The Origin, Evolution and Diagnosis of Seneca Valley Virus, a New Vesicular Disease-Causing Picornavirus of Pigs

Nick J. Knowles, Katarzyna Bachanek-Bankowska, Jemma Wadsworth, Veronica L. Fowler & Donald P. King

Vesicular Disease Reference Laboratory Group

OS'16 Open Session of the EuFMD - Cascais –Portugal 26-28 October 2016

European Commission for the Control of Foot-and-Mouth Disease

BBSRC bioscience for the future

The Pirbright Institute receives strategic funding from BBSRC.

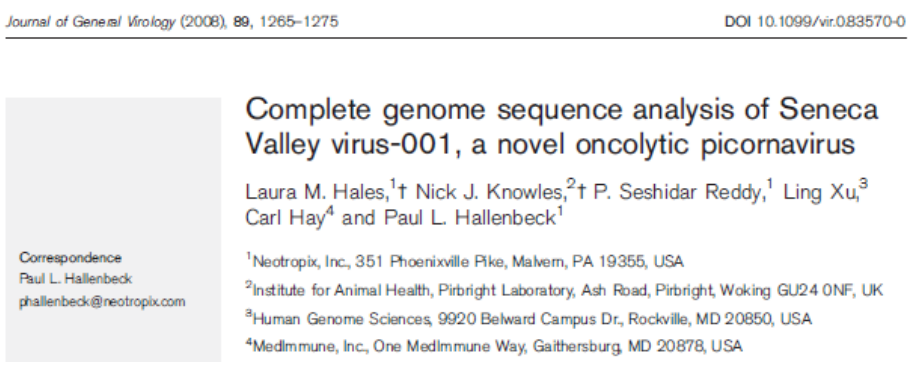
www.pirbright.ac.uk

http://www.picornaviridae.com/senecavirus/senecavirus.htm

Discovery of Seneca Valley Virus

Between 1988 and 2001, at least 10 unidentified viruses were isolated from pigs by the National Veterinary Services Laboratory, Ames, Iowa (John Landgraf, pers. comm.) and one by the Plum Island Animal Disease Center (Jim House, pers. comm.). Seven virus isolates were received at Pirbright in Jan 1999 (PIADC) and Sep 2003 (NVSL). These were partially sequenced at Pirbright in 2003 and shown to be the same novel picornavirus.

A virus was identified as a contaminant of PER.C6® cells at NeoTropiX Inc. and named **Seneca Valley virus**. The complete genome was sequenced in 2004 (Hales et al., 2008). Sequence comparisons showed the NVSL/PIADC isolates and SVV were the same virus. SVV has also been used as an anti-cancer agent (Reddy *et al*., 2007).



Journal of General Virology (2008), **89**, 1265–1275 DOI 10.1099/vir.0.83570-0

Complete genome sequence analysis of Seneca Valley virus-001, a novel oncolytic picornavirus

Laura M. Hales,[1]† Nick J. Knowles,[2]† P. Seshidar Reddy,[1] Ling Xu,[3] Carl Hay[4] and Paul L. Hallenbeck[1]

Correspondence
Paul L. Hallenbeck
phallenbeck@neotropix.com

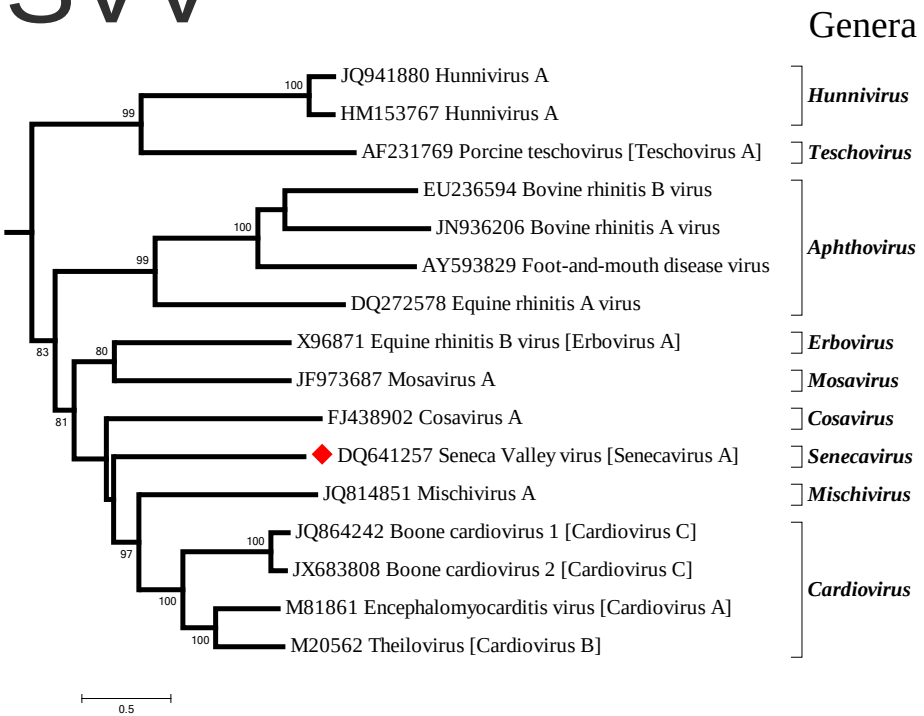
[1]Neotropix, Inc., 351 Phoenixville Pike, Malvern, PA 19355, USA
[2]Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking GU24 0NF, UK
[3]Human Genome Sciences, 9920 Belward Campus Dr., Rockville, MD 20850, USA
[4]MedImmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA

Reddy PS, Burroughs KD, Hales LM, Ganesh S, Jones BH, Idamakanti N, Hay C, Li SS, Skele KL, Vasko AJ, Yang J, Watkins DN, Rudin CM, Hallenbeck PL. (2007). **Seneca Valley virus, a systemically deliverable oncolytic picornavirus, and the treatment of neuroendocrine cancers.** J. Natl. Cancer Inst. 99:1623-33.

http://www.picornaviridae.com/senecavirus/senecavirus.htm

Classification of SVV

P1 Capsid Region
ML tree



Order: *Picornavirales*

Family: *Picornaviridae*

Genus: *Senecavirus*

Species: *Senecavirus A*

Virus (common) name: Seneca Valley virus 1 (single serotype)

SVV genome organisation:

VPg+5'UTR$^{IRES-IV}$[L/1A-1B-1C-1D-2A^{npgp}/2B-2C/3A-3B^{VPg}-3C^{pro}-3D^{pol}]3'UTR-poly(A)

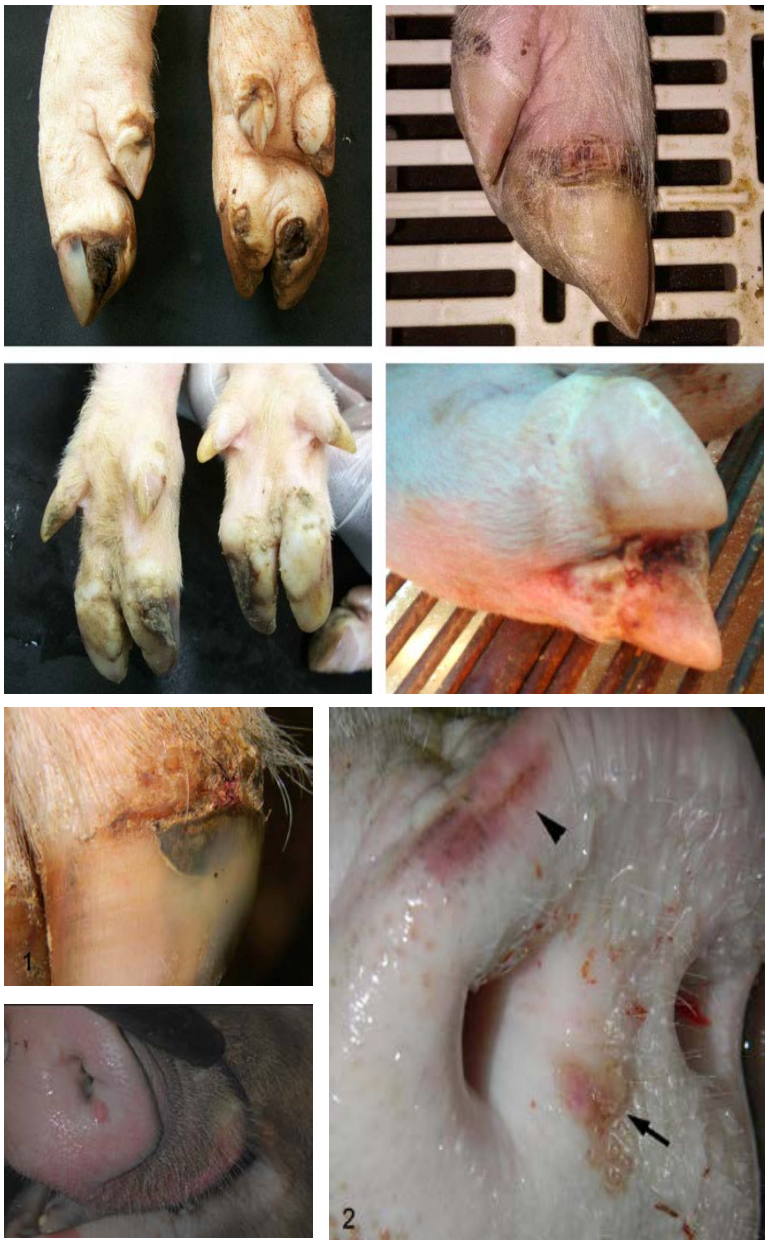
http://www.picornaviridae.com/senecavirus/senecavirus.htm

SVV Disease

- Inoculation of pigs with 2 early SVV isolates failed to produce any disease.
- However, there has been a strong association with idiopathic vesicular disease since the early 2000's.

Symptoms include:

- Vesicles of the snout, mouth, and/or just above the hoof
- Lameness, fevers, lack of energy and/or appetite
- 4-10 day increase in piglet mortality with/without diarrhoea



Amass *et al*., 2004

Experimental infection of pigs

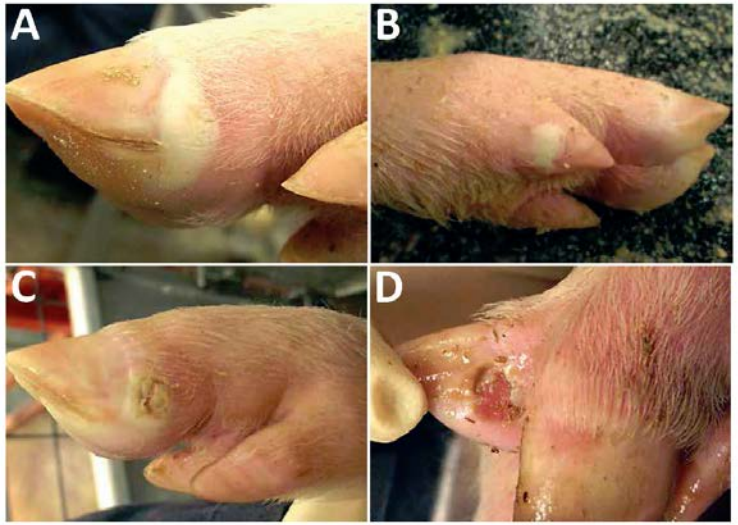


Figure 1. Vesicular lesions on feet of pigs experimentally infected with Senecavirus A. A) Blanched, intact, fluid-filled vesicle on lateral coronary band of toe. B) Intact vesicle on coronary band of medial dewclaw. C) Ruptured vesicle on coronary band of toe. D) Ruptured vesicle with ulceration and erosion in interdigital space.

US Department of Agriculture, Ames, Iowa &
Iowa State University College of Veterinary Medicine, Ames

DISPATCHES

Emerg. Infect. Dis. (2016) 22: 1246-1248.

Vesicular Disease in 9-Week-Old Pigs Experimentally Infected with Senecavirus A

Nestor Montiel,[1] Alexandra Buckley,[1] Baoqing Guo,[1] Vikas Kulshreshtha, Albert VanGeelen, Hai Hoang, Christopher Rademacher, Kyoung-Jin Yoon, and Kelly Lager

Senecavirus A has been infrequently associated with vesicular disease in swine since 1988. However, clinical disease has not been reproduced after experimental infection with this virus. We report vesicular disease in 9-week-old pigs after Sencavirus A infection by the intranasal route under experimental conditions.

The Study

We purchased 17 conventionally raised weaned pigs and housed them until 9 weeks of age at the campus of the Agricultural Research Service, National Animal Disease Center, US Department of Agriculture (Ames, IA, USA), in accordance with Institutional Animal Care and Use Committee protocols (protocol ACUP 2867). At this time, each pig received an intranasal inoculation of a cell culture–propagated SVA isolate (SVA15–41901SD, third passage) (B. Guo, unpub. data) at a dose of 5×10^7 PFU/animal. Challenge virus was grown in a swine testicular cell line (CRL-1746; American Type Culture Collection

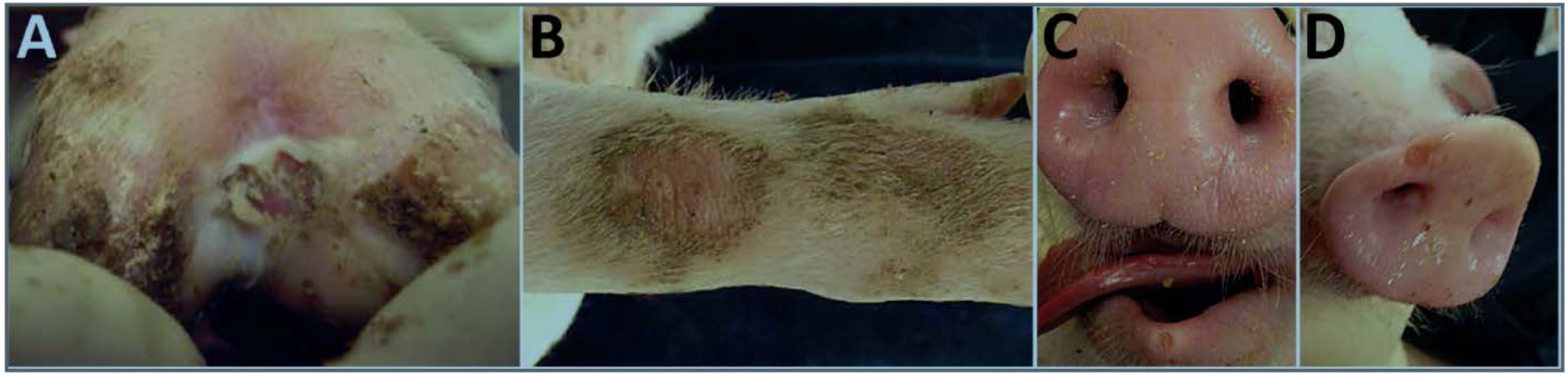


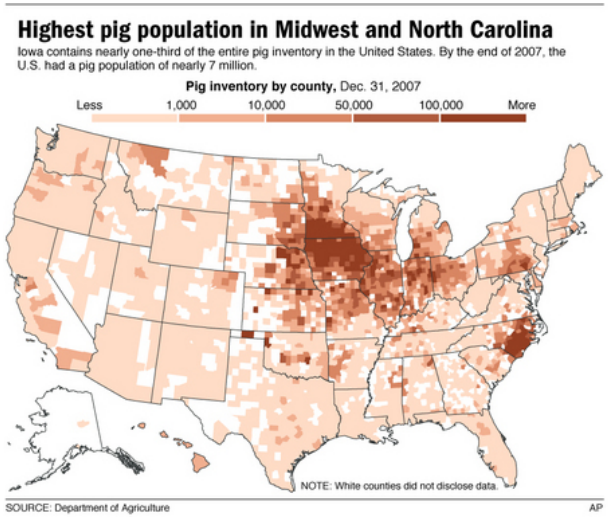
Figure 2. Vesicular and skin lesions on feet and snout of pigs experimentally infected with Senecavirus A. A) Ruptured vesicle with deep ulceration, necrosis, and crusting in interdigital space. B) Skin abrasion on carpus. C) Vesicle and erosion on lower lip. D) Vesicle on snout.

http://www.picornaviridae.com/senecavirus/senecavirus.htm

SVV Occurrence in Pigs (1988-2016)

USA

1988
2001
2015
2015
1989
2015
2016
1992
1994
2000
2010
2015
1990
2001
2015
1988
2015
2005
1999
1997



Canada

2007
2016
2011
(unknown location)

Brazil

2015
2015
2015
2015

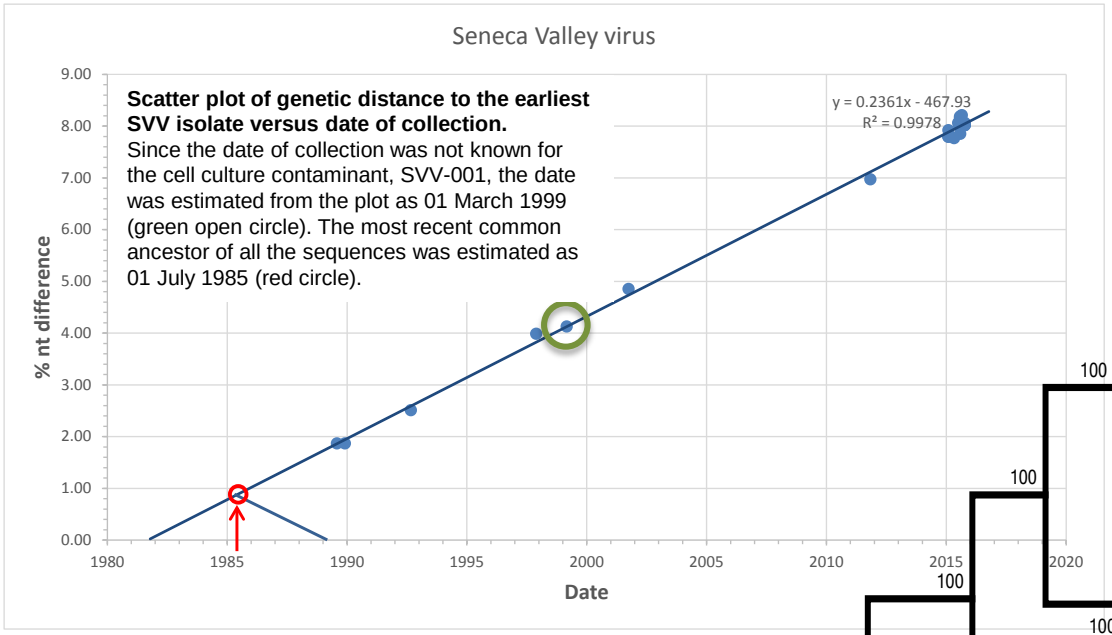
400 km
200 mi
© d-maps.com

China

2016
2015

http://www.picornaviridae.com/senecavirus/senecavirus.htm

SVV Evolution



7172 sites

All isolates from pigs, except…

- cell culture contaminant
- mouse
- environment

Current diagnostic assays

Current laboratory methods include:

- Virus isolation (IB-RS-2, PK-15, LK, Vero, LLC-MK2, RK-13)
- Virus neutralisation
- Competitive ELISA
- Conventional reverse transcriptase (RT) PCR (RT-PCR): targeting 3D
- SYBR Green-based real-time RT-PCR assay (rRT-PCR): targeting VP1

There are no rRT-PCR assays which which target 3D

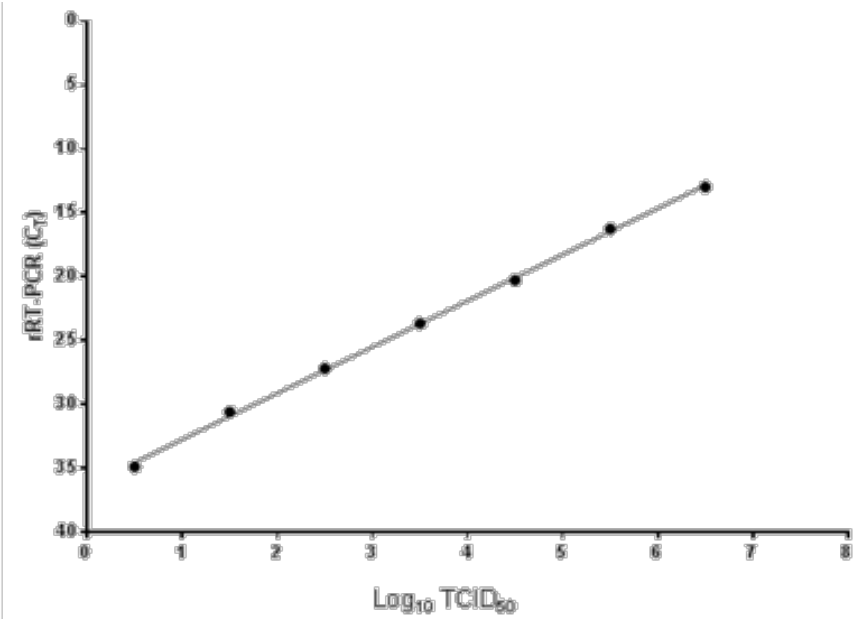
- With the increase in full genomes available on GenBank, the aim of this study was to design a TaqMan® based rRT-PCR assay based on highly conserved regions of the SVV genome (3D) that complement the front-line rRT-PCR tests used for other vesicular diseases.

(Knowles et al., 2005; Yang et al., 2011; Leme et al., 2015; Bracht et al., 2016)

rRT-PCR Design & Analytical Performance

- Ten published SVV full genomes (DQ641257, KC667560, KR063107, KR063108, KR063109, KT757280, KT757281, KT757282, KT321458 and KX349733) and seven unpublished SVV partial genome sequences (EU271766-EU271772) were used for the design of the primers and probe.

- The analytical sensitivity of the rRT-PCR is 0.79 $TCID_{50}$/ml corresponding to a C_T value of 36.8 as determined by parallel virus isolation using a 2-fold dilution series of a SVV isolate (KS15-01).



- The rRT-PCR assay detected:
  - 8/8 SVV isolates collected over the period 1988-2015;
  - 11/12 + nasal (a) and 10/12 + rectal (b) swab pools.
  - No false positives were reported for nasal swabs.
  - 4 false positives were reported for rectal samples but were above the diagnostic cut off (C_T 36).

- Diagnostic specificity was 100% when using RNA extracted from FMDV, SVDV, VSINV and VSNJV) and RNA or DNA extracted from other 14 other common swine viruses.

rRT-PCR Summary

- This new rapid and sensitive rRT-PCR could be adopted by reference laboratories to promptly detect SVV in vesicular disease cases that have been negated for notifiable diseases such as FMD.

- Since the assay design targets the highly conserved 3D region, this SVV rRT-PCR assay is expected to be more robust than other assays targeting more variable regions of the SVV genome.

- In addition, this assay could be used as a research tool for pathogenesis and epidemiological studies, including investigations into the periods and route of virus excretion, the existence of virus carrier status, and the presence of virus in non-porcine hosts.

Conclusions/observations

- Seneca Valley virus was probably introduced into US pigs circa 1985 from an unknown source.
- In the US, SVV has occurred in most of the major pig-producing areas.
- Spread to Canada occurred from Minnesota in 2007.
- Recently SVV has spread to Brazil and China.
- Outbreaks of idiopathic vesicular disease have been associated with SVV in all these countries.
- Recently eight truckloads of pigs from Ontario were turned away at the US border due to SVV vesicular lesions.
- Diagnostic assays, both molecular and virological are in now place to allow the laboratory identification of SVV.

Future

- We predict that SVV will spread around the world in the next few years. This may present problems with differential diagnosis with other vesicular disease causing viruses of pigs (foot-and-mouth disease virus, swine vesicular disease virus, vesicular stomatitis virus and vesicular exanthema of swine virus).

- Searching for the origin of SVV should be concentrated on animals associated with pig farms in the USA. It is highly probable a rodent source may be found.

http://www.picornaviridae.com/senecavirus/senecavirus.htm

Acknowledgements

Jim House (retired), PIADC

John Landgraf (retired), NVSL, Ames

Susanna Williamson

Jianfa Bai
Russell H. Ransburgh
Elizabeth G. Poulsen
Xuming Liu
Gary A. Anderson
Ying Fang

Valerie Mioulet

The Pirbright Institute receives strategic funding from BBSRC.