**Institution:** University of Bristol

**Unit of Assessment:** UoA6 – Agriculture, Veterinary and Food Science

**Title of case study:** Improving feline health through the worldwide application of infectious and genetic disease polymerase chain reaction assays

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

Bristol University's School of Veterinary Sciences, a global leader in feline medicine, was the first UK centre to develop and commercially offer polymerase chain reaction (PCR) and quantitative (q) PCR assays to detect a range of feline infectious and genetic diseases. Since 2008 there has been a dramatic increase in the number of qPCR tests performed, with over 35,000 tests carried out between 2008 and 2013. The results of genetic testing have informed breeding programmes and resulted in a reduced prevalence of genetic disorders such as polycystic kidney disease (PKD). The results of testing for infectious diseases have informed diagnosis and treatment modalities and, together with the genetic testing, have contributed to **significant improvements in feline health and welfare**. This work has also generated commercial income in excess of £1.7M, which has been used to further research into feline infectious and genetic diseases.

**2. Underpinning research** (indicative maximum 500 words)

The underpinning research was conducted by Dr Chris Helps (Research Fellow 2001-2007; Senior Research Fellow 2007-present) and Dr Séverine Tasker, (PhD Student 1999-2002; Lecturer in Small Animal Medicine 2002-2006; Senior Lecturer in Small Animal Medicine 2006-2013; Reader in Feline Medicine 2013-present).

Early industry funded studies (2001-2003) by Dr Chris Helps resulted in the development of feline herpesvirus [1,4], feline calicivirus [4], *Chlamydophila felis* [1,4] and *Bordetella bronchiseptica* [4] qPCR assays and their use in samples from over 1700 cats from 218 European catteries. Subsequent studies by Dr Séverine Tasker (2001-2002), funded by charitable trusts, led to the development of qPCR assays to detect two species of feline haemoplasmas [2]. More recent studies (2002-2013) by Drs Chris Helps and Séverine Tasker, funded by industry and charitable trusts, led to the development of qPCR assays for the detection of feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV). To date 12 qPCR assays have been developed for a range of important feline infectious diseases including the agents causing cat 'flu', conjunctivitis and anaemia, and 10 PCRs have been developed to detect a range of genetic diseases such as kidney [5] and metabolic disorders [6].

Many of the PCR assays described above have been used in national and international research projects to study the pathogenesis and determine the prevalence of infectious organisms in cat populations. Other research studies using these PCR assays have identified risk factors for infection and described the *in vivo* kinetics of infection with feline haemoplasmas, monitored responses to antibiotic treatment [3] and evaluated the potential routes of transmission. Many of the PCRs to detect inherited genetic diseases have been used to evaluate their prevalence in cat populations [5,6] and to monitor the change in prevalence over time. From 2002-2013, over 30 papers have been published in peer-refereed scientific journals.

**Important findings from this research**

- PCRs are very sensitive and specific methods for the diagnosis of feline infectious and genetic diseases [1,2,4].
- Feline herpesvirus, feline calicivirus, *Chlamydophila felis* and *Bordetella bronchiseptica* are significantly correlated with upper respiratory tract disease in cats [4]. Risk factors associated with these diseases in cats were poor hygiene, contact with dogs with upper respiratory tract disease and large numbers of cats housed together.
- From 2005-2013, due to genetic testing and selective breeding, there has been a 90% reduction in the prevalence of the mutation causing PKD in the cat population tested.
- Older male non-pedigree cats are more likely to be infected with haemoplasmas.
- The feline haemoplasma, *Mycoplasma haemofelis*, shows cycles of infection *in vivo.*
- Marbofloxacin is an effective antibiotic treatment for feline haemoplasma infection.

- Doxycycline is an effective antibiotic treatment for feline chlamydophila infection
- Saliva is a possible mechanism of transmission of feline haemoplasmas.

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


[1] Helps, C.R., Reeves N.A., Egan, K., Howard, P. and Harbour, D.A. (2003) Detection of *Chlamydophila felis* and feline herpesvirus by multiplex real-time PCR. Journal of Clinical Microbiology. 41:2734-2736. DOI: 10.1128/JCM.41.6.2734-2736.2003

[2] Tasker, S., Helps, C.R., Day, M.J., Gruffydd-Jones, T.J. and Harbour, D.A. (2003) Use of real-time PCR to detect and quantify *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' DNA. Journal of Clinical Microbiology. 41:439-441. DOI: 10.1128/JCM.41.1.439-441.2003

[3] Tasker, S., Helps, C.R., Day, M.J., Harbour, D.A., Gruffydd-Jones, T.J. and Lappin, M.R. (2004) Use of a Taqman PCR to Determine the Response of *Mycoplasma haemofelis* Infection to Antibiotic Treatment. Journal of Microbiological Methods. 56:63-71. DOI: 10.1016/j.mimet.2003.09.017

[4] Helps, C.R., Lait, P., Damhuis, A., Björnehammar, U., Bolta, D., Brovida, C., Chabanne, L., Egberink, H., Ferrand, G., Fontbonne, A., Grazia Pennisi, M., Gruffydd-Jones, T., Gunn-Moore, D., Hartmann, K., Lutz, H., Malandain, E., Möstl, K., Stengel, C., Harbour, D.A. and Graat, E.A.M. (2005) Factors associated with upper respiratory tract disease caused by FHV, FCV, *C. felis* and *B. bronchiseptica* in cats - experience from 218 European catteries. The Veterinary Record, 156:669-673. DOI: 10.1136/vr.156.21.669

[5] Helps, C.R., Tasker, S., Barr, F.J., Wills, S.J. and Gruffydd-Jones, T.J. (2007) Detection of the single nucleotide polymorphism causing feline autosomal-dominant polycystic kidney disease in Persians from the UK using a novel real-time PCR assay. Molecular and Cellular Probes. 21:31-34. DOI: 10.1016/j.mcp.2006.07.003

[6] Grahn, R.A., Grahn, J.C., Penedo, M.C.T., Helps, C.R. and Lyons, L.A. (2012) Erythrocyte Pyruvate Kinase Deficiency Mutation Identified in Multiple Breeds of Domestic Cats. BMC Veterinary Research. 8:207. DOI: 10.1186/1746-6148-8-207.



Grants : In addition to a number of small grants from charities, the following larger grants have supported research in this area.

[i] 2011-2014 PFIZER ANIMAL HEALTH. £105,000. Tasker, S., Helps, C.R. & Stokes, C.R. Feline haemoplasmas: cultivation & defining immunological responses.

[ii] 2007-2010 PFIZER ANIMAL HEALTH. £37,650. Tasker, S. & Helps, C.R. Evaluation of haemoplasma pathogenic determinants.

[iii] 2007-2010 UNIVERSITY OF BRISTOL STUDENT PHD SCHOLARSHIP for Barker E. £48,600. Tasker, S & Helps, CR. Evaluation of haemoplasma pathogenic determinants.

[iv] 2006-2010 WELLCOME TRUST. £261,712. Tasker, S. & Helps, C.R. Pathogenicity, in vitro culture requirements, transmission and phylogeny of haemotropic mycoplasmas.

[v] 2001-2003 INTERVET, The Netherlands. £50,000. Harbour, D.A. and Helps, C.R. Prevalence of feline pathogens in European catteries detected by real-time PCR.


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

Accurate diagnosis of infectious and genetic diseases is important for the treatment and management of cats by vets, as well as to inform breeding policy to reduce the prevalence of inherited genetic diseases. The infectious agents for which qPCR assays have been developed result in serious and welfare-compromising clinical signs in cats, which can be fatal if these agents are not detected and thus not treated appropriately. Upper respiratory tract disease associated with feline herpes virus, feline calcivirus, *C. felis* and *B. bronchiseptica* cause ocular and nasal discharge, pyrexia and anorexia, whilst haemoplasmas cause haemolytic anaemia. The retroviruses FIV and FeLV are immunosuppressive agents that are associated with secondary infections and neoplasia. The pioneering PCR assays described in the underpinning research section have led to significant commercial activity within Langford Veterinary Services (LVS, a wholly owned subsidiary of the University of Bristol), in excess of **£1.7M** to date, and the following clinical benefits for affected cats:

### An increase in the number of PCR tests performed leading to specific treatments

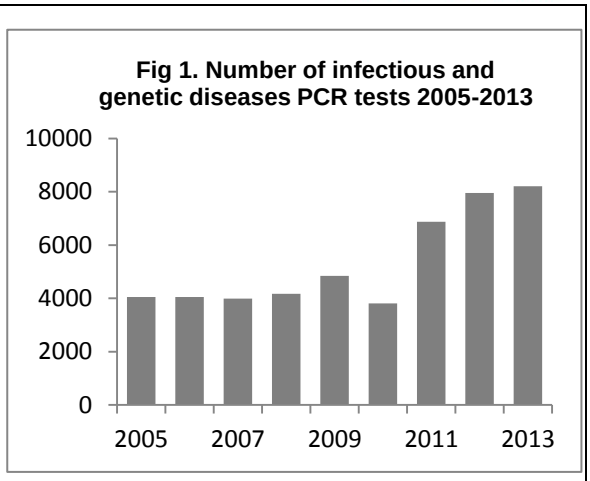


**Fig 1. Number of infectious and genetic diseases PCR tests 2005-2013**

The PCR assays are offered to vets and cat breeders throughout the UK and Europe [a]. By the end of 2013, more than **53,000 PCR tests** will have been performed on over **40,000 cats** since testing began in 2002. We have seen a significant increase in the number of PCR tests run, from ~4000 per annum in 2005-2008 to over 8000 per annum in 2013 [b] (Figure 1). Accurate detection of feline infectious agents leads to correct treatment protocols (e.g. doxycycline for *C. felis*, antivirals for FHV infection, marbofloxacin for haemoplasmas) and significantly improves health outcomes for infected cats. It also allows for appropriate management (e.g. isolation of cats, barrier nursing, keeping cats indoors, vaccination of in-contact cats) to prevent disease transmission [j].

### Reduction in the prevalence of inherited genetic diseases

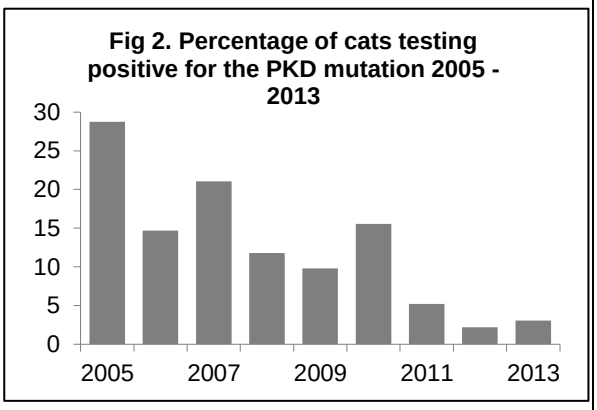


**Fig 2. Percentage of cats testing positive for the PKD mutation 2005 - 2013**

By genetic testing and selective breeding the prevalence of inherited genetic disorders can be reduced. Figure 2 shows the prevalence of the PKD mutation in 4085 breeding cats, from 735 breeders, tested between 2005 and 2013 [b]. There has been a **90% reduction in the number of cats testing positive for the PKD mutation** over the 8 years Bristol has been running the genetic test, such that less than 5% of submitted cat samples were PKD positive in 2013. This indicates that fewer cats will develop chronic kidney disease due to PKD and results in a positive impact on feline health and welfare [k].

As a result of the Bristol genetic testing service for cat breeders, Dr Chris Helps has been asked by the Governing Council of the Cat Fancy (GCCF), the main registration body for pedigree cats in the UK, to collaborate on developing breed group genetic disease test panels [c]. These will form part of the GCCF Breeder Scheme, which aims to promote responsible cat breeding and reduce the prevalence of inherited genetic diseases in pedigree cats.

### Number of vets and breeders using the PCR testing service

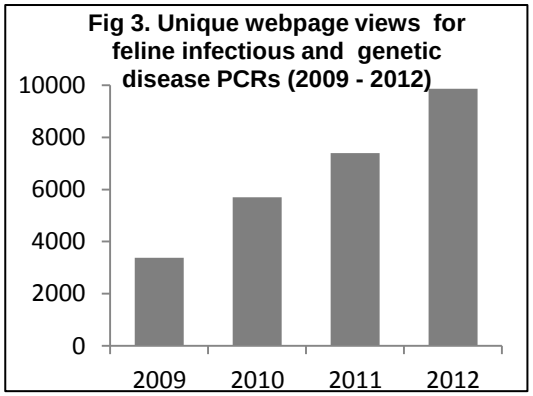
Since 2009, 705 veterinary practices and 30 referral and University diagnostic laboratories, both in the UK and Europe [d], have submitted samples for feline infectious disease qPCR testing, showing that a significant proportion (735 out of 4562 (16%, as of 2011)) of UK veterinary practices use these diagnostic tests. In addition, since 2005 over 1900 [e] cat breeders across Europe have submitted samples for genetic disease PCR testing and have used the results to inform their breeding policy, with the ultimate aim of reducing or eliminating inherited genetic diseases in their breeding lines and increasing feline health. The increase in the number of infectious disease samples submitted clearly shows that vets are using these assays, and hence value the results for diagnosing and treating cats. Worldwide Guidelines, which include this Bristol research, are now available for vets to use to direct therapy and management on the basis of qPCR results [l,m].

### Advice calls and dissemination of information

An audit of advice calls and emails undertaken over a typical week in November 2012 showed that 32 vets and 10 cat breeders contacted the Diagnostic Laboratory and Feline Centre regarding feline infectious and genetic disease PCR testing [f]. This equates to **over 2000 advice calls per year** and shows that a significant number of vets and breeders are aware of and/or use the PCR assays, value the results they obtain and benefit from talking to clinicians and geneticists who can help them interpret the results.

**National and international interest in the infectious and genetic PCRs**

Figure 3 shows unique page views for feline infectious and genetic disease PCRs taken from the Langford Veterinary Services website. Between April 2009 and November 2012 there were over **26,000 unique page views** on 15 webpages, with the average number of views increasing from ~3500 in 2009 to ~10,000 in 2012; a 285% increase in 3 years [g]. Looking at the source of these unique page views shows that 41% originated outside of the UK, with visits from 126 countries being logged [g]. This level of interest in feline infectious and genetic disease PCRs shows both the national and international impact of this work.




Through sharing reagents derived from the PCR assay development, a number of other international laboratories are now offering infectious disease testing by PCR assays e.g. Iran, Israel, New Zealand, Portugal, Sweden, Thailand, Turkey, Indonesia, France, Australia, The West Indies and USA, enabling cats worldwide to be tested for infectious agents [h,i].
In conclusion, thanks to Bristol research, qPCR tests for feline infectious and genetic diseases are now widely used to benefit feline health and welfare both in the UK and internationally. Clinical guidance targeted at veterinary surgeons working in the UK and internationally now also includes recommendations to utilise these testing procedures as part of standard practice [l,m] and the GCCF is introducing breed-panel genetic testing in collaboration with Bristol [c].

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

[a] LVS website, http://www.langfordvets.co.uk/diagnostic-laboratories/diagnostic-laboratories/pcr-acarus *(Corroborates molecular diagnostic testing service available in UK and Europe)*
[b] Excel spread sheets for Fig 1 and Fig 2 data, *(data for Figs1& 2, corroborating large number of tests run and their change over time and decrease in cats testing positive for PKD. Full spreadsheet available via RED, Bristol University)*
[c] Letter from the GCCF regarding collaboration and breed specific genetic testing. *(Corroborates national importance of genetic testing and of Dr Helps' recognised role)*
[d] List of veterinary practices, referral laboratories (including Universities) and cat breeders sending samples to LVS for disease testing. *(Corroborates large number of users of infectious disease PCR testing in UK and Europe)*
[e] Spreadsheet of cat breeders using the genetic testing service *(Corroborates impact on behaviour of over 1900 breeders who have used genetic testing to screen their cats)*
[f] Spreadsheet showing advice calls in a typical week *(Shows the importance of qPCR testing and result interpretation, and its impact on both the vets and breeders who use the service*)
[g] Unique views of the LVS website for the feline infectious and genetic disease PCR tests *(Data for Fig 3, showing worldwide interest in PCR testing)*
[h] Statements from commercial labs, local and international collaborators. *(Responses to questionnaire describing the international impact of developing tests with Bristol reagents)*
[i] Contact 1, Universiti Putra, Malaysia. *(Contact for testimonial regarding international impact of PCR test development)*
[j] Contact 2, Field Veterinary Officer, Cats Protection, National Cat Centre *(Contact for impact of infectious disease qPCR testing on cat health, disease treatment decisions and transmission)*
[k] UFAW website. http://www.ufaw.org.uk/POLYCYSTICKIDNEYDISEASEPERSIAN.php *(Describes impact of genetic testing on disease reduction, quotes Bristol research)*
[l] European Advisory Board on Cat Diseases (ABCD) Guidelines published in Journal of Feline Medicine and Surgery (2009) 11: Feline herpes virus (547-555), Feline calicivirus (556-564), FeLV (565-574) and *C. felis* (605-609). *(Impact via dissemination of information about PCR testing as a recommended method)*
[m] Canine and Feline Infectious Diseases by Jane E Sykes (2013). Elsevier, Health Sciences Div. Ch 5 Nucleic acid detection assays, Ch 22 FeLV, Ch 23 Feline respiratory viral infections, Ch 33 Chlamydial infections, Ch 41 Hemoplasma infections. SBN 1437707955 *(Impact as for [l])*