Institution: University of Cambridge

Unit of Assessment: 6

Title of case study: Development of diagnostic techniques to reduce incidence of strangles infection worldwide

Period when the underpinning research was undertaken: 2000-2009

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Duncan Maskell	Marks & Spencer Professor of Farm Animal Health, Food Science & Food Safety	1996 - 2018

Period when the claimed impact occurred: August 2013 – date

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact (indicative maximum 100 words)

Strangles is a highly contagious upper respiratory tract infection caused by the bacterium *Streptococcus equi*, which affects horses, donkeys and ponies worldwide, with more than 600 outbreaks in the UK alone each year. After initial infection, persistence can result in horses becoming long term carriers. Rapid identification of infected/carrier horses allows suitable quarantine and treatment measures to be implemented. Research undertaken by Professor Duncan Maskell at the University of Cambridge, in collaboration with researchers at the Wellcome Trust Sanger Institute and Animal Health Trust (AHT), led to the subsequent development by the AHT of two rapid diagnostic tests for strangles. These tests are now available in eight countries and have prevented outbreaks by identifying horses that have been exposed to *S. equi*, and which may be infectious, and quarantining them before they can transmit the infection to naïve animals. In the UK, reported cases reduced from 1262 in 2010 to 243 by September 2020, a reduction of 80%.

2. Underpinning research (indicative maximum 500 words)

It would be difficult to exaggerate the importance of whole genome sequencing to the development of vaccines and diagnostics for infectious diseases such as strangles. A completely assembled genome sequence provides all the genetic information contained within a pathogen, greatly facilitating comprehensive studies of its physiology, biochemistry, and genetic organisation. When coupled with other powerful 'functional genomics' techniques (such as signature-tagged mutagenesis, whole genome array technologies, subtractive hybridisation technologies), an extensive understanding of the molecular basis of the infectious disease caused by the bacterium being sequenced is possible. This enables rational choices to be made in selecting the best potential vaccine candidates ('reverse vaccinology'), and identifying targets for DNA- and serology-based diagnostics. Sequencing technology has progressed with increasing rapidity over the last ten years, but at the beginning of the century was an expensive and time-consuming endeavour.

Reverse vaccinology

Prof. Maskell, (employed at the University of Cambridge until 2018), is a pioneer in the field of reverse vaccinology. He was one of the first to comparatively analyse the pathogen species *Bordetella* in 2003 [R1]; to identify the molecular reasoning behind pathogen niche dynamics in 2006 [R2]; to undertake a whole genome intraspecies comparison in *Salmonelle typhi* in 2008 [R3] and to undertake a comparative analysis of the complete genome of *Streptococcus uberis*

in 2009 [R4]. This early research led Prof. Maskell and collaborators to revolutionise sequencing techniques and their use within veterinary medicine.

***Streptococcus equi* and strangles disease**

Building on this research, Prof. Maskell established the Veterinary Strep Interest Group at the University of Cambridge, to promote the importance of genome sequencing *Streptococci* of veterinary and zoonotic importance, and seek funding to sequence the genomes of a range of pathogens of particular importance to veterinary microbiology, and horses in particular.
One of the group's focus points was *Streptococcus equi. S. equi* is the causative agent of strangles, one of the most frequently diagnosed and serious infectious diseases of horses, found worldwide. Strangles symptoms vary enormously, but in severe cases the lymph nodes can become so swollen that horses struggle to breathe properly, hence the name 'strangles'. Transmission is by direct contact between animals or by contact with fomites, and prevention of the disease is complicated by carrier horses which look completely healthy, but intermittently shed *S. equi*, triggering new outbreaks. The identification and treatment of carrier horses is, therefore, extremely important if new outbreaks of strangles are to be prevented.

Genome sequencing strongly suggests that *S. equi* evolved from the very closely related but usually less virulent *S. zooepidemicus,* which is associated with inflammatory airway disease in young Thoroughbred horses. This discrimination between species allowed Prof. Maskell and collaborators at the Animal Health Trust (AHT) and Wellcome Trust Sanger Institute (WTSI) to apply their expertise to conceive and design a project that sequenced and analysed the whole genomes of isolates of *S. equi* and *S. zooepidemicus* from around the world [5]. Published in 2009, the completion of the *S. equi* strain 4047 (Se4047) and *S. zooepidemicus* strain H70 (SzH70) genome sequences enabled the identification of *S. equi*-specific targets suitable for multiplex diagnostic PCR-based tests, that are discriminatory, and reduce the risk of false-negative reporting [5]. The study identified the genes associated with synthesis of the molecule 'equibactin', which enhances the ability of *S. equi* to acquire iron and was present in all of the *S. equi* isolates, but in none of the diverse collection of *S. zooepidemicus* isolates examined [R5].

This finding, with other observations, led to the subsequent development by a research group led by Dr Andrew Waller at the AHT, in correspondence with Prof. Maskell, of a triplex qPCR test for strangles, able to detect *S. equi* at levels below the threshold of the culture assay, even in the presence of contaminating bacteria, and a substantial improvement on other diagnostic tests. The same output was also exploited to identify fragments of surface exposed or secreted proteins that were restricted to *S. equi* and could be used to develop an indirect ELISA (iELISA*)* antibody test.

3. References to the research (indicative maximum of six references)

R1. Parkhill, J., Sebaihia, M., Preston, A., Murphy, L. D., Thomson, N., Harris, D. E., . . . **Maskell, D. J**.. Comparative analysis of the genome sequences of *Bordetella pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica*. *Nat Genet*, 2003. *35*(1), 32-40. doi:10.1038/ng1227 *

R2. Sebaihia, M., Preston, A., **Maskell, D. J**., Kuzmiak, H., Connell, T. D., King, N. D., . . . Temple, L. M. Comparison of the genome sequence of the poultry pathogen Bordetella avium with those of *B. bronchiseptica, B. pertussis,* and *B. parapertussis* reveals extensive diversity in surface structures associated with host interaction. *J Bacteriol*, 2006. *188*(16), 6002-6015. doi:10.1128/JB.01927-05 *

R3. Holt, K. E., Parkhill, J., Mazzoni, C. J., Roumagnac, P., Weill, F. -X., Goodhead, I., . . . **Maskell, D. J.,** Dougan, G. High-throughput sequencing provides insights into genome variation and evolution in *Salmonella* Typhi. *Nat Genet*, 2008 *40*(8), 987-993. doi:10.1038/ng.195 *

R4. Ward, P. N., Holden, M. T. G., Leigh, J. A., Lennard, N., Bignell, A., Barron, A., . . . **Maskell, D. J.**, Parkhill, J. Evidence for niche adaptation in the genome of the bovine pathogen *Streptococcus uberis.*. *BMC Genomics*, 2009.*10*, 54. doi:10.1186/1471-2164-10-54 *

R5. Holden, M. T. G.; Heather, Z.; Paillot, R.; Steward, K. F.; Webb, K.; Ainslie, F.; Jourdan, T.; Bason, N. C.; Holroyd, N. E.; Mungall, K.; Quail, M. A.; Sanders, M.; Simmonds, M.; Willey, D.; Brooks, K.; Aanensen, D. M.; Spratt, B. G.; Jolley, K. A.; Maiden, M. C. J.; Kehoe, M.; Chanter, N.; Bentley, S. D.; Robinson, C.; **Maskell, D. J.**; Parkhill, J.; Waller, A. S. Genomic evidence for the evolution of *Streptococcus equi*: host restriction, increased virulence, and genetic exchange with human pathogens. *PLoS Pathog* 2009 **5**(3):e1000346. *

* All research outputs have been published in peer-review journals.

Competitive funding secured
Funding for this research was secured from the following agencies with Prof. Maskell as co-Investigator on all (details unavailable due to data protection):

- Horserace Betting Levy Board
- The Horse Trust (Home of Rest for Horses)

4. Details of the impact (indicative maximum 750 words)

The research described in section 2 by Professor Maskell at the University of Cambridge and colleagues at the Animal Health Trust (AHT) and Wellcome Trust Sanger Institute (WTSI) has generated wide-reaching impact by developing two diagnostic tests and providing testing facilities to eight countries, improving management and thereby significantly reducing the prevalence of strangles infection. The sale of over 90,000 tests also generated over GBP 1.1 million income for the AHT in the UK, where samples are analysed.

Identifying infected horses
Based on the findings of the research [R5], two blood tests were developed by researchers at the AHT [E1,E2], which enabled the identification of both infected horses and horses which had been exposed to the pathogen:

- The triplex qPCR assay [E1] was published in March 2013, with patents obtained in Europe in 2014 (qPCR) and in 2017 (an additional control strain) [E3,E4]. The test allowed a rapid turnaround for the detection of infection, with an overall sensitivity of 93.9% and specificity of 96.6%, detecting *S. equi* at levels below the threshold of the culture assay, even in the presence of contaminating bacteria. Since its release as a diagnostic assay, 38,627 qPCR assays have been undertaken in the UK between 2014 and 2019 [E5].

- An iELISA test was also developed and published in August 2013 [E2] with both high sensitivity (93.3%) and specificity (99.3%), that provided a robust assay for identifying horses exposed to *S. equi*. Over 56,000 iELISA assays were run in the UK between August 2013 and 2018, after which a pre-made kit was launched and disseminated worldwide: Netherlands (1,000 samples, 40 kits), UAE (4,400 samples, 176 kits), Republic of Ireland (2,200 samples, 88 kits), France (1000 samples, 40 kits) and Germany (3,000 samples, 120 kits). Since 2019 the tests have also been available in Argentina and Australia [E5].

The tests have generated income for the AHT. Between January 2019 and September 2020 the AHT generated approximately GBP 285,575 for analysing PCR tests and GBP854,837 for analysing iELISA tests [E5].

Preventing transmission

The PCR and iELISA tests have prevented outbreaks by identifying horses with mild symptoms that have been exposed to *S. equi*, and which may be infectious before they can transmit the infection to naïve animals. The test can also identify horses post-infection which have become asymptomatic carriers of the disease (approximately one in ten). The value of these tests to the end-user can be inferred from their uptake and use. For example, following the introduction of strangles in the UAE, the iELISA test has helped to control the disease, and now all imported horses are tested. Since starting to use the test, 13,312 tests have been analysed by the Central Research Laboratory in Dubai, with 1,126 positive test results (to 17th May 2020) [E6]. The Scientific Director at the Central Research Laboratory said *"We never had Strangles before… but some years ago it was introduced ... The disease spread rapidly to many farms in the UAE, which was a disaster. The antibody Strangles ELISA helped us a lot to control the disease"* [E6]. The iELISA is also used to provide assurance to trading partners in the United Arab Emirates that horses that are travelling to UAE are *Streptococcus equi* antibody negative [E6].

In Australia, both iELISA and qPCR tests have been successfully used to control and manage responses to outbreaks of strangles in riding schools, hunt clubs and racing stables [E7]. A professor of veterinary microbiology at the University of Melbourne, where the iELISA and PCR tests have been licenced for over 5 years said: *"Use of these tests has made an extraordinary difference in how quickly we can resolve these outbreaks, which means that the financial impact of this disease on the affected premises is minimised. Use of these diagnostic tests also has a positive benefit in terms of horse welfare, as testing has enabled us to quickly identify infected horses and limit spread to other susceptible horses."* [E7].

Within the UK, the Horserace Betting Levy Board (an executive non-departmental public body, sponsored by the Government's Department for Digital, Culture, Media & Sport) recommends the routine use of the strangles ELISA blood test during the isolation period, when horses are introduced to new premises, to identify previously infected or potentially infectious horses quickly, before the disease can spread [E8].

Reducing case numbers

The two strangles tests developed are supporting efforts to reduce the incidence of strangles. The number of positive diagnoses in the UK is showing a downward trend: 1262 in 2010 vs. 426 in 2019 and 243 in 2020 up to end of September. However, the majority of positive samples in 2018/2019 were from healthy horses that were identified during screening, suggesting that the number of sick animals is in decline [E5].

Prof. Maskell's research has had a direct impact on the health and wellbeing of horses internationally through reduction in strangles cases; it has supported businesses to control and manage outbreaks in at least 8 countries, reduced the animal health risk associated with international horse trade, and generated GBP 1.1 million sales to the AHT.

5. Sources to corroborate the impact (indicative maximum of 10 references)

E1. Journal publication demonstrating development of new qPCR test based on original research undertaken by Maskell in collaboration with AHT and WTSI:
Webb K., Barker C., Harrison T., Heather Z., Steward KF., Robinson C., Newton JR., Waller AS., Detection of *Streptococcus equi* subspecies *equi* using a triplex qPCR assay, 2013, The Veterinary Journal, 195, 300 – 304

E2. Journal publication demonstrating development of new iELISA test based on original research undertaken by Maskell in collaboration with AHT and WTSI:
Robinson C., Steward KF., Potts N., Barker C., Hammond T., Pierce K., Gunnarsson E., Svansson V., Slater J., Newton JR., Waller AS. Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. 2013. The Veterinary Journal, 197, 188 - 191, doi: 10.1016/j.tvjl.2013.01.033

E3. Patent application citing original research EP 2 344 673 B1 published in 2014
E4. Patent application citing original research EP2794920B1 published in 2017
E5. Testimonial from Animal Health Trust
E6. Testimonial from Scientific Director, Central Veterinary Research Laboratory, UAE
E7. Testimonial from Centre for Equine Diseases, University of Melbourne
E8. Horserace Betting Levy Board Guidance for Strangles in the UK