Institution: University of Cambridge

Unit of Assessment: UoA 6

Title of case study: Novel diagnostics informing pig herd management on a global scale

Period when the underpinning research was undertaken: 2011 - present

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:**
Dr Alexander (Dan) W Tucker	Reader in Veterinary Public Health	2003 - present
Dr Lucy A Weinert	Sir Henry Dale Fellow	2012 - present
Professor Duncan J Maskell	Marks & Spencer Professor of Farm Animal Health, Food Science & Food Safety	1996 - 2018

Period when the claimed impact occurred: 2017 - 2020

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

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

The bacterium *Glaesserella parasuis (formerly known as Hemophilus parasuis)* is one of the leading causes of pig mortality and morbidity worldwide. Catastrophic disease outbreaks can occur when pig populations with different variants of the pathogen are mixed. Research undertaken at the University of Cambridge led to a multiplex PCR test which substantially improves the ability to identify circulating variants. This allows more effective targeting of vaccination to control outbreaks and better-informed pig flows to prevent spread and minimise the use of antibiotic treatment. It is being used by a US-based pig producer that also operates in China and Mexico to inform management and vaccination strategies for their 365,000 sows. As secondary impact to the improvement in pig health, the test has generated commercial revenues of EUR 35,200 for a partner in Germany, and is also available as a diagnostic in Canada, China and the USA.

2. Underpinning research (indicative maximum 500 words)

Globally, pig meat is the largest consumed individual animal protein: in 2019, 11.1 kg pig meat was consumed *per capita* globally (source: Organisation for Economic Co-operation and Development). This reality drives intensive pig farming practices. Intensification that increases herd population size and density supports the emergence and spread of infectious disease due to increased contact between animals. This enables pathogen transfer, which can disrupt finely balanced meat supply chains and allied industries, with potentially high animal mortality rates. *Glaesserella parasuis* is a bacterium found in the upper respiratory tract of swine that can cause severe systemic disease, primarily affecting young animals (4–8 weeks old). Endemic bacterial infections such as *G. parasuis* are prevalent throughout all pig supply chains and present a major challenge for pig health, welfare and for antibiotics stewardship. Outbreaks of *G. parasuis* have led to pig mortality rates of between 5-10%, particularly in intense pig producing countries such as Canada, the USA, Europe, China and the Philippines. *G. parasuis* is classed into 15 different serotypes (distinct variation within a species of bacteria or virus, which do not offer cross-protective immunity). Catastrophic disease outbreaks can occur when pig populations with divergent resident serotypes of the pathogen are mixed, or when replacement breeding stock are introduced. Knowing the serotype within a given herd allows introduction of commercial or autogenous bacterin vaccines (vaccines created to target outbreak of a specific serotype of pathogen) which provides protection within that serotype. Producers may further mitigate the risk of *G. parasuis* disease by using prophylactic antibiotics; this practice has been officially banned in the EU since 2018, so better ways to manage infection are important to establish.

Distinguishing between serotypes of G. parasuis

Researchers at the University of Cambridge built collaborations with established academic and commercial veterinary diagnostic bacteriologists in key pig producing countries – UK, Denmark,

Spain, Germany, China, USA and Canada – enabling them to build a collection of pure colonies of previously serotyped isolates of *G. parasuis* for whole genome sequencing. Research undertaken by Tucker, Maskell and Weinert in 2013 then analysed the whole genome sequences of these reference strains along with >100 field isolates to identify an extremely high level of association between the sequence of genes encoding enzymes responsible for bacterial cell wall synthesis (capsule loci) and the traditional serotyping results (R1).

Serotyping of *G. parasuis* previously relied on access to panels of reference strains and, by 2014, was available only in single laboratories in Germany, USA and Australia. The research conducted at Cambridge enabled them to develop a multiplex PCR in 2015, based on variations within the capsule loci, able to simultaneously detect multiple serovars of *G. parasuis* from a single clinical sample (R2). The multiplex PCR method has the advantage of being fast, simple, and transferable to molecular diagnostic laboratories with basic equipment, and it can be performed on crude DNA derived directly from bacterial colonies.

Since publication of the methodology for the multiplex PCR in 2015, testing has been instituted in commercial and research laboratories, improving differential diagnosis of *G. parasuis* serotypes, allowing better targeting of treatment, and minimising use of antibiotics while maintaining pig health.

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

R1. Howell KJ, Weinert LA, Luan SL, Peters SE, Chaudhuri RR, Harris D, Angen Ø, Aragon V, Parkhill J, Langford PR, Rycroft AN, Wren B, **Tucker AW** (equal senior author), Maskell DJ. Gene content and diversity of the loci encoding biosynthesis of capsular polysaccharides of the 15 serovar reference strains of *Haemophilus parasuis*. Journal of Bacteriology. 2013 Sep 15;195(18):4264-73, DOI: 10.1128/JB.00471-13

R2. Howell KJ, Peters SE, Wang J, Hernandez-Garcia J, Weinert LA, Luan SL, Chaudhuri RR, Angen Ø, Aragon V, Williamson SM, Parkhill J, Langford PR, Rycroft AN, Wren B, Maskell DJ, **Tucker AW**. Development of a multiplex PCR assay for rapid molecular serotyping of *Haemophilus parasuis*. Journal of Clinical Microbiology. 2015 Dec 1;53(12):3812-21, DOI: 10.1128/JCM.01991-15

All references have been subject to peer review

Competitive funding secured
BBSRC Longer and Larger grants scheme, 2009-2015, A multivalent vaccine and single platform diagnostic for bacterial respiratory diseases of pigs, GBP2,303,325

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

G. parasuis is endemic worldwide, but outbreaks in naïve populations can be catastrophic for the pig farming industry. For example, for one pig provider an outbreak resulted in more than 1200 high-value pigs being lost in ten days, and four years on, the mortality doubled for pigs from weaning to market, in addition to the impact of the isolate entering downstream commercial flows [E1].

The cost implications of this are substantial, as shown by an example cost calculation from the UK National Animal Disease Information Service of a typical *G. parasuis* outbreak. For a six-week outbreak in a farm of 300 sows, they estimated a cost of GBP8000, with an ongoing vaccination cost of GBP6000 per year. Use of Cambridge University research has improved diagnosis of this disease, allowing better targeting of treatment, minimising use of antibiotics and reducing costs such as these for producers.

Impact on disease diagnosis
Since publication of the methodology for the multiplex PCR in 2015, testing has been instituted in commercial and research laboratories. Currently the methodology is offered commercially in

Germany (IVD gmbh), Canada (University of Montreal Veterinary Diagnostic Laboratory) and the USA (Iowa State University).

IVD GmbH serves a population of 40,000 customers in 28 European countries, primarily in Germany and the Netherlands. The test has replaced their previous serological method, and, since its introduction in April 2017, has shown a rapid increase in tests, from just under 100 in 2017, to just under 300 in 2019. Approximately 880 different pig populations have been tested to date; at a charge of EUR40 per test, this equates to revenue of EUR35,200 for the company. A managing partner at IVD GmbH says: *"The development of this serotyping multiplex PCR is of great value for the veterinary diagnostic industry, as it is indeed for us…"* [E2]

In Canada, via the University of Montreal Veterinary Diagnostic Laboratory, the test has been used to serotype 452 strains since 2017, reducing the number of un-typeable strains from almost 50% to 2%, thereby allowing practitioners to implement more effective vaccination strategies. The lab has also helped laboratories from Chile, Mexico and Colombia to use the test for research purposes, and also transferred the technique to Iowa State University, USA. The professor who heads the diagnostic service states that the test *"has been an incredible advancement in science and it has significantly helped the swine industry"*. [E3]

Impact on pig health and welfare

Rapid serovar identification using the multiplex *G. parasuis* PCR determines whether multiple virulent serovars or a mixture of virulent and avirulent serovars are present in a sample. This knowledge allows earlier introduction of prophylactic vaccination, or strategies that prevent the mixing of 'pig flows' with incompatible serotype profiles; both approaches minimise the use of antibiotics while maintaining pig health.

For the past four years Tucker has used his expertise to provide technical advice to Genus plc's Pig Improvement Company (PIC) in the capacity of Adviser for Global Health Assurance. PIC specialises in the genetic improvement of pigs, and has more than 300 units around the world; they lead the market with a share of 24% and are the only listed porcine genetics company (Genus, About Us). Their units are supplied from an elite gene pool held at nucleus units in Canada and the USA; more than 120 million slaughter pigs each year contain PIC genetics. Innovations that support achieving the highest possible health status are important in enabling rapid, unhindered dissemination of genetics around the world but also, once the pigs are on-farm, in enabling pigs to grow in line with genetic potential and ensure efficiency of resources.

Tucker worked with Genus PIC to implement the PCR test developed at Cambridge to support practical preventive medicine approaches at farm level. Genus PIC is currently employing the multiplex PCR to characterise the array of *G. parasuis* serotypes present in its key supply pyramids, with the goal of identifying those breeding herds carrying serotypes more frequently associated with disease [E4]. Given that the most severe disease outbreaks arise when pigs are exposed to strains of *G. parasuis* to which they have no prior exposure or immunity, this has enabled a more preventive approach to matching sources when mixing of piglets from multiple farms is unavoidable within the PIC production pyramids. It also supports better matching of supply farm to customer farm to minimise outbreaks of disease after the sale of replacement breeding stock. Genus PIC is also now coordinating an unprecedented analysis of more than 600 isolates of *G. parasuis* from around the world to determine which serotypes are most strongly associated with disease in the key pig producing countries, thereby informing the design of more relevant vaccines in future.

A Technical Services Specialist for Genus PIC states [E4] that the multiplex PCR tool developed by Maskell and Tucker has been highly valuable for a breeding stock company such as PIC for the following reasons:

1. It improves/supports source selection decisions,
2. Allows them to identify proper vaccine candidates
3. Enables them to refine pig movements; and
4. Allows them to identify sources of new introductions of infection

Their use of the test was presented at the American Association of Swine Veterinarians conference 2019, referencing R2. The presentation described that these “rapid and reliable tools can be useful for pathogen surveillance, identification of appropriate autogenous vaccine candidates, to improve pig flow management by commingling sources with the same serotype and to prevent introduction of pigs with potentially virulent HPS strains”. [E5]

Another major pig producer, Pipestone (USA) manages 280,000 sows in the US, 60,000 sows in China and 25,000 sows in Mexico. Their Health Assurance Director says that they have found “tremendous value” from the Cambridge work on *G. parasuis*. They have used the multiplex PCR test to screen during clinical outbreaks, and confirm presence of specific serotypes to decide gilt (young female pig yet to have a litter) flow changes. The tool has also been used to select isolates for autogenous vaccines, and to measure the efficacy of control strategies on specific *G. parasuis* serotypes. The Cambridge test allowed them to take more accurate and opportune decisions on gilt sourcing, preventing considerable outbreaks or instability that would have needed high antibiotic use. They have been able to implement a more strategic use of antibiotics (timing, class, route), and a more accurate selection of commercial or autogenous vaccines [E1].

Disease and mortality from *G. parasuis* infection is a significant issue for the pig farming industry, and by consequence, for the food supply chain and cost of protein that plays a key role in nutrition. The test developed based on research from the University of Cambridge is recognised as an important progression in tackling this pathogen [E6, R1 and R2 referenced page 3], generating revenue for diagnostic services, improving pig management and enabling more effective vaccination and antibiotic use strategies.

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

E1. Testimonial from Health Assurance Director, Pipestone, USA
E2. Testimonial from commercial diagnostic laboratory: GmbH, Germany
E3. Testimonial from diagnostic services, University of Montreal
E4. Corroboration from Technical Services Specialist, Genus PIC
E5. Presentation from American Association of Swine Veterinarians conference 2019
E6. Mar Costa-Hurtado, Emili Barba-Vidal, Jaime Maldonado, Virginia Aragon, Update on Glässer’s disease: How to control the disease under restrictive use of antimicrobials, Veterinary Microbiology, Volume 242, 2020, 108595, ISSN 0378-1135, DOI: 10.1016/j.vetmic.2020.108595.