Institution: University of Exeter

Unit of Assessment: UoA 5 Biological Sciences

Title of case study: Biosystems Technology Ltd - Providing Alternatives to Vertebrate Animals for Life Sciences R&D

Period when the underpinning research was undertaken: 2007-2015

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 Rick Titball	Professor of Molecular Microbiology	2007 – present
Dr Olivia Champion	Research Fellow	2007 – 2016

Period when the claimed impact occurred: July 2015 - 31July 2020

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

1. Summary of the impact

Reduction, refinement and replacement of vertebrates in R&D is a global priority. Research at Exeter led to the **establishment of a spin-out company, BioSystems Technology Ltd** (BST), which has developed a new global product, TruLarv™, supplying laboratory-grade insects (genetically engineered *G. mellonella* larvae) as alternatives to vertebrates for R&D including drug efficacy studies. BST has had **commercial and economic impact** during the period 2015-2020, [text removed for publication]. It has **changed working practices and drug testing protocols** around the world, providing cost-savings for customers and reducing the use of vertebrates. BST plays a prominent role in raising awareness of alternatives to vertebrate animal testing, and its work has been cited by over 500 journalists and broadcasters around the world since 2015.

2. Underpinning research

In 2007 Titball and Champion initiated pilot projects to explore alternative infection models for studying bacterial pathogens. These models included *in vitro* cell culture, zebra-fish, plants, and insects. Larvae of the wax moth (*Galleria mellonella*) had advantages over the other systems including ease of handling, survival at 37ºC (mammalian body temperature), and the ability to precisely dose by injection. Initial studies showed the potential for the model to be used to study virulence of *Yersinia pseudotuberculosis* (the cause of enteric infection in humans, animals and birds) **[3.1]**, *Campylobacter jejuni* (the most common cause of food poisoning in Europe and the USA) **[3.2]**, and *Burkholderia pseudomallei* (the cause of melioidosis in humans and animals) **[3.3]**. We also carried out pioneering studies which showed that the pharmacokinetics of antibiotics were similar in wax moth larvae and in humans **[3.4; 3.5]**. Despite this, we encountered problems with experimental reproducibility, which delayed or prevented the publication of research. Discussions with other members of the research community confirmed that our experiences with poor reproducibility of the model were common.

Against this background, we investigated the cause of experimental variability. The only *G. mellonella* larvae available at that time were produced on an industrial scale, as fishing bait or food for captive animals such as fish and reptiles. The age, weight and health status of the larvae were variable. Secondly, the larvae possessed a microbial flora on the cuticle which often included pathogenic microbes and these were often introduced into the larvae during injection. Finally, the larvae were bred with antibiotics added to feedstuffs (to limit infection) and hormones (to limit pupation).

To reduce variability, we developed, and patented, a method for decontamination of the insect cuticle that did not affect the overt health of larvae. We showed that larvae that were age and weight defined, bred without added antibiotics or hormones, and surface decontaminated,

provided a robust and reproducible model for infection studies **[3.6]**. This finding coincided with the increased awareness of *G. mellonella* larvae as a model system for microbial infection and antimicrobial drug discovery. We therefore set out to make our standardised insect larvae (trade marked by us as TruLarv™) available to the scientific community through the establishment in 2015 of University of Exeter spin-out company, Biosystems Technology (BST). Since then, we have expanded our product range to include earlier instar stages of larvae (ProuLarv™), since these can be used to study oral infections, the effects of probiotics and gut microflora. BST also runs training courses to train researchers in the handling and use of *G. mellonella* larvae.

BST has so far secured funding totalling approximately £600k from the National Centre for the Replacement, Refinement and Deduction of Animals in Research, from Innovate UK and from the Eurostars programme to further develop this model [text removed for publication] .

3. References to the research

3.1 Champion OL, Cooper IA, James SL, Ford D, Karlyshev A, Wren BW, Duffield M, Oyston PC, **Titball RW**. *Galleria mellonella* as an alternative infection model for *Yersinia pseudotuberculosis*. Microbiology. 2009 May;155(Pt 5):1516-22. doi:10.1099/mic.0.026823-0. PubMed PMID: 19383703.

3.2 Champion OL, Karlyshev AV, Senior NJ, Woodward M, La Ragione R, Howard SL, Wren BW, **Titball RW**. Insect infection model for *Campylobacter jejuni* reveals that O-methyl phosphoramidate has insecticidal activity. J Infect Dis. 2010 Mar;201(5):776-82. doi: 10.1086/650494. PubMed PMID: 20113177.

3.3 Wand ME, Müller CM, **Titball RW**, Michell SL. Macrophage and *Galleria mellonella* infection models reflect the virulence of naturally occurring isolates of *B. pseudomallei*, *B. thailandensis* and *B. oklahomensis*. BMC Microbiol. 2011 Jan 17;11(1):11. doi: 10.1186/1471-2180-11-11. PubMed PMID: 21241461; PubMed Central PMCID: PMC3025829.

3.4 Thomas RJ, Hamblin KA, Armstrong SJ, Müller CM, Bokori-Brown M, Goldman S, Atkins HS, **Titball RW**. *Galleria mellonella* as a model system to test the pharmacokinetics and efficacy of antibiotics against *Burkholderia pseudomallei*. Int J Antimicrob Agents. 2013 Apr;41(4):330-6. doi:10.1016/j.ijantimicag.2012.12.009. Epub 2013 Feb 8. PubMed PMID: 23402703.

3.5 Benthall G, Touzel RE, Hind CK, **Titball RW**, Sutton JM, Thomas RJ, Wand ME. Evaluation of antibiotic efficacy against infections caused by planktonic or biofilm cultures of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in *Galleria mellonella*. Int J Antimicrob Agents. 2015 Nov;46(5):538-45. doi: 10.1016/j.ijantimicag.2015.07.014. Epub 2015 Aug 31. PubMed PMID: 26364845.

3.6 Champion OL, Titball RW, Bates S. Standardization of *G. mellonella* larvae to provide reliable and reproducible results in the study of fungal pathogens. J Fungi (Basel). 2018 Sep 6;4(3). pii: E108. doi: 10.3390/jof4030108. PubMed PMID: 30200639; PubMed Central PMCID: PMC6162639.

4. Details of the impact

Government directives and public awareness of animal welfare are drivers for the reduction, refinement and replacement of vertebrate animals in research and development in the Life Sciences. There are also significant ethical, financial and legislative drivers compelling businesses to seek alternatives to existing vertebrate testing systems. Research at the University of Exeter led to the creation of a spin-out, Biosystems Technology Ltd (BST) in 2015 **[5.1]** which has become a successful life sciences venture with worldwide sales in 18 countries. Its new products have changed working practices and protocols leading to cost savings and reductions in use of mammals. The Company has led an international campaign to reduce use of vertebrates in experiments.

Commercial and Economic Impact
With an innovative product of laboratory grade *G. mellonella* larvae (TruLarv™) produced using a patented method **[5.1; 5.2]**. BST is a profitable **[5.3]** and growing UK SME, founded with the support of the Innovate iCURE programme [text removed for publication]. For investigators who are newly adopting the TruLarv™ BST provides associated supporting services, such as training in the use of this model **[5.1]**. BST is also developing new products for this market [text removed for publication].

Changes to working practices and protocols
By producing TruLarv™, and selling to early stage adopters, BST is providing an alternative to vertebrate animals in life sciences R&D, whilst ensuring experiments are carried out to a rigorous standard. *G. mellonella* larvae are a cost effective and ethical alternative to vertebrates for modelling microbial infection, for antimicrobial drug screening, to understand host responses to infection and for assessing toxicity. The ethical value of the model derives from the reported lack of pain fibres in insects and the general view that insects do not feel pain like vertebrates (Bateson, 1991; Sneddon et al., 2014). Consequently, the experimental use of insects is not regulated. In 2019 the UK National Centre for the Replacement, Refinement & Reduction of Animals in Research identified further work on *G. mellonella*, as a strategic priority **[5.4]** and subsequently awarded funding to the University of Exeter.

Some customers of BST are SMEs involved in antibiotic discovery [text removed for publication] . TruLarv™ have enabled these customers to screen drug candidates rapidly and at low cost for toxicity and efficacy at an early stage in the drug discovery progress **[5.5; 5.6]**. A Project Lead [text removed for publication] noted: “By using this early-stage selection we were able to increase the value of our programmes and progressed only the most promising molecules into more sophisticated vertebrate models” **[5.6]**. The contribution of BST to drug discovery is recognised by the Director of Science and Technology at the National Centre for the Replacement, Refinement and Reduction of Animals in Research, who describes *G. mellonella* as having a ‘pivotal role’ in the development of drugs and non-pharmaceutical chemicals **[5.7]**.

The replacement of vertebrate infection models by TruLarv™ has also provided significant cost savings to customers. One of our customers, at the University of Surrey, calculated that by using TruLarv™ for initial screening of candidate probiotics, the number of regulated animals that would ordinarily be used in infection and intervention trials has been reduced by 75% [text removed for publication] **[5.8]**.

Raising Awareness of alternative disease models
BST supports UK Government policy to reduce and replace animals used for pre-clinical research and has led a campaign to raise awareness of alternative disease models. The work of BST has been cited extensively by journalists and broadcasters around the world in the domestic and overseas press since 2015 **[5.9]**. Olivia Champion, CEO of BST, has presented the work of the company in a number of forums including, for example, TedX and with BST winning the Exeter Living Award for Technology and Innovation in 2018 **[5.10]**.

5. Sources to corroborate the impact

5.1 Biosystems Technology web site; https://web.archive.org/web/20201127142025/https:/biosystemstechnology.com/about.

5.2 Titball RW, Champion O. Method for preparation of research organisms. International patent number: WO/2017/029496 (published 23/02/2017). Also published as GB2557778 (United Kingdom, 27/06/2018), EP3337521 (European Patent Office, 27/06/2018) and US20190084712 (United States Of America, 21/03/2019) https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017029496.

5.3 Audited company accounts for 2018/19.

5.4	NC3Rs 2019 funding priority areas. www.web.archive.org/web/20200312155835/https://www.nc3rs.org.uk/funding-scheme-priority-areas Funding award listed as: Professor Richard Titball (University of Exeter) Galleria mellonella as an infection model for viral pathogens.
5.5	[text removed for publication]
5.6	[text removed for publication]
5.7	Letter of testimony from the Director of Science and Technology at the National Centre for the Replacement, Refinement and Reduction of Animals in Research. Saying '***G. mellonella will continue to play a pivotal role in understanding human health and disease, and in the development of safe and efficacious pharmaceutical and non-pharmaceutical chemicals.***"
5.8	Letter of testimony from a Professor of Veterinary Microbiology and Pathology and Head of the Department of Pathology and Infectious Diseases at the University of Surrey. Saying that the introduction of TruLarv™ has reduced '***the number of mammals and birds used in Infection and intervention trials in our laboratory by 75%.***'
5.9	Selected Press and Media Coverage. https://web.archive.org/web/20200728182857/https://phys.org/news/2017-01-mammal-moth-larvae.html)
5.10	Exeter Living Awards – past winners. BST won the Technology and Innovation award in 2018. http://web.archive.org/web/20210310142236/https://www.exeterlivingawards.co.uk/wp-content/uploads/2019/11/sponsor-category-square-dark-2.png