developing this story.

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Photo: Shutterstock.com

Progress towards eradication of Bovine Viral Diarrhoea Virus in Scotland

Among the countries of the UK, Scotland has led the way in applying current technologies and knowledge exchange in efforts to eradicate the important endemic disease of cattle, caused by Bovine Viral Diarrhoea Virus (BVDV), a pestivirus.

The BVD Eradication Scheme was started in Scotland in 2010-2011 with a voluntary phase where farmers arranged herd tests for BVDV. Mandatory testing started in February 2013 with financial contributions from the Scottish Government and from livestock farmers.
The Eradication Scheme was based on testing bulk milk tanks on dairy farms and blood samples from beef and dairy cattle and their calves for antibody against the virus (by an ELISA test) and for the virus itself, by polymerase chain reaction (PCR) or by an ELISA which detects the virus antigen. By January 2014, a BVDV herd status was required to market calves, followed by individual testing of calves in herds which were deemed to have "not-negative" status, at the beginning of Phase 4 in June 2015. This meant that cattle found to be positive for BVDV were declared "not fit for sale" and affected cattle could only move off-farm direct to slaughter.
Phase 5 of the Eradication Scheme was introduced in January 2019 with continued herd testing, including use of official tags (that provide tissue samples) for detection of virus in young cattle. Other measures included banning movement of cattle into BVDV-infected herds and the introduction of compulsory BVD investigations (CBI) of herds with long-term not-negative status. This has resulted in a very low number of cases of BVDV in Scotland, reducing from more than 40% of farms being "not-negative" in 2010 to 9% in 2020, making eradication within the next few years a real possibility.

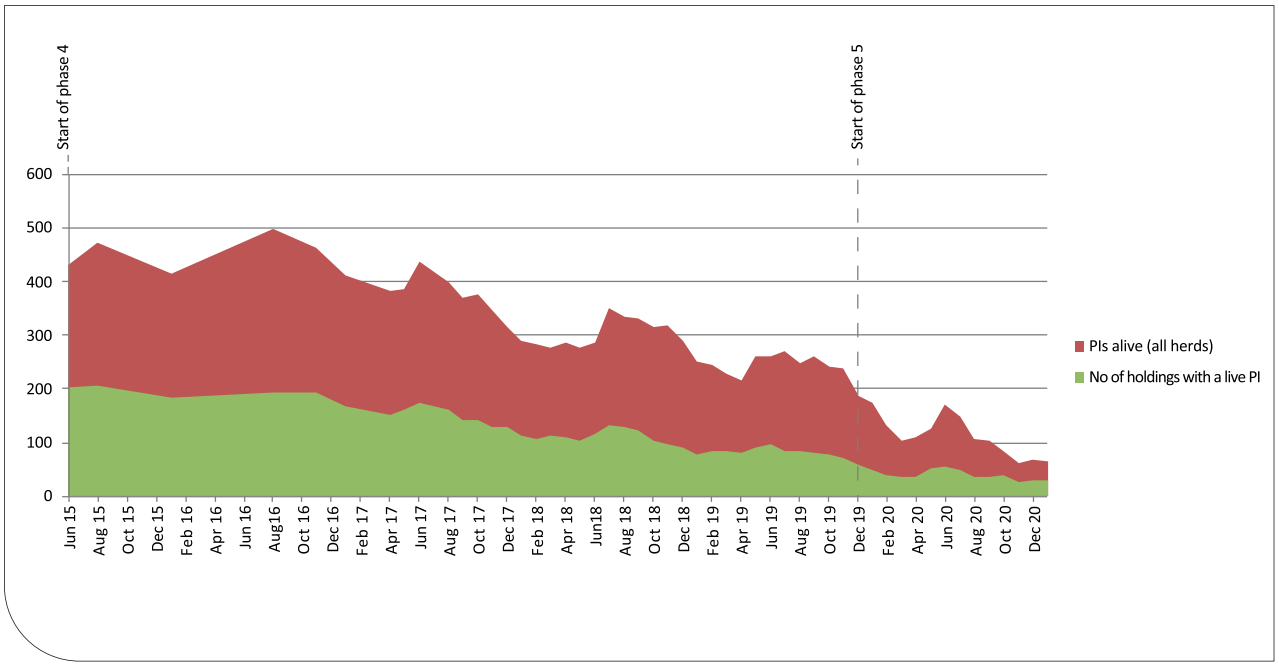
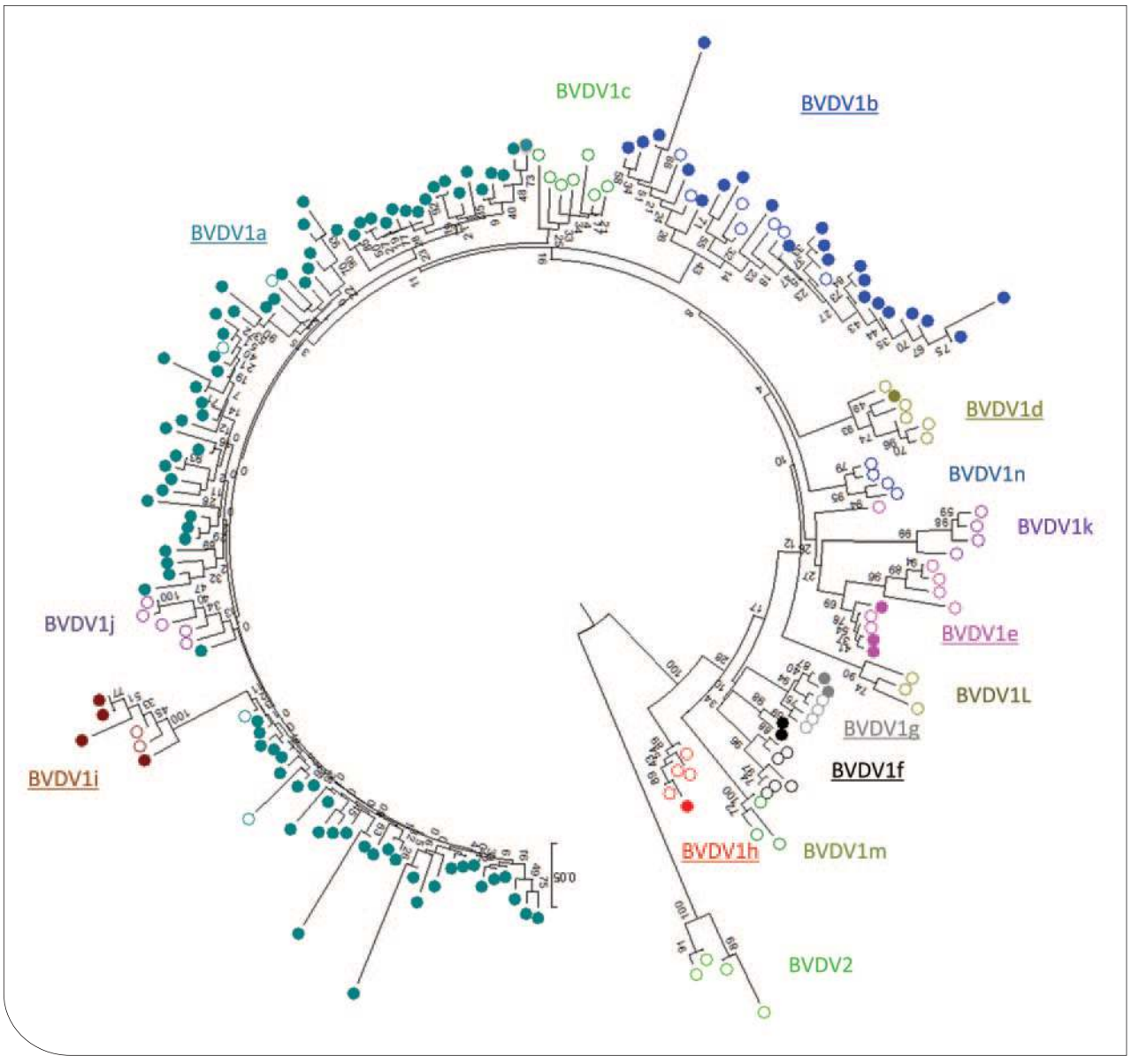


Figure courtesy of the Scottish Government BVD policy team.

Progress of the BVDV eradication in Scotland. At the end of 2020 a total of 6,179 PIs had been identified, with 66 live PIs remaining in 30 herds (8,349 breeding herds).

Moredun's contribution to BVDV research has been considerable. Dr Peter Nettleton, for many years the lead virologist at Moredun, played an important role in understanding the pathogenesis of BVD (how the virus infects and damages organs and tissues) and developing improved diagnostic tests. This included work on BVDV and a closely associated sheep disease, called Border Disease (BD), and caused by Border Disease Virus (BDV). The virus antigen ELISA relied on monoclonal antibodies applied in a double layer in the laboratory-based test. This approach increased the sensitivity of the test. In other words, it was better at diagnosing disease when it was present, with few false negatives ([1]Entrican et al, 1994).

Peter Nettleton and Moredun scientists were also involved in the molecular analysis of BVDV and BVD strains which led on to the development of a real-time one step polymerase chain reaction (RT-PCR) assay which can detect and identify BDV and BVDV types 1 and 2 in blood samples. This research showed that the RT-PCR test was more sensitive than virus isolation and that the typing of the virus by this method agreed with whole genome sequencing ([2]Willoughby et al, 2006).



This figure shows a "genetic tree" of BVDV types and subtypes from Biobank samples. Different colours are used to differentiate these. Open circles represent reference sequences and closed circles are Biobank samples. BVDV types found in the Biobank are underlined. Each dot represents a virus isolate and the closer they are to each other on the tree, the more similar they are. The predominance of BVDV1a (teal colour) in the UK is clear from this example.

Image courtesy of NADIS and accredited to Phil Scott, DVM&S, DipECBHM, CertCHP, DSHP, FRCVS.

Young calf persistently infected with BVD (right) compared to similarly-aged normal herd mate.

BVDV causes multiple clinical signs and syndromes in infected cattle. When cattle are infected via contact with other cattle, usually by close nose-to-nose interactions, the signs are usually short in duration but can include immunosuppression, diarrhoea, abortion, and poor fertility. In dairy cattle, a sudden drop in milk yield can be seen at the bulk tank level, especially if a number of cows are infected simultaneously. BVDV, however, causes its most devastating effect when pregnant cows are infected with the virus in the first trimester of pregnancy. Calves born to these cows are usually persistently infected with BVDV because they were unable to mount an immune response against the virus when they were early foetuses. These PI calves act as "virus factories" and are the main source of continuing infection in herds. Persistently infected cattle may appear normal but some show poor growth and a few develop Mucosal Disease, with clinical signs such as oral and interdigital ulcers and diarrhoea, ultimately leading to death. Peter Nettleton was often heard to say, rather in awe, "How can a small virus with only six genes manage to cause so much damage to cattle".

A "Biobank" of samples from BVDV infected cattle was created at Moredun by collaboration between the EPIC Centre Of Expertise for animal disease outbreaks and Scottish approved laboratories, in support of the Scottish BVD Eradication Scheme ([3]Russell et al, 2017). These samples were sequenced to identify viral types and subtypes present. From more than 5000 samples sequenced to date, 8 different BVDV type 1 subtypes have been identified while no BVDV type 2 samples were detected. Many samples carried virus with identical sequence in the region analysed and these were often found in single geographical locations, suggestive of outbreaks of a single virus strain. In contrast, a wide variety of sequences were detected among samples collected across Scotland and the UK, illustrating the diversity of BVDV strains.

Performing this analysis allows monitoring for any changes in BVDV strains as the eradication scheme progresses and can potentially track BVDV strains that might appear in new outbreaks. It is interesting that while the BVDV1a strain remains the most commonly identified, the BVDV1b strain has increased in frequency by 30-40% since 2016. These sequencing technologies provide a resource that can be used to analyse the movement of BVDV strains both within Scotland and between Scotland and other nations, particularly in the latter stages of the Scottish Eradication Scheme, and so inform the advice available to both livestock keepers and policymakers.

I would like to thank Dr George Russell and Dr Jenny Purcell (Scottish Government) for their help in developing this story.

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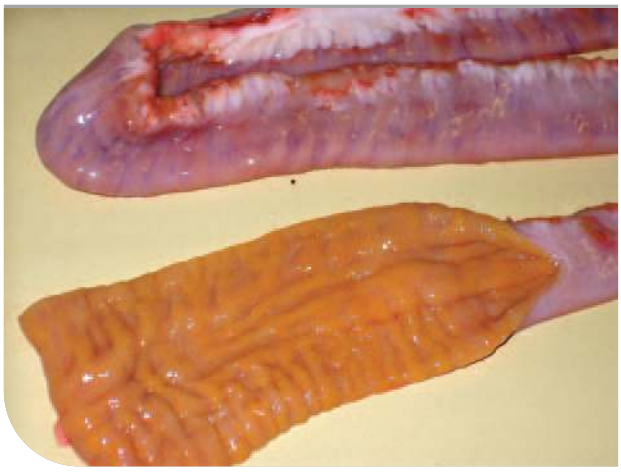
Johne's Disease

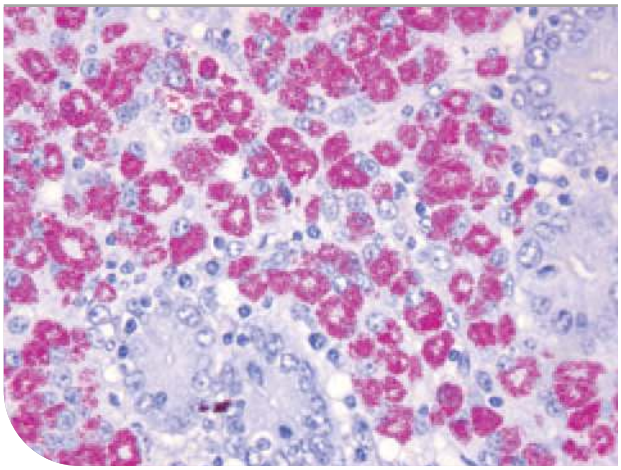
When Moredun scientists attend agricultural events or roadshows, the most common question they are asked is "when will there be a better way of diagnosing and preventing Johne's disease?" Farmers with cattle herds are first in line to ask, followed closely by those keeping sheep and goats, and Dr Karen Stevenson and colleagues are always ready to provide advice. In spite of many years of research endeavour and much knowledge gained on the establishment and progress of Johne's disease in animals, and of the pathogenic bacteria itself, diagnosis and prevention remain challenging and difficult problems.

Johne's disease is caused by *Mycobacterium avium paratuberculosis* (MAP), a thick-walled bacterium, which is very successful at surviving in the environment. Johne's is a disease principally of ruminants, spread primarily by the faecal-oral route, especially to young offspring. The infection causes severe thickening of the lining of the gut resulting in poor absorption of food and severe diarrhoea.

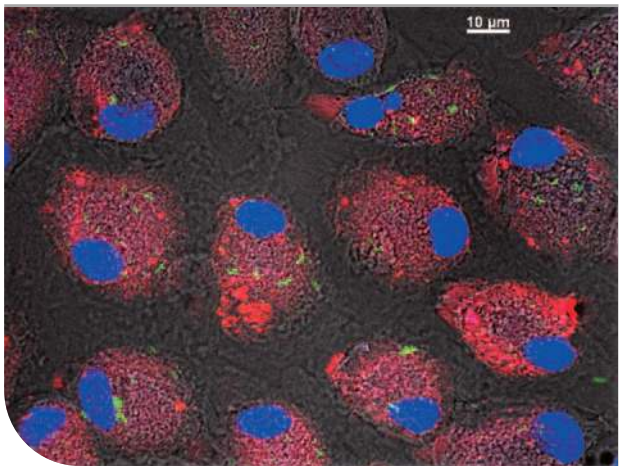


This image is of bovine intestine showing a thickened, reddened and ruched lining associated with Johne's disease.

Moredun provided the first confirmation that there are two closely related organisms responsible for Johne's disease in sheep ([1]Taylor, 1945). These were not genetically distinguished until the 1990s by a New Zealand group and, subsequently, Moredun scientists conducted many studies over the years to fully characterise these different strain types. Various protein-based and molecular techniques were deployed including most recently whole genome sequencing, which identifies the DNA components of the organism ([2]Bryant et al., 2016). This strain characterisation is very important for epidemiological studies including understanding transmission routes and tracing sources of infections. Other epidemiological studies conducted by Moredun investigated MAP infections in free-living deer and, in collaboration with SRUC, provided the first evidence of MAP causing paratuberculosis in rabbits. This led to numerous studies of MAP infection in a variety of wildlife, emphasising the importance of wildlife reservoirs when considering control options on farm.



This image shows MAP bacteria, stained with fluorescent dye, packed inside macrophage cells.



This is a microscope image of MAP bacteria, stained green, invading macrophage cells in the tissues of the gut. This inflammatory cellular response disrupts absorption of food resulting in diarrhoea and wasting.

Neil Gilmour and his Moredun colleagues published several key papers regarding the pathogenesis of Johne's disease following experimental oral challenge of calves ([3]Gilmour et al, 1965). Since then, Moredun has continued to develop experimental animal models for MAP infection in deer, sheep, cattle, rabbits, mice, and currently a gut-loop model in calves, expanding knowledge on interactions between the bacterium and animal host particularly the host immune responses.

Another area of research in which Moredun scientists have been active for decades is vaccination. MAP vaccines comprise inactivated whole bacterial cells, which induce partial immunity measured by reduced bacterial shedding and clinical lesions. One key Moredun study showed that the level of immunity was correlated with the hypersensitive response induced by the vaccine. Re-vaccination was expected to boost immunity in animals with waning hypersensitivity and was widely practised. In 1973, [4]Gilmour and Angus carried out re-vaccination experiments in sheep and found that re-vaccination 11 months post initial inoculation did not increase immunity to experimental challenge with MAP and could in fact result in deterioration of the immunological and clinical status of the animal. Today, Moredun continues working in the field of vaccination by constructing targeted knock-out mutants (removing specific elements of MAP DNA in the laboratory) in an attempt to produce a live attenuated vaccine.

Farmers have access to Johne's disease screening programmes, which offer different control options, allowing the appropriate control strategy to be implemented for individual farms. Control can only be achieved through a combination of biosecurity measures, improved hygiene and regular testing. Testing is generally based on identification of antibodies against MAP in either milk or blood samples to identify infected animals. Although considerable progress has been made in reducing disease levels on many farms, it is recognised that antibody tests have poor sensitivity (the ability to detect disease when it is present) for identifying subclinically infected animals (those with no visible abnormal signs). To detect these animals, a more effective diagnostic test that can be used early in the infection process is a priority.

Moredun started research in the area of molecular diagnosis of Johne's disease in the 1990s, developing methods for extraction of genetic material and molecular polymerase chain reaction (PCR) detection of MAP in tissues and faeces. Novel technologies such as screening expression libraries (collections of clones each expressing a single protein or part of a protein) and comparing the proteomes (the complete set of proteins produced by an organism) of MAP with closely related mycobacteria were used to identify MAP-specific genes and proteins. This enabled development of a diagnostic test to detect the early cell-mediated immune response (the immune response of cells not involving antibodies) to MAP infection in sheep and cattle ([5]Hughes et al., 2017). While this test is currently not suitable for commercial or field purposes, it continues to provide information to increase scientific understanding of this complex disease. Johne's disease remains a key priority for research at Moredun.

Moredun scientists have also applied their expertise to other mycobacterial diseases including tuberculosis caused by *Mycobacterium tuberculosis* in humans and *Mycobacterium bovis* in cattle. In collaboration with the University of Edinburgh, they were the first to diagnose leprosy caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis* in red squirrels in the British Isles.

I would like to thank Dr Karen Stevenson and Dr Craig Watson for their help in developing this story.

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Ovine Pulmonary Adenocarcinoma - "Jaagsiekte"

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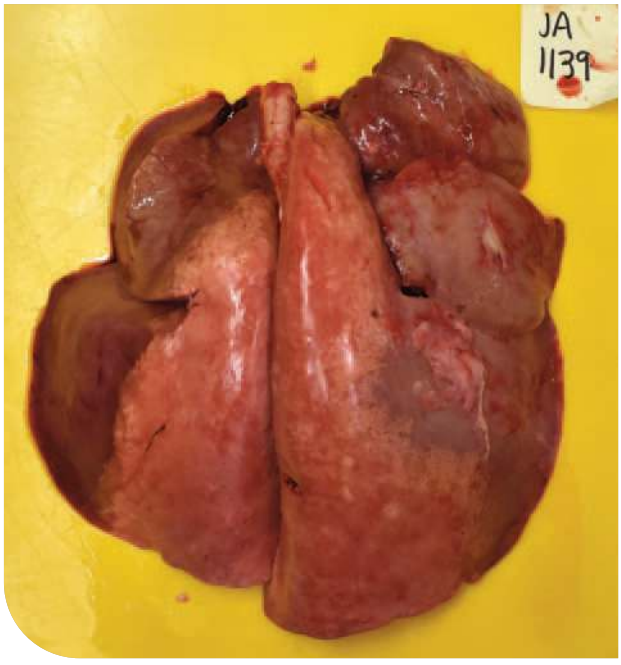
Ovine Pulmonary Adenocarcinoma (OPA) remains one of the most important and fascinating diseases of sheep in the UK and in many sheep rearing countries across the world. Shepherds have long recognised that the disease was transmitted among individual sheep within flocks and that the outcome of clinical disease was inevitably death with post-mortem examinations identifying tumour formation in the lungs.

The hypothesis that the disease could be produced experimentally by injecting the liquid fraction of the tumour tissue, or a reverse transcriptase producing (RTP)-agent, into the lungs of sheep was demonstrated by [1]Martin et al., (1976). The identification of the RTP-agent suggested the presence of a retrovirus which was subsequently identified, a number of years later, as Jaagsiekte sheep retrovirus (JSRV).

Moredun's scientists demonstrated that JSRV, external to the host genome (exogenous virus), was specifically associated with the OPA tumours by showing that the virus was consistently detected in tumours and lung secretions from affected animals ([2]Palmarini et al., 1999). This was difficult technically due to the presence of closely-related host-genome associated retroviruses (endogenous virus). Moreover, the team showed that the infectious JSRV21 clone was sufficient and necessary to induce OPA, providing unequivocal evidence that JSRV causes OPA ([3]Palmarini et al., 1999).



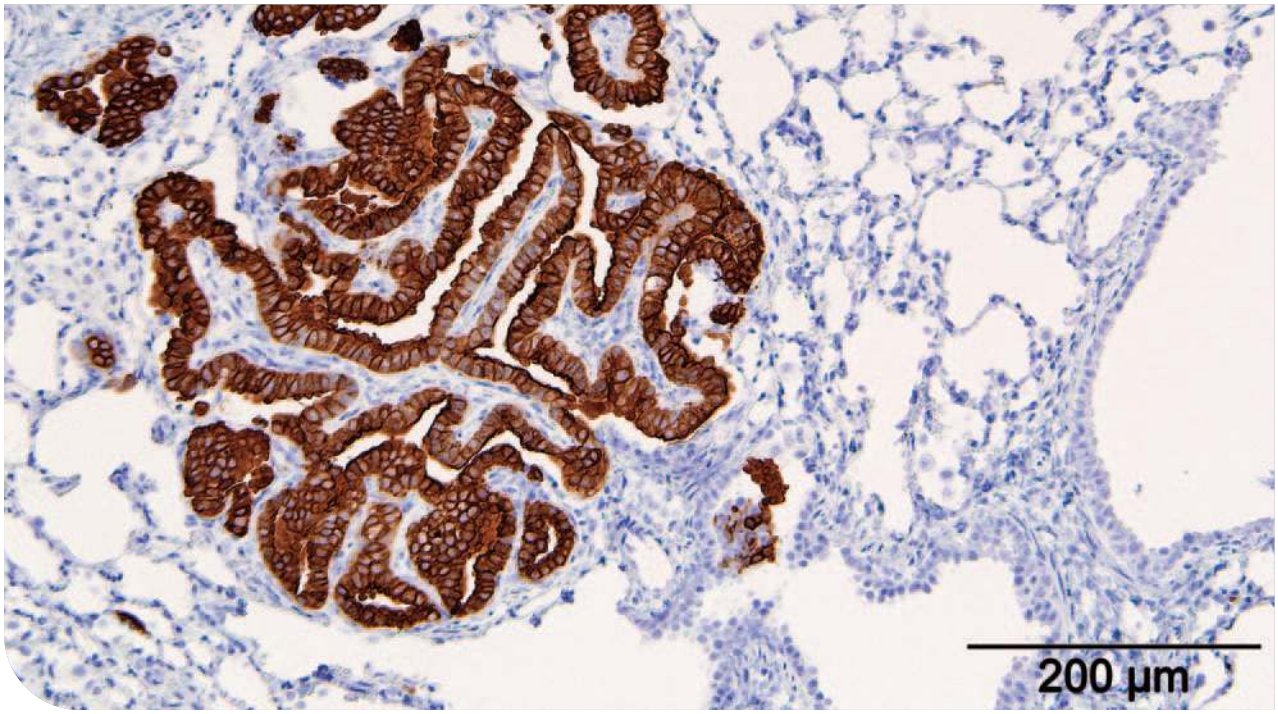
OPA-affected lungs: the dark areas on both sides are tumour taking over the lung tissue.

Dr Mike Sharp and colleagues at Moredun identified that an unusual aspect of OPA is that affected sheep fail to produce significant levels of antibodies to JSRV, essentially making early serological diagnosis impossible and contributing significantly to disease spread among farms with flock owners unable to ensure their stock are free of OPA prior to mixing or trading. Development of polymerase chain reaction (PCR) testing, a diagnostic method which identifies very small fractions of viral genomes, indicated the presence of JSRV proviral DNA in infected tissues and in blood ([4]González et al., 2001). For the first time, it could therefore be concluded that JSRV was detectable in naturally infected sheep before the onset of clinical disease, and even before the development of discernible tumours. Detection of proviral DNA in blood was an important step forward in understanding the progression of disease; however, the proviral DNA in blood fluctuates over time and is not always detectable, making the test unsuitable for testing individual animals.

Sheep lungs infected with JSRV were investigated to identify the pattern of gene expression in the infected tissues. Many differences were identified between infected and control lungs, with JSRV associated with an increase in genes linked to the innate, or non-specific, immune responses and to cancer pathways identified previously in humans ([5]Karagianni et al., 2019). While fortunately humans are not known to be affected by viruses similar to JSRV, lung adenocarcinomas do occur in people. Interestingly, the Moredun study showed that there was a considerable degree of overlap in gene expression between OPA tumours and human lung adenocarcinomas, suggesting similar pathways to disease and indicating the importance of veterinary species for comparative medical studies in humans. This work is a major focus of Dr David Griffiths and the Moredun team in the Strategic Research Programme supported by RESAS and Scottish Government.

While work is ongoing to identify biomarkers of OPA which may help in future diagnosis, ultrasound scanning of the lungs of sheep, which can detect tumours greater than 1 cm in diameter ([6]Scott and Cousens, 2018), is currently helping in farmers' decisions to cull individual animals early to reduce transmission of JSRV in flocks. This is showing significant promise in reducing the levels of OPA in flocks scanned over consecutive years. A short video about ultrasound scanning for OPA is available on the Moredun website https://www.moredun.org.uk/research/diseases/opa-jaagsiekte.

This image is of a tumour in lung tissue of a naturally infected sheep. The brown staining shows the JSRV Env protein.



The Moredun Foundation has conducted annual roadshows across the UK and Ireland for many decades, and scientists and communications staff spend much time at the major national agricultural events, including the Royal Highland Show, discussing the focus and progression of livestock research. OPA is one of the diseases which most concerns sheep farmers in terms of health, welfare and sustainability of food production and remains a key policy priority for Moredun and funding bodies.

Dr Phil Scott performing ultrasound scanning of a tup's chest to identify OPA tumours.

I would like to thank Dr Chris Cousens and Dr David Griffiths for their help in developing this story.

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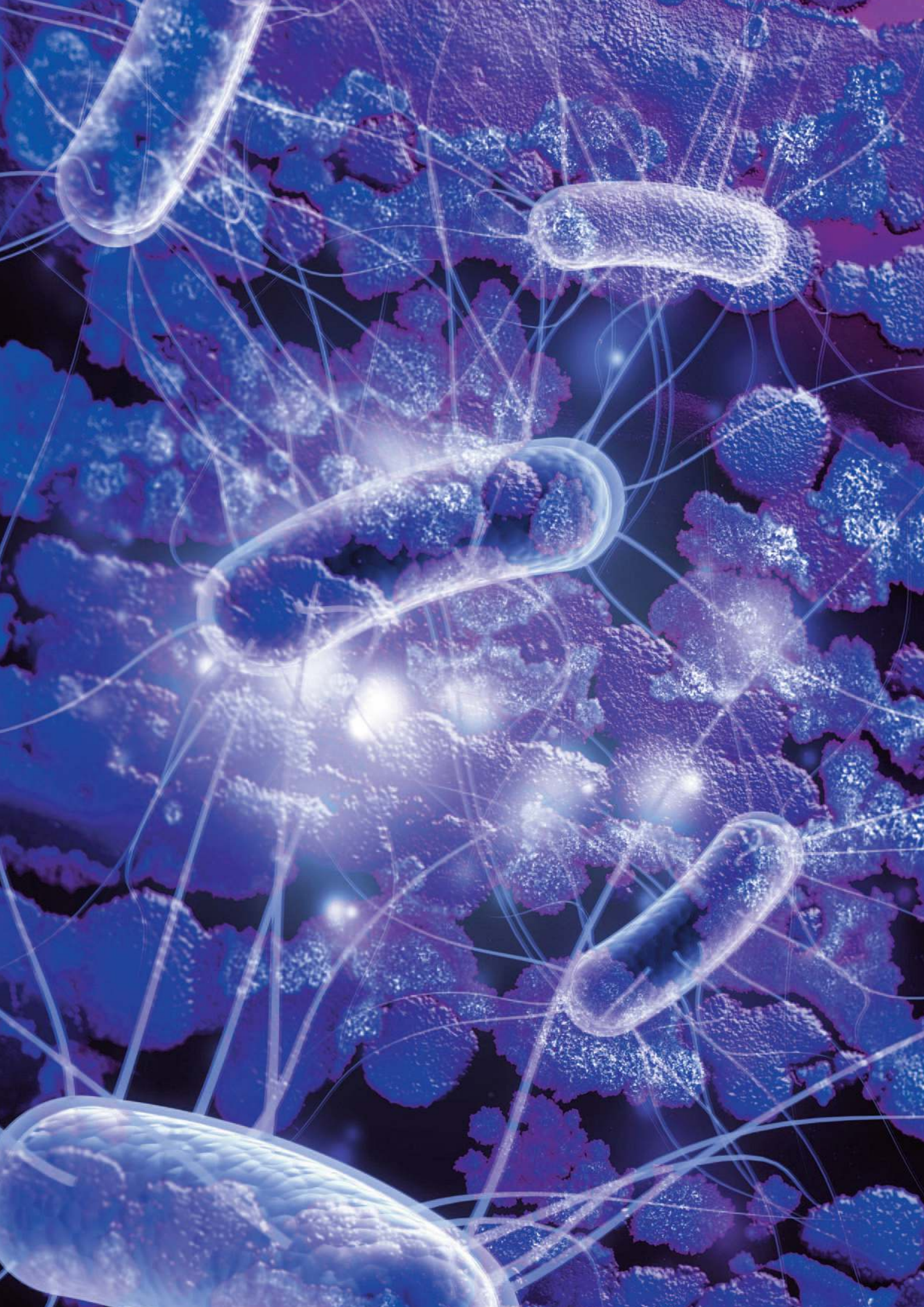

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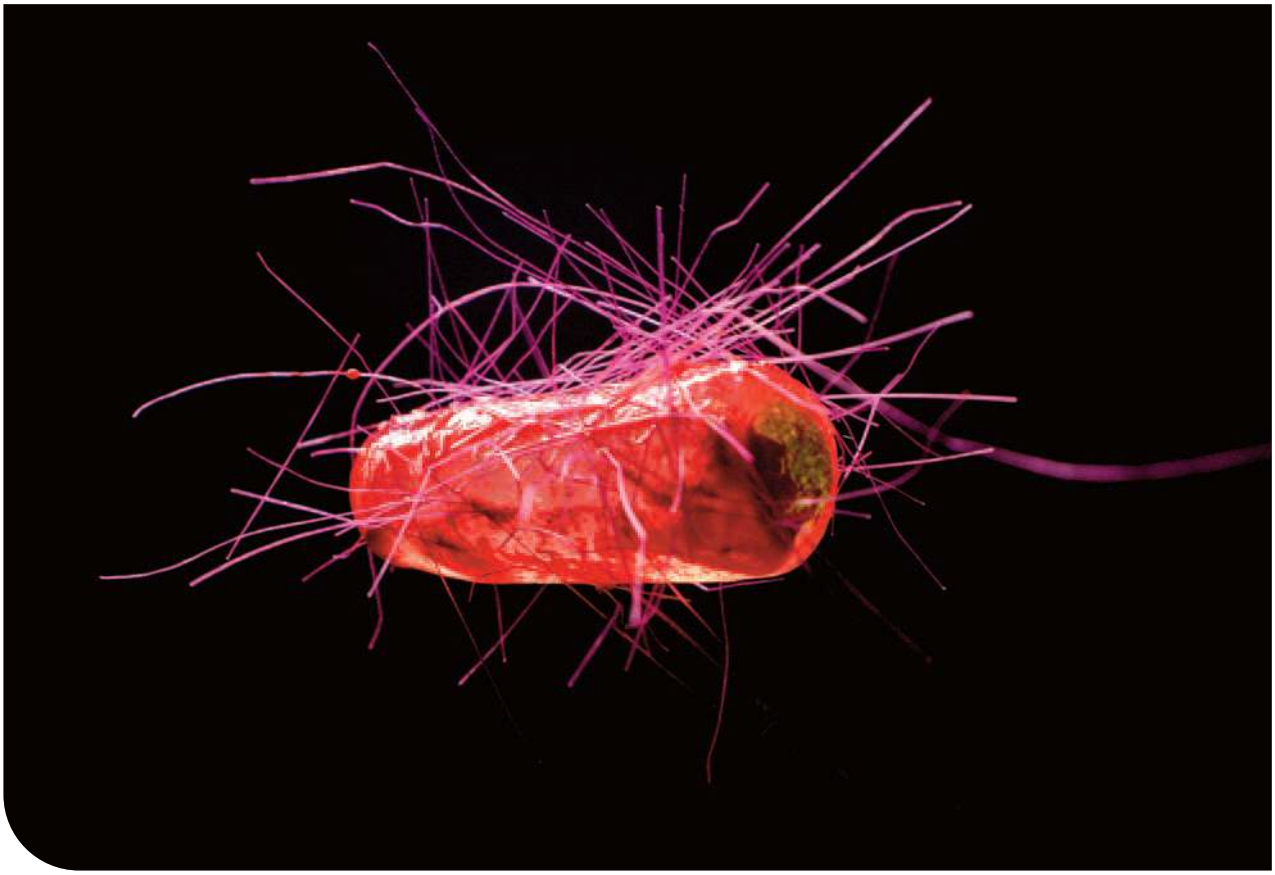
Developing a "Food Safety" Vaccine for *Escherichia coli* O157

The genus Escherichia comprises Gram negative bacteria, including *Escherichia coli,* abbreviated to *E. coli,* which are harmless residents of the lower gut in animals and humans, with some virulent strains causing diarrhoea and other clinical signs usually in neonatal or the young across the host species. This means that multiple *E. coli* strains are very common in the environments which surround people, animals and plants in all parts of the world.

E. coli O157 became infamous in Scotland in 1996 when cross-contamination of cooked and raw meats in a retailer's premises caused a large outbreak of disease in humans resulting in hundreds of cases of severe gastroenteritis, with many patients requiring hospitalisation, and some deaths. Disease in humans is linked to the production of Shiga toxin by *E. coli* O157 which causes damage to organs and especially the kidneys. Fatalities as a result of *E. coli* O157 infections usually occur in older people, the young, or immunocompromised individuals. Ruminants, and in particular cattle, are the main reservoir of human *E. coli* O157 infections. Interestingly, ruminants infected with these bacteria show no signs of disease, and infection does not appear to affect animal production.

In Scotland, the prevalence of *E. coli* O157 infections in humans was found to be higher than in the other geographical areas of the UK. Moredun scientists were involved in studies, with a number of collaborating organisations, which showed that beef cattle in Scotland had similar levels of infection with *E. coli* O157 to beef cattle in England and Wales and that approximately 90% of isolates were Shiga toxin positive. The study also showed that Scottish cattle had fewer isolates which were Shiga toxin negative than England and Wales, and that Scottish cattle also had a higher proportion of "super-shedders" (individuals with a higher than normal excretion levels of the bacteria) ([1]FSA/FSS, 2018). While it is possible that observations of high prevalence of Shiga toxin-producing *E. coli* O157 in Scottish cattle and high levels of human disease caused by *E. coli* O157 are linked, it is also possible that human infections may have arisen from exposure to *E. coli* O157 from other sources/ foodstuffs and/or from other food-producing regions. Whatever the source of infection, it was clear that preventing infection in cattle would potentially break the transmission cycle and reduce the risk of infection. in people. A vaccine was, therefore, needed to prevent human disease, as the infected cattle remain asymptomatic and unaffected by the bacterial infection - a significant issue to address in numerous aspects, and hence the title of this story - a "Food Safety" vaccine.



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E. coli O157 with hair-like flagella.

Work started at MRI in 2005 to develop a vaccine to control *E.coli* O157 in cattle, the main reservoir of human infection. Initially the vaccine targeted the flagella used by the bacteria to attach to epithelial cells lining the bovine gut.

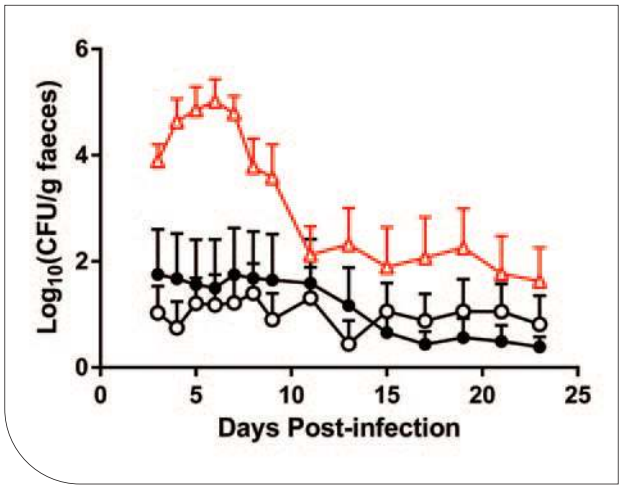
Preparations of H7 flagellin, the main structural component of *E. coli* O157 flagella, were used to vaccinate cattle which were subsequently challenged with the bacteria to assess the protective effects of the vaccine. While this resulted in fewer animals becoming infected by the bacteria, those animals that were infected, shed similar levels of bacteria in their faeces. Therefore while showing some promise, the vaccine was not thought to be sufficiently effective for it to be useful at reducing human infections ([2]McNeilly et al., 2008).

Subsequently, combinations of H7 flagellin together with proteins involved in the bacteria's type III secretion system, also involved in attachment to the bovine gut, were tested in experimental trials. These vaccines proved to be highly effective at reducing shedding of bacteria from cattle challenged with the bacteria ([3]McNeilly et al., 2010). Further work showed that both H7 flagellin and type III secretion proteins were required for optimal protection ([4]McNeilly et al., 2015).

These initial studies focused on vaccinating cattle and then challenging them with a large single dose of bacteria. Moredun scientists subsequently developed a better way of testing the vaccine in a way which better reflected the way cattle are exposed to the bacteria in the field. Through extensive studies at the high biological containment facilities at Moredun, a key factor, Shiga toxin 2a, involved in the ability of *E. coli* O157 to transmit between cattle was identified ([5]Fitzgerald et al., 2019). Using a highly transmissible strain of the bacteria, it was demonstrated that the vaccine was able to significantly reduce cattle-to-cattle transmissions. Mathematical modelling of the data predicted that the vaccine would be highly effective at reducing cattle *E. coli* O157 infections in the field ([1]FSA/FSS, 2018).

Based on these highly promising results, a commercially manufactured version of the vaccine is now being evaluated in a large scale field trial within a US feedlot which is known to be infected with *E. coli* O157. This work is supported by Roslin Technologies Ltd. who have a license to commercialise the vaccine (http://roslintech.com/safeguarding-food-products/). If this trial is successful, it is hoped that the vaccine will be commercially available in the near future.

This story covers a significant period of time, not only due to the complexity of identifying animals infected with *E. coli* O157 and conducting novel experimental vaccination approaches in high containment facilities, but also due to the lack of funding sources for this type of research. While *E. coli* O157 is clearly a very important zoonosis, a disease which passes from animals to humans via food or the environment, there is little incentive for animal health companies to invest in animal vaccines when infection does not cause any disease - infected cattle are healthy, grow and mature normally and are indistinguishable from uninfected cattle by the farmer. Currently, farmers have an obligation to only present "clean" cattle for slaughter which reduces the chances of contamination of carcasses via dirty hides. Furthermore, processes in abattoirs are designed to reduce contamination of the carcass with gut contents. Despite these control measures, rates of *E. coli* O157 infections in humans have remained static in the UK over the last decade.



The graph shows shedding of E. coli O157 from vaccinated (black and white circles) or unvaccinated cattle (red triangles). Vaccinated cattle shed fewer bacteria in faeces and do not have the clear peak shedding period seen during the first 10 days after infection.

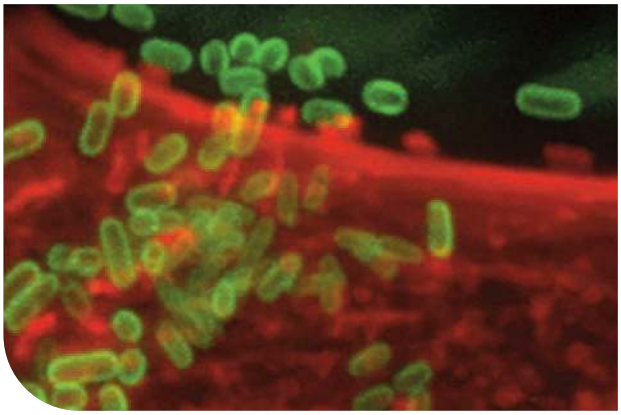


Photo: Mahajan et al 2009 Cellular Microbiology

Fluorescent image showing E. coli O157 forming tight attachment to cells lining the bovine gut.

Clearly the time has come for a more technologically-based approach for preventing infection in the first place, through vaccinating the reservoir hosts, cattle. Lack of investment in solutions for some of the "One Health" diseases remains a real conundrum for researchers.

I would like to thank Dr Tom McNeilly for his help in preparing this story.

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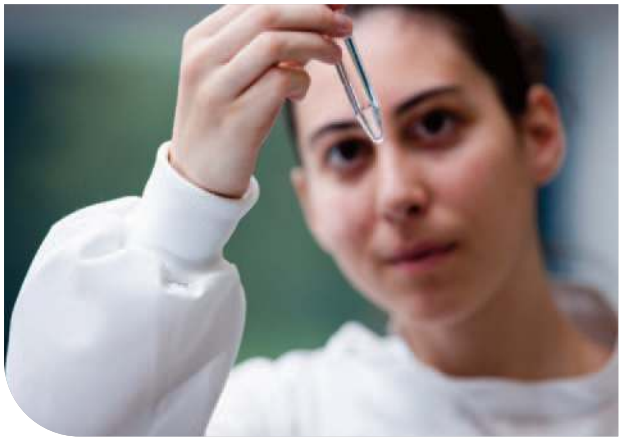
Moredun's role in the past, present and future in livestock vaccines and diagnostics

The thoughts of Al Nisbet, Head of Department, Vaccines and Diagnostics

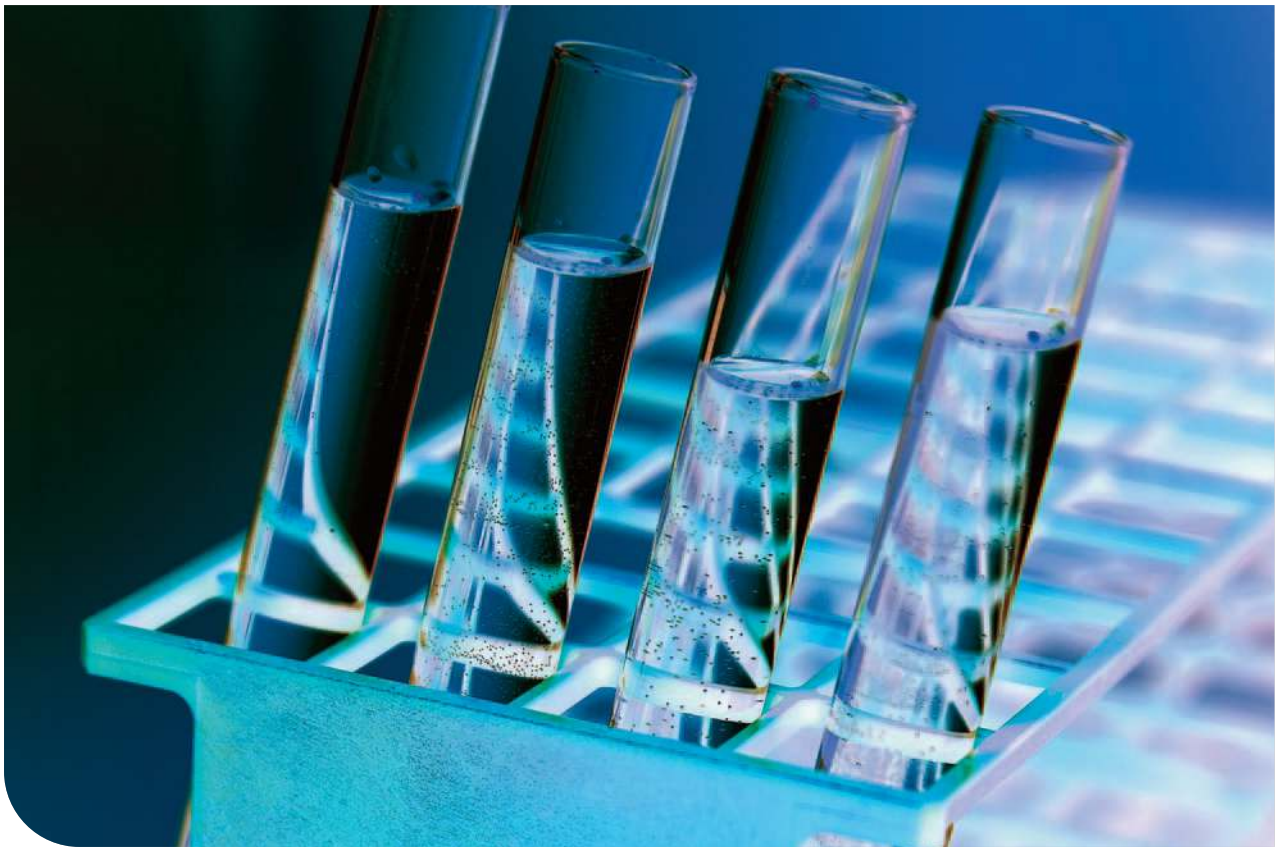
In my lifetime there has never been such an intense worldwide public focus on the development of vaccines and diagnostic tests, and their power to restore our personal freedoms, as we have seen in Moredun's centenary year. You only have to listen to the chatter in social media and the press to hear how the scientists' language of "R" numbers, PCR tests, lateral flow tests, neutralizing antibodies etc. has become everyone's language as we all seek to understand what's happening and what's in store. I have to say, instead of being jealously protective of our secret scientific jargon, I'm delighted that people are able to freely and competently use it to understand complex issues around disease diagnosis and vaccination. After all, that's what being a Moredun scientist is all about - "Make something useful, make sure people know how to use it and that you know what you're talking about" to paraphrase our Mission Statement.

That same mission has formed the foundation of Moredun's science right from the outset and now we're addressing some of the most challenging livestock diseases with some really sophisticated tools which weren't available to our predecessors. The same technologies that are being employed against COVID-19 are in our arsenal for tackling livestock diseases and have been developed, or are in various stages of development, by Moredun scientists for this purpose. Let's look at a few examples: PCR tests to identify multiple respiratory pathogens in cattle simultaneously; immunological and lateral flow tests for the diagnosis of sheep scab; a range of vaccines under development for controlling some of our most intractable production problems like gastrointestinal nematodes, for example. Many people will be aware that the Oxford/Astra Zeneca COVID-19 vaccine is a "viral vectored" vaccine – i.e. genetic information which tells the body to make an immune response to the vaccine is actually delivered as part of another, harmless, virus. At Moredun we are using exactly this technology to use animal viruses ("vectors") to deliver vaccines against livestock diseases. The vaccines being developed here encompass tick-borne and reproductive diseases of sheep and cattle respiratory diseases but could be deployed against a number of other diseases. This work may also lead to the production of new vaccines against the virus which causes louping ill (in sheep), for example, for which our stakeholders have expressed great interest.



Other work on novel vaccine delivery methods includes the use of new technologies to deliver vaccines on the surface of microscopic "nanoparticles" and this work also showed that the components of a prototype sheep scab vaccine could be delivered to animals in this way and induce strong immune reactions. Work on the prototype sheep scab vaccine has also demonstrated the ability to produce a diagnostic blood test which can discriminate between animals which were vaccinated and animals which were infected. This tool is a key component in the development of the vaccine if it is to be used in conjunction with effective diagnosis.

Every project that we perform at Moredun is aimed at improving animal health and welfare which leads to benefits for the farmer and the environment. We are able to attract and retain internationally recognised scientists purely because of this focus and it's no surprise that so many of our staff have close family links with the livestock industry. As a final note, although our science in the Vaccines and Diagnostics Department is focussed on these areas, we all know that disease control can really only be effective when we combine diagnosis, vaccination, nutrition, breeding, management, the strategic use of medicines and biosecurity - so our scientists interact with the relevant collaborators both within our Institute and internationally to deliver that ambition. When humans produce animals for food we need to be doing it in the highest welfare manner and most sustainable way possible – that's what our stakeholders are all about and that's why we work to deliver them the tools to do just that.



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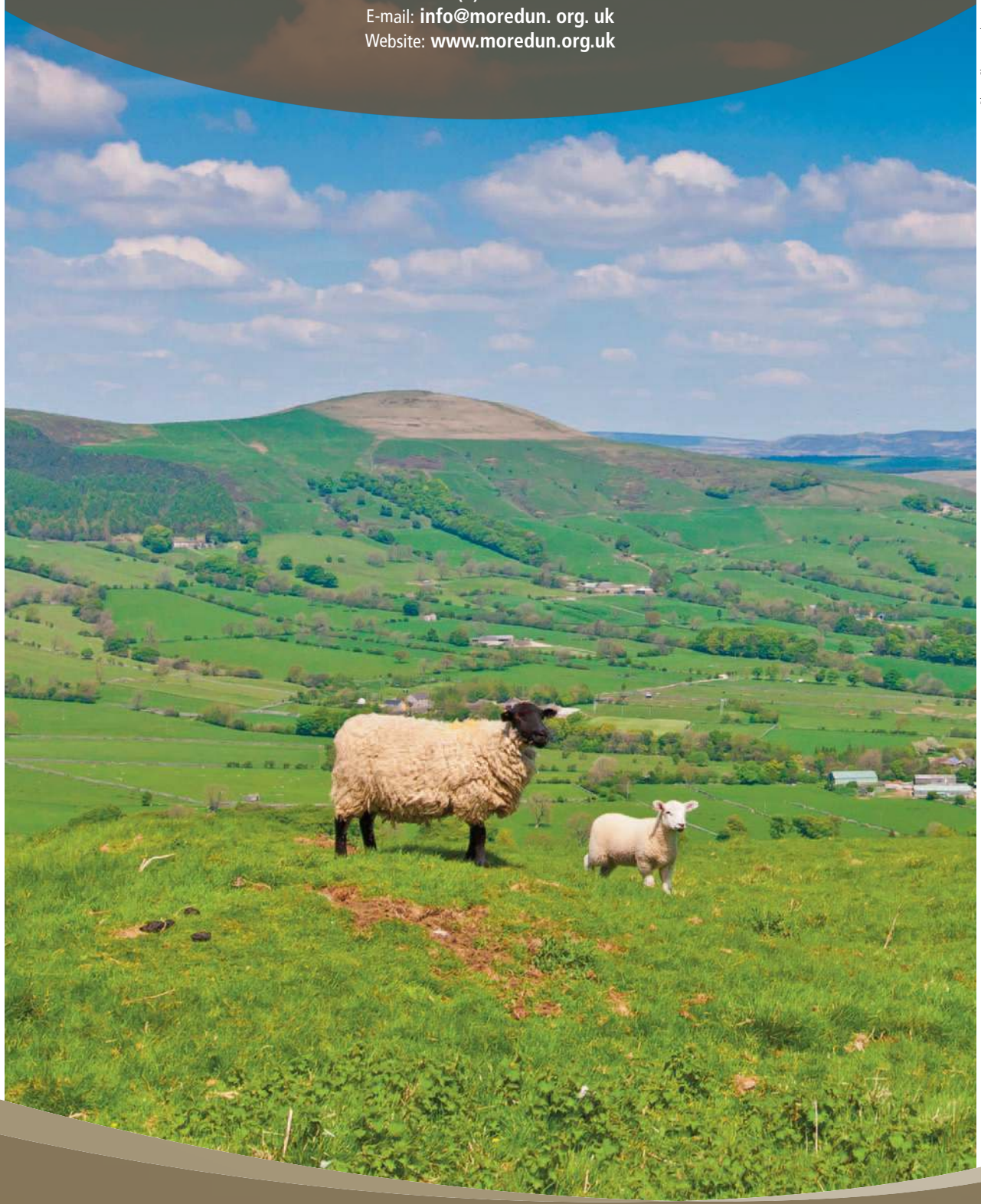

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