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Breeding for disease resistance & reduced disease transmission

Insights from experimental, field & modelling studies

Andrea Doeschl-Wilson

Professor in Infectious Disease Genetics & Modelling

Andrea.wilson@roslin.ed.ac.uk

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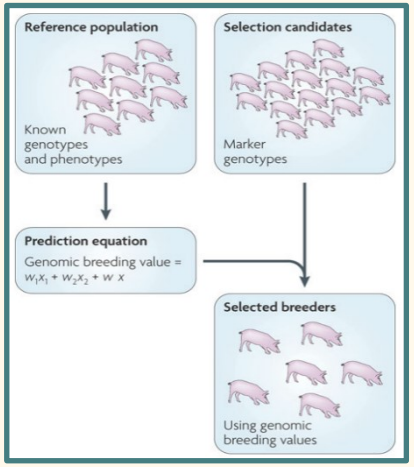
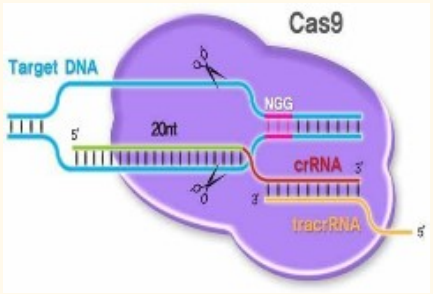
Outline

1. Principles of genetic disease control in the context of novel technologies: genomic selection vs gene editing
2. Case study PRRS
   a. exploiting natural host genetic variation in PRRS resistance
   b. gene editing solutions?





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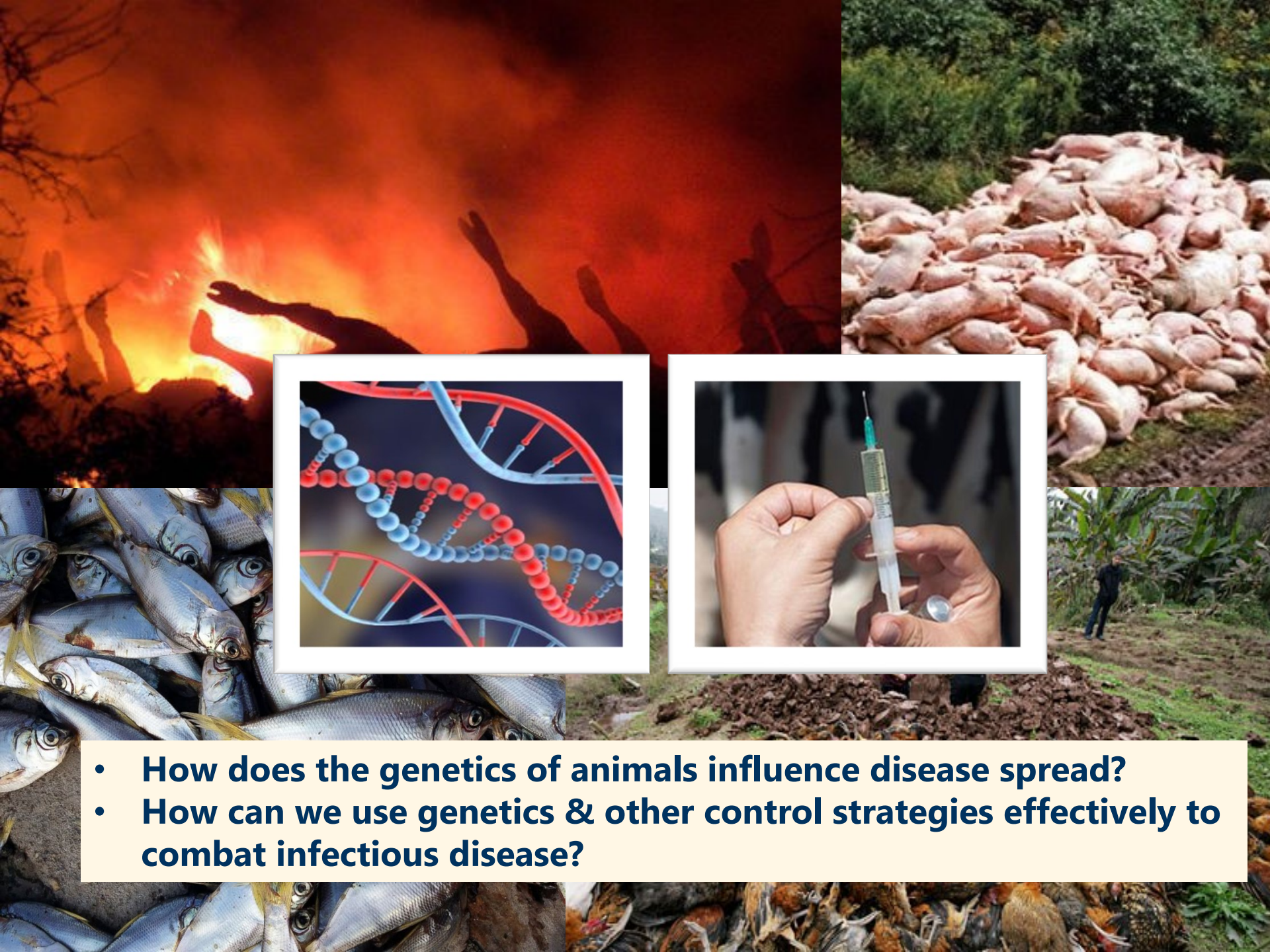
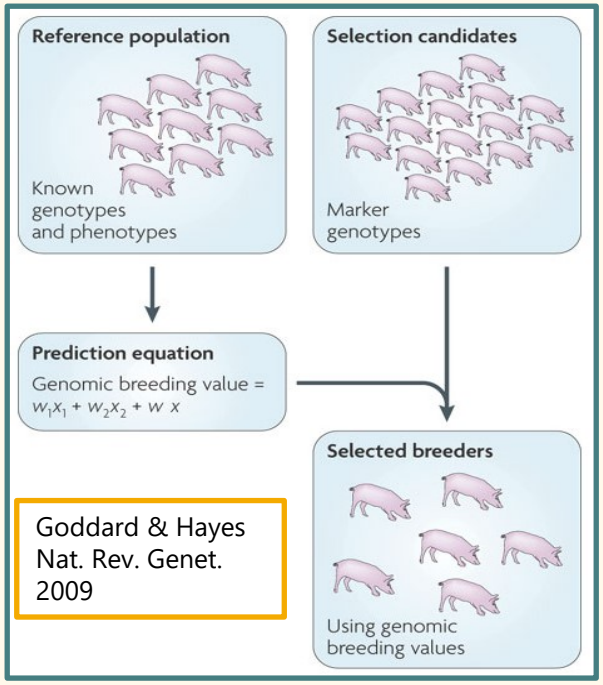


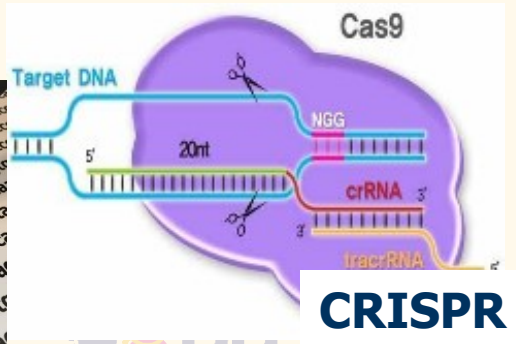
- How does the genetics of animals influence disease spread?
- How can we use genetics & other control strategies effectively to combat infectious disease?

2 approaches for genetic disease control

- **Exploit existing natural genetic variation** in host response to infections
  - Genomic selection



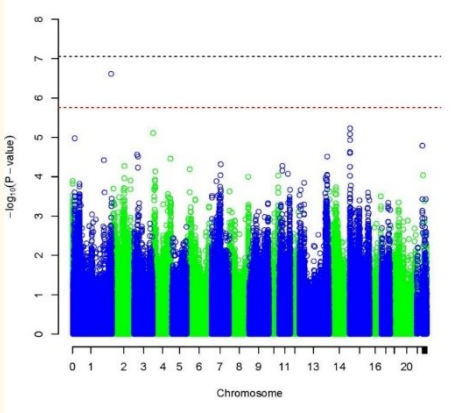
- **Purposefully change the genome**
  - Genome editing

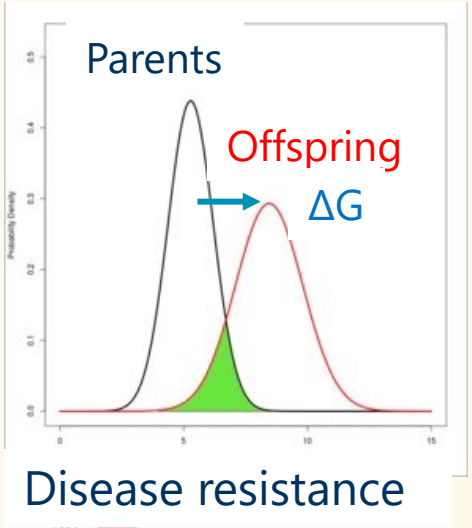


CRISPR Cas9

Key principles of genetic disease control

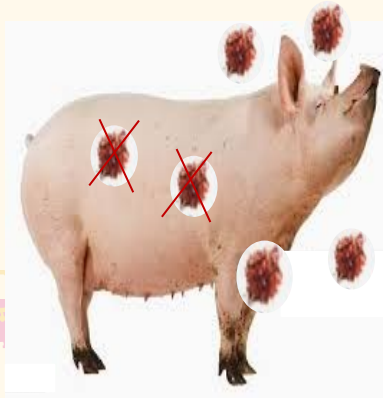
- Identify which genomic loci / genes are associated with better responses
- Identify the animals that either naturally have the beneficial variants at these loci, or introduce beneficial variant by editing the target gene
- Select these animals as parents for the next generation
  - Offspring generation will have on average better response (ΔG)





‘Breeding for disease resistance’ What does it mean & how to measure it?

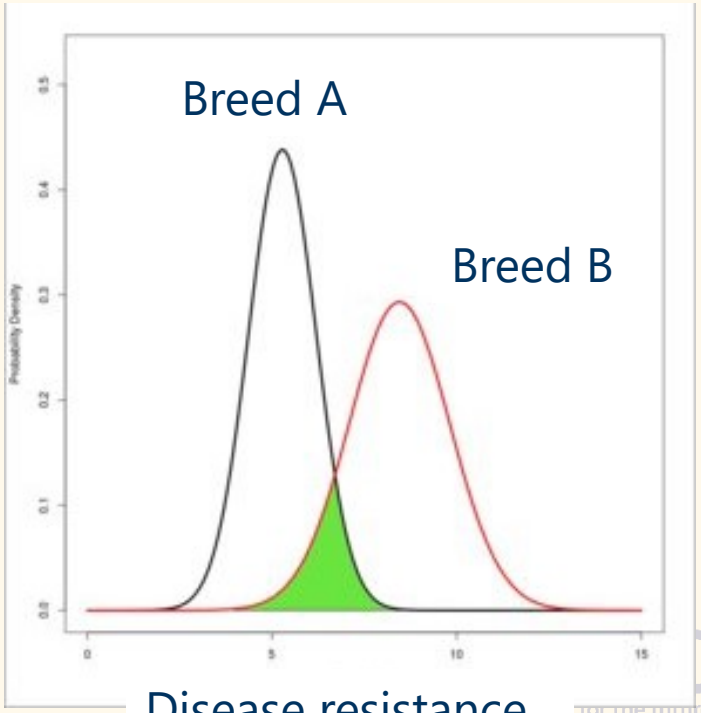
- Often poorly defined in animal breeding, due to large data demand for quantitative genetic analyses
- Resistance
  - to becoming infected (diagnostic test results; pathogen load)
  - to developing disease (signs / symptoms)
  - to dying (alive / dead; time of death)
- Breeding for disease resistance does not necessarily reduce disease prevalence



Some common ‘genetic’ myths

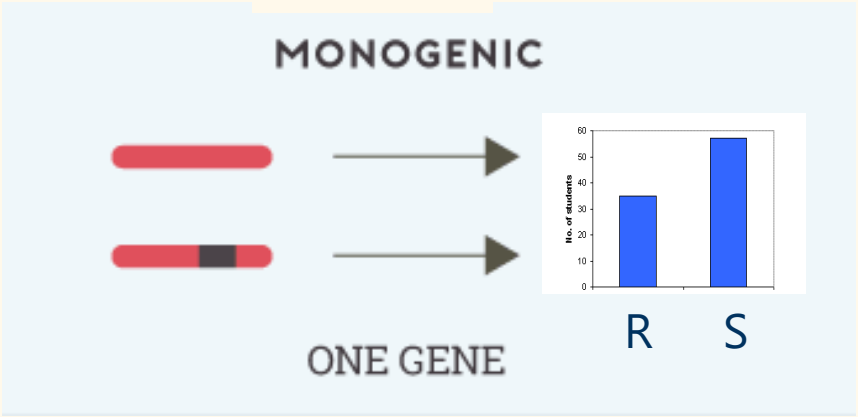
Myth 1: “Genetic differences occur only between breeds but not within breeds”

- Evidence for **between breed** differences in disease resistance
- There is also strong exploitable **within breed** variation

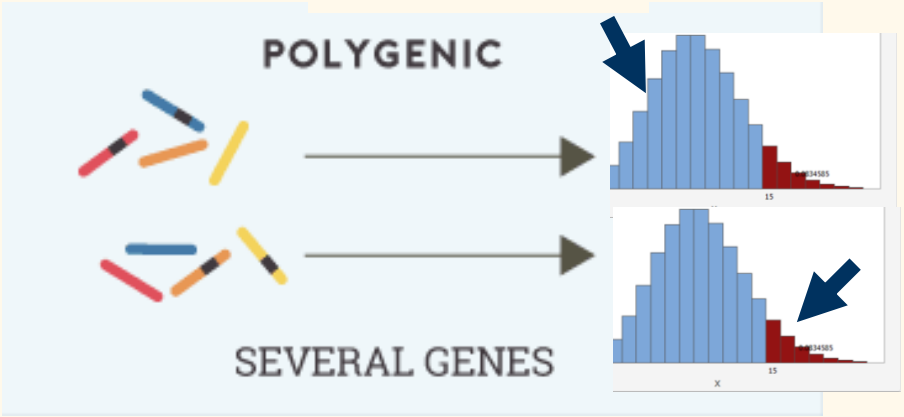


Myth 2: “Disease resistance is controlled by a single gene and animals are either resistant or not”

RARE



COMMON



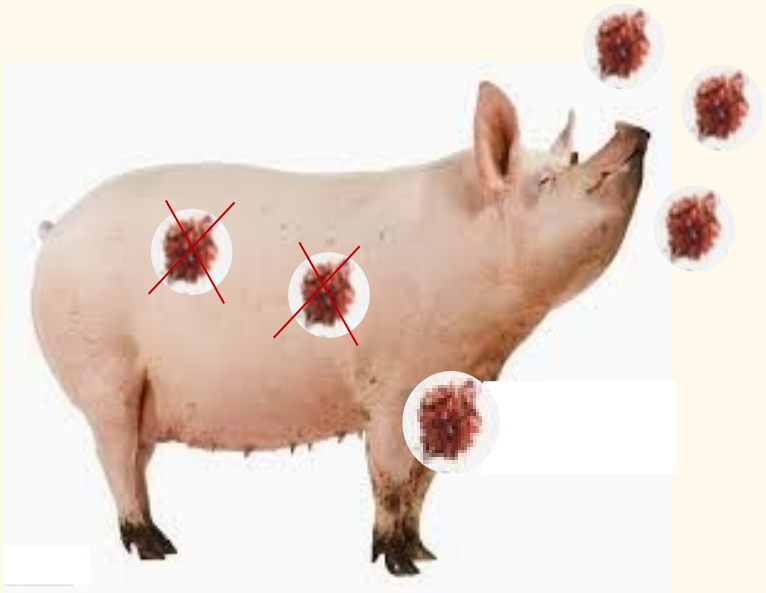
Individual genes explain usually < 5% of the total variation

Different implications on breeding strategies and on pathogen evolution

Myth 3: “Resistance is the only trait that matters for genetic disease control”

Reduce incidence	Mitigate impact
• Eliminate pathogens • Improve host resistance / infectivity	• Decrease pathogen virulence • Improve host tolerance

Resistance



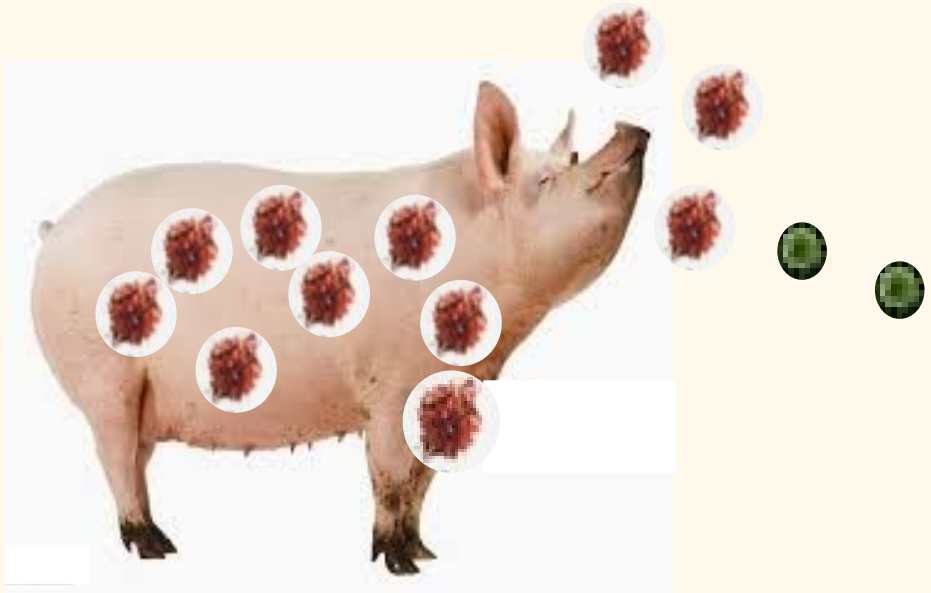
Resistance:
= ability to block pathogen entry or restrict pathogen replication

High resistance corresponds to:

- Low pathogen burden
- High health and production
- Low risk of transmission

Desirable target trait to maintain high individual health & performance

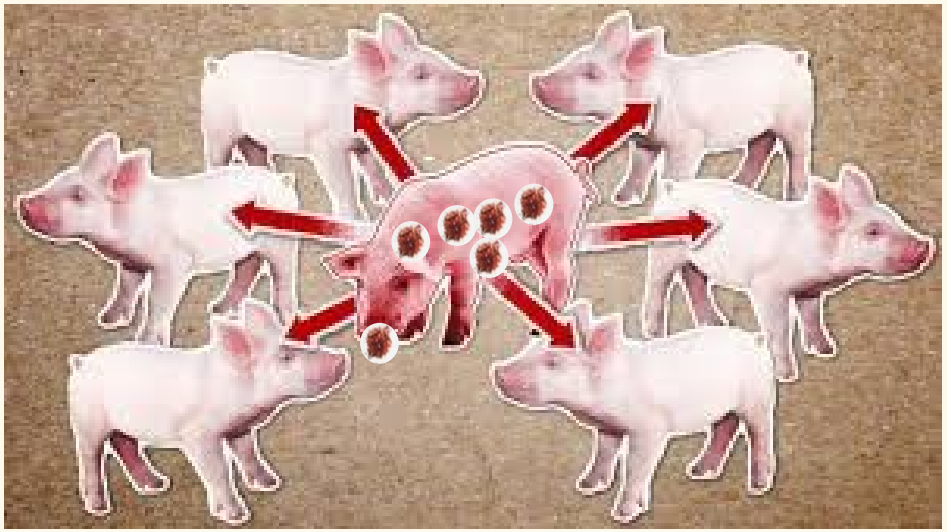
Tolerance



Tolerance:
= ability of a host to limit the detrimental impact of infection on health / performance,
without necessarily affecting pathogen burden per se

- **Desirable target trait to maintain high performance in the face of constant exposure to infection**
- **But how does high tolerance affect transmission?**

Infectivity



Early identification & removal of the most infectious individuals would be a very effective disease control

Infectivity:
= ability of an infected individual
to transmit the infection

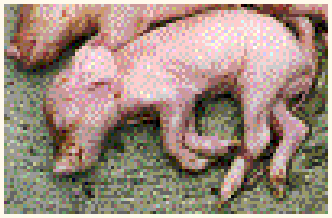
- Many recent epidemic outbreaks attributed to '**super-spreaders**':
  - 20% individuals responsible for 80% of transmissions

Myth 3: “Resistance is the only trait that matters for genetic disease control”

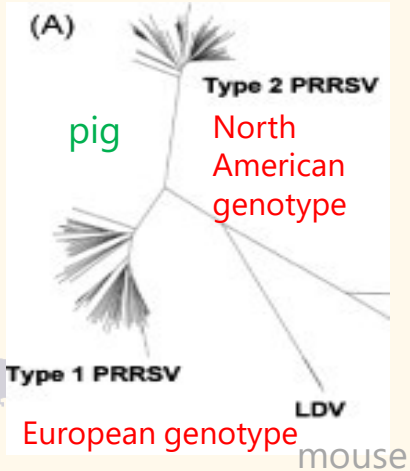
Reduce incidence	Mitigate impact
• Eliminate pathogens • Improve host resistance / infectivity	• Decrease pathogen virulence • Improve host tolerance

Can we produce animals with greater genetic resistance & tolerance & lower infectivity?

Case study PRRS



- **Costly, endemic viral disease in pigs**
  - **Estimated production losses in US / Europe: €1.5 Billion per year**
- **Health & production effects**
  - **Morbidity & reduced growth in piglets**
  - **Reproductive loss in sows**
  - **Secondary infections → Increased use of antibiotics**
- **Conventional control failing**
  - **Vaccination & biosecurity failing**
  - **RNA virus with very high mutation & recombination rate**



The PRRS Host Genetics Consortium & partners

PHGC
PRRS Host Genetics Consortium

Academic Partners

Funding

Industry Partners

Mostly North-America!

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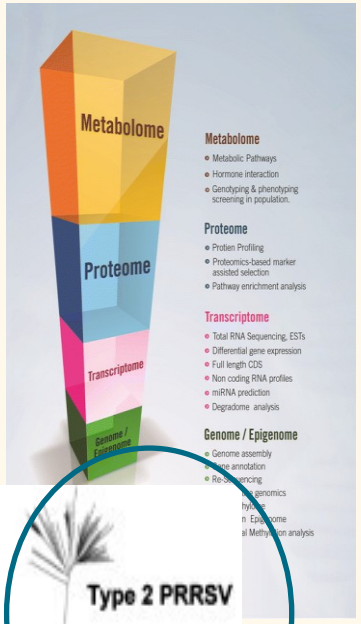
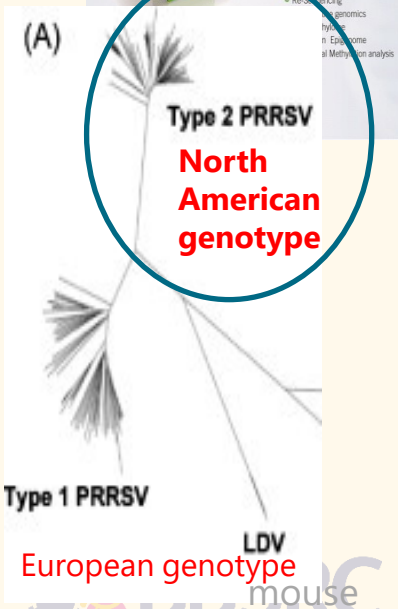
PHGC studies (2007 – present)

Overall aim: Explore the role of host genetics as an avenue of PRRS control

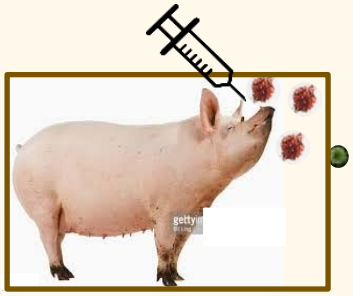
- Large scale PRRSV challenge experiments & polymicrobial natural disease challenge
  - Different commercial pig breeds
  - Different PRRSV strains (type 2!)
  - With / Without vaccination
  - Co-infections (esp. PCV2)
- Phenotypes & -omic data from thousands of commercial pigs

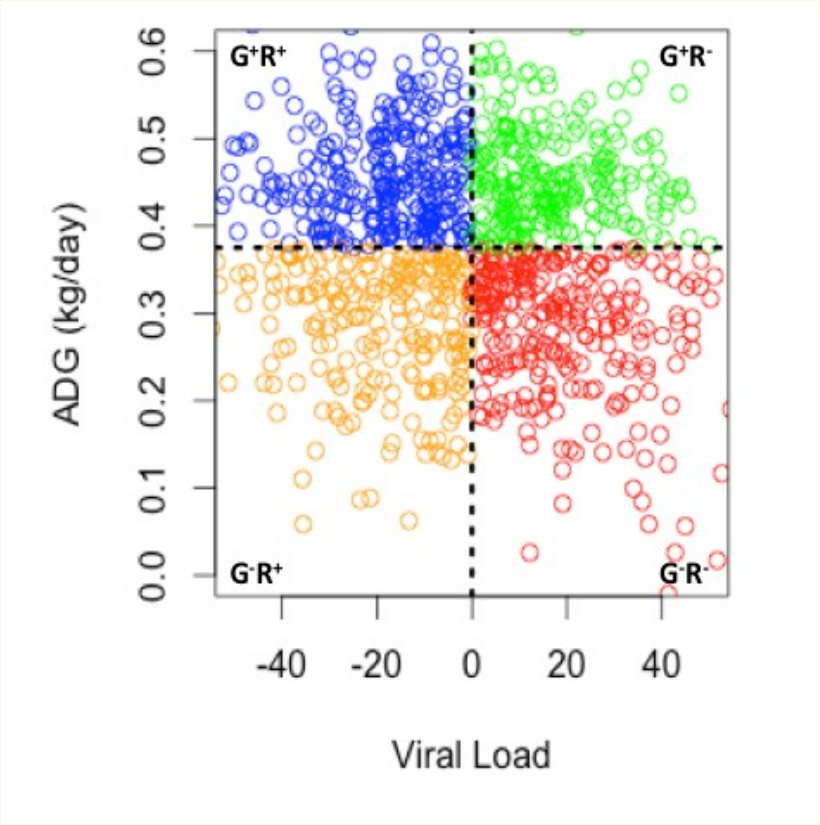




Rowland et al. Front. Genet 2012; 3:260

Insights from large scale PRRSV challenge experiments





- All >1500 challenged pigs became viraemic
- Much genetic and phenotypic variation in response to PRRSV infection
- Response is mostly controlled by many genes, each with small effect:
  h2 (Viral Load) = 0.31
  h2 (Weight gain) = 0.30
  rg (VL, WG) = -0.45

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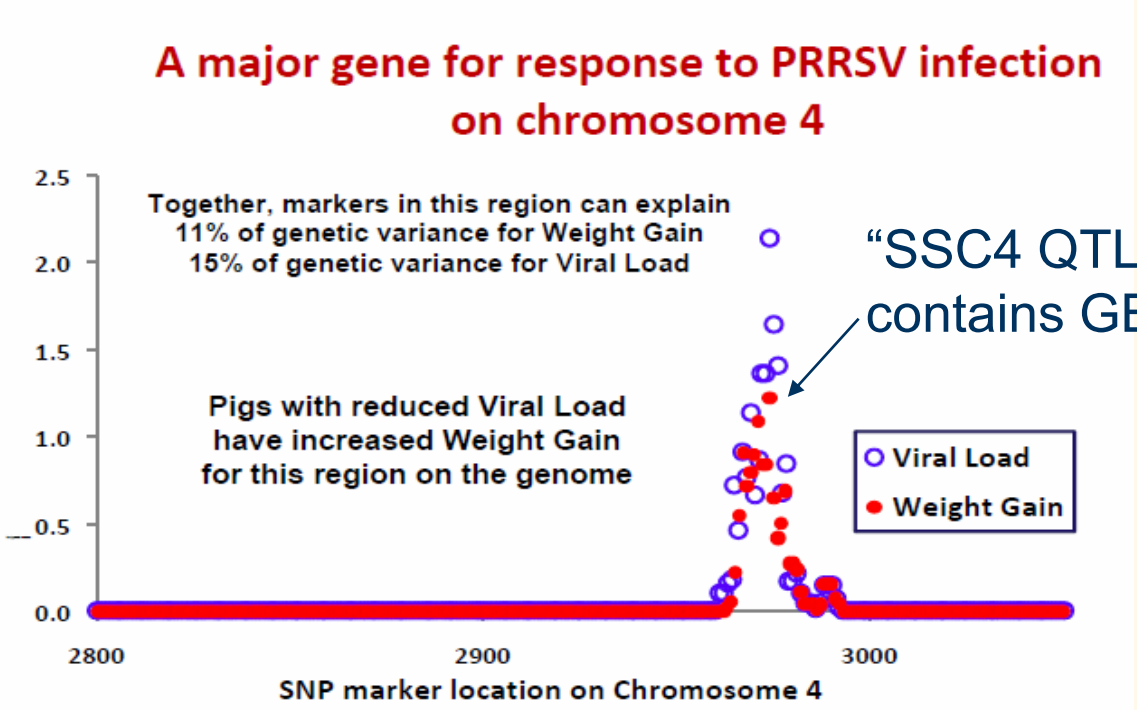
The ‘natural’ PRRSV resistance gene



“SSC4 QTL” contains GBP5 gene

(Boddicker et al. JAS 2012; GSE 2014)

Koltes et al. BMC Genomics (2015) 16:412
DOI 10.1186/s12864-015-1635-9

BMC Genomics

RESEARCH ARTICLE Open Access

Identification of a putative quantitative trait nucleotide in guanylate binding protein 5 for host response to PRRS virus infection

James E. Koltes[1†], Eric Fritz-Waters[1†], Chris J. Eisley[1,2], Igseo Choi[3], Hua Bao[4], Arun Kommadath[4], Nick V. L. Serão[1], Nicholas J. Boddicker[5], Sam M. Abrams[3], Martine Schroyen[1], Hyelee Loyd[1], Chris K. Tuggle[1], Graham S. Plastow[4], Leluo Guan[4], Paul Stothard[4], Joan K. Lunney[3], Peng Liu[2], Susan Carpenter[1], Robert R. R. Rowland[6], Jack C. M. Dekkers[1] and James M. Reecy[1*]

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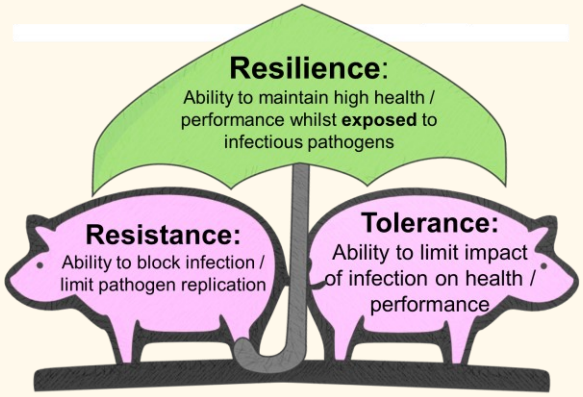
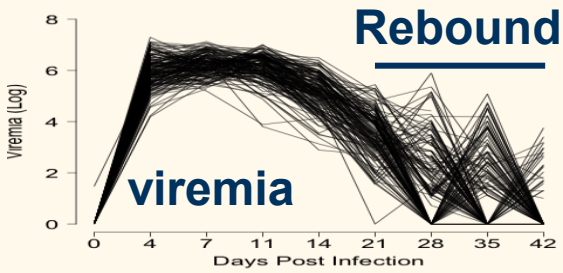
How does this gene affect individual and herd health?





Pigs that carry the beneficial gene variant have:

- Lower virus load when infected (Boddicker et al. JAS 2012; GSE 2014)
- Faster growth when infected (Boddicker et al. JAS 2012, Lough et al. GSE 2018)
- Less prone to experience viremia rebound (Go et al., BMC Sys. Biol. 2018)
- Lower farrowing mortality during PRRS outbreaks (Orrett PhD thesis 2018)
- More effective vaccine response (Dunkelberger et al. JAS 2017)
- Higher resistance to PCV2 when vaccinated (Dunkelberger et al. JAS 2017)
- Faster growth & fewer number of treatments in a polymicrobial natural disease challenge (Jeon et al. Livest. Sci 2021)

→ **Candidate resistance gene to be included in breeding programmes?**

Some important remaining questions

- Are the results valid for European (UK) pig populations and European (UK) PRRSV strains & vaccines?
- Does the GBP5 gene have any negative effects on production traits?
- Does the GBP5 gene affect PRRSV transmission?

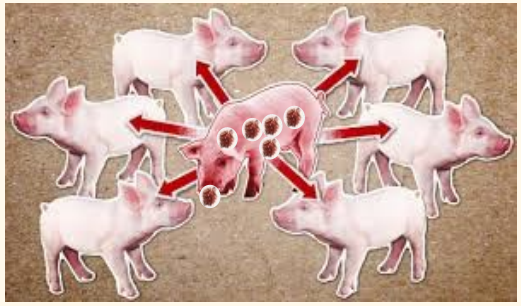
Are the results valid for European (UK) pig populations and European (UK) PRRSV strains & vaccines?

- Inconclusive results from a small PRRSV challenge & vaccination study in Spain: PRRS resistance marker had a beneficial effect on growth rate in vaccinated pigs, but no effect in infected pigs (Abella et al., Res. Vet Sci 2015)
- **Urgent need to validate the results from the North-American studies**

Does the GBP5 gene have any negative effect on production traits?

No evidence based on published results from large scale genetic evaluations carried out by the pig industry or from academic research projects

Does the GBP5 gene affect PRRSV transmission?



- Results from a recent small-scale genetic transmission experiment (~400 pigs):

  no significant effect of the GBP5 gene on the susceptibility of pigs to PRRSV infection nor on their infectivity

  → No indication that selection for GBP5 would reduce the transmission of PRRSV

- Analogous results from a vaccine transmission experiment (Chase-Topping et al., Vaccine 2020)

Genome editing solutions to PRRS

RESEARCH ARTICLE

Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function

Christine Burkard[1], Simon G. Lillico[1], Elizabeth Reid[2], Ben Jackson[2], Alan J. Mileham[3], Tahar Ait-Ali[1], C. Bruce A. Whitelaw[1], Alan L. Archibald[1]*

1 The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, **2** The Pirbright Institute, Ash Road, Pirbright, Woking, United Kingdom, **3** Genus plc, DeForest, Wisconsin, United States of America

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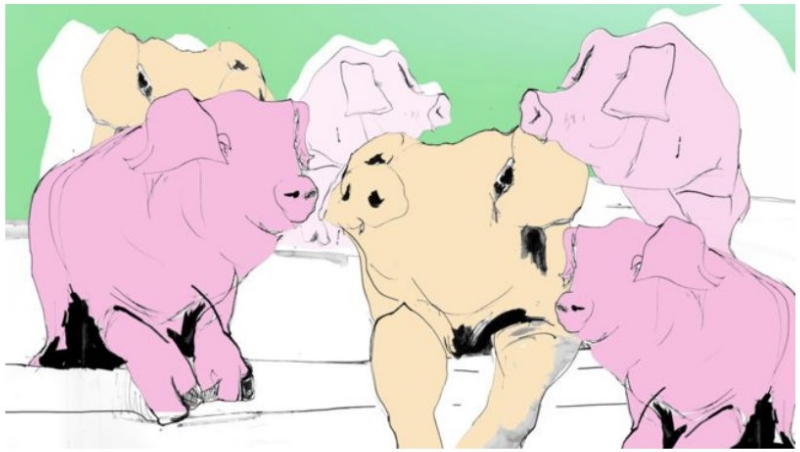
Gene-edited farm animals are on their way

By Pallab Ghosh
Science correspondent, BBC News

20 June 2018 Share

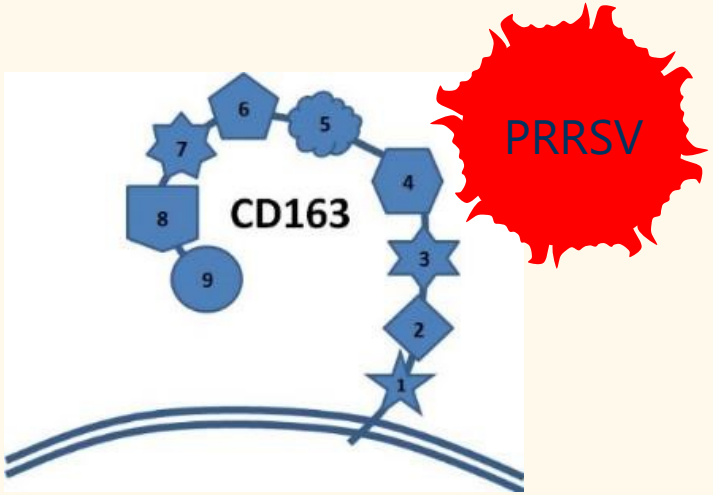


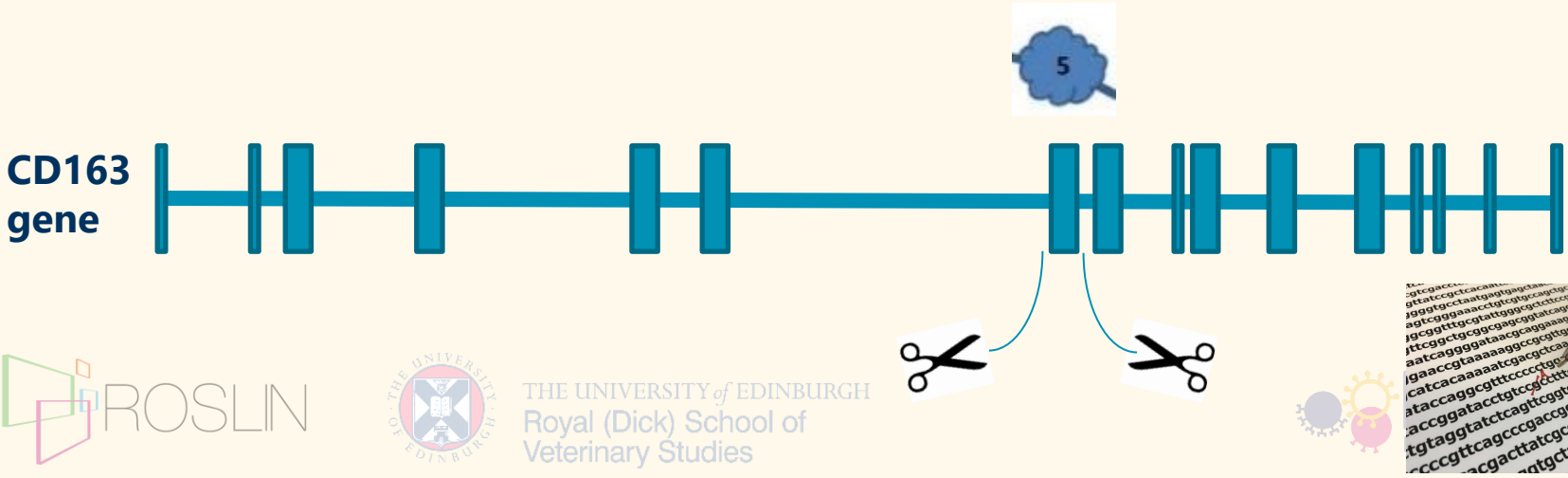
Scientists have created pigs that are immune to one of the world's costliest livestock diseases.

BREAKING

Genome editing solutions to PRRS

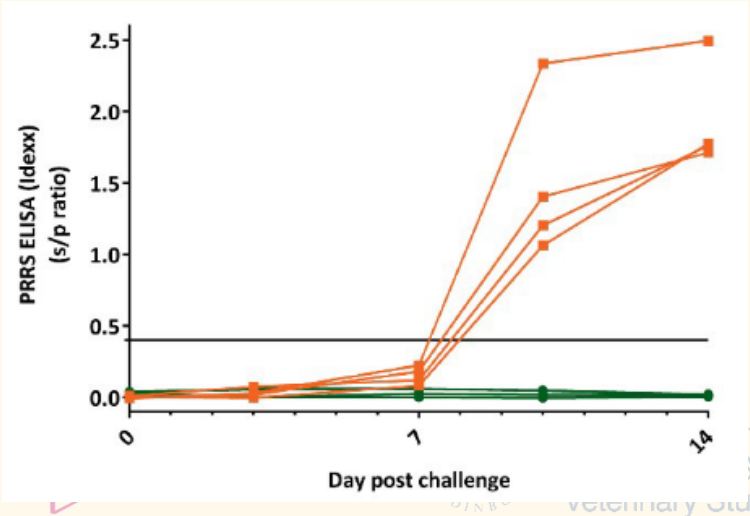
- PRRSV binds to the CD163 receptor on cell surface
- There is no natural genetic variation in the host CD163 gene
- But it is now possible to edit the CD163 host gene





Genome editing outcome

"Edited pigs" without the CD163 domain 5



Edited pigs

- Look normal
- Seem to function normally
- **But are COMPLETELY resistant to PRRSV infection (PRRSV1 & 2!)**

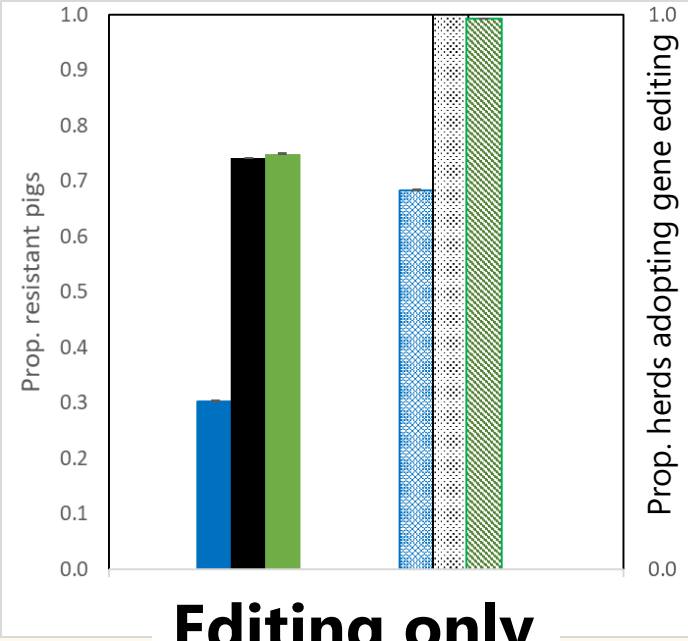
Questions

- **Is it possible to nationally eradicate PRRS with gene editing?**
- **Is it practically feasible?**
  - How many genetically resistant pigs are needed?
  - How would they need to be distributed across herds?
  - How long will it take to produce sufficient commercial pigs with the resistance gene?
  - ….

Petersen et al., PNAS 2022

What does it take to eliminate PRRS?

Average baseline disease risk R_0 = 1.5



Disease eradication through gene editing alone requires large number of genetically resistant pigs, good disease surveillance & large scale adoption

Distribution of genetically resistant pigs across herds:

- Optimal
- Comprehensive
- Concentrated
- Unregulated

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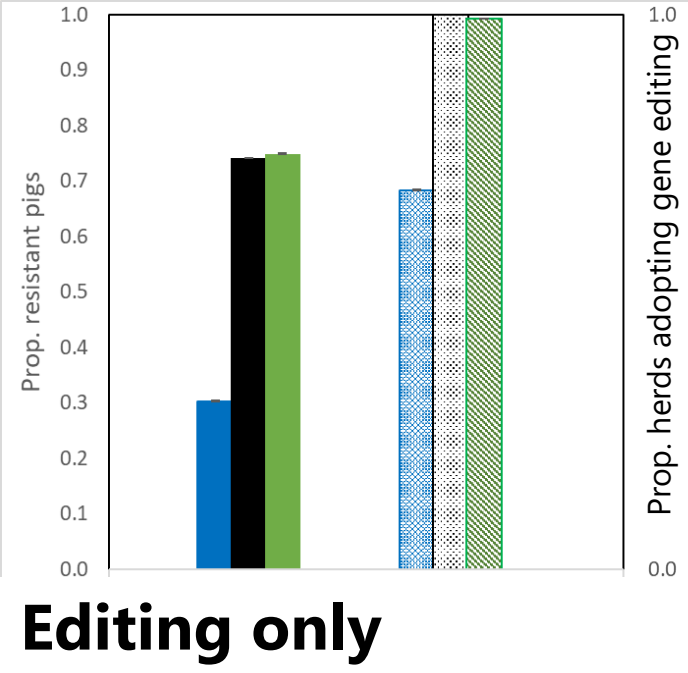
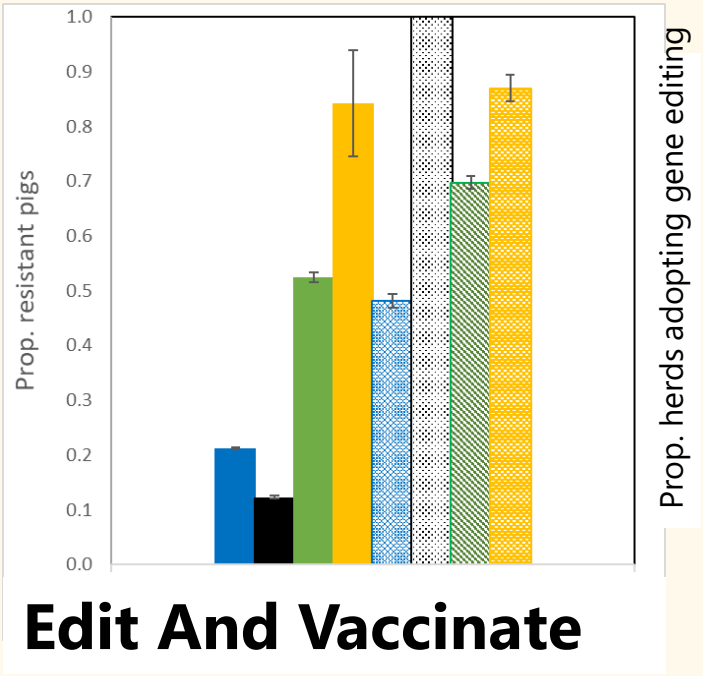
Petersen et al., PNAS 2022

Combine gene editing & vaccination

Average baseline disease risk R_0 = 1.5



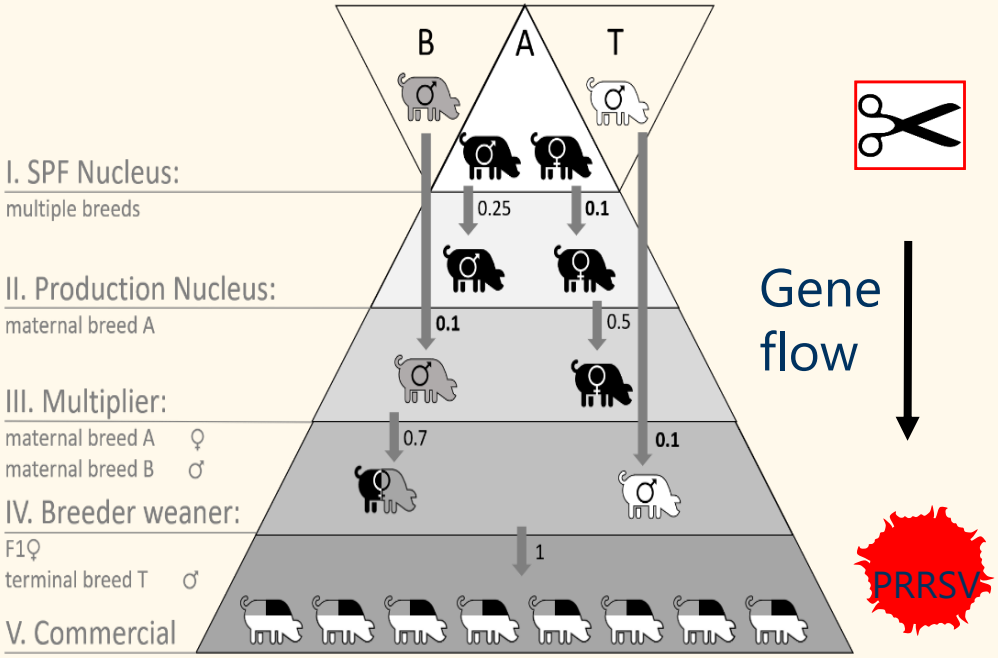


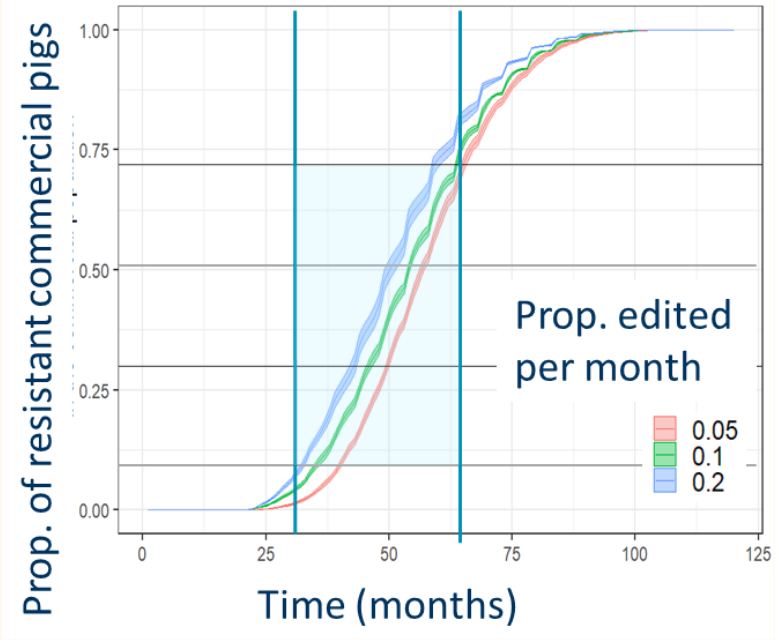
Distribution of genetically resistant pigs across herds:

- Optimal
- Comprehensive
- Concentrated
- Unregulated

PRRS eradication achievable if complemented with vaccination

Timeframe for generating sufficient genetically resistant commercial pigs





Sufficient genetically resistant commercial pigs could be produced within < 6 years

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Petersen et al., PNAS 2022

Conclusions from gene editing modelling studies

Key modelling predictions:

- Eliminating PRRS through gene editing alone is unlikely
- But could be possible within 6 years if combined with sufficiently **effective vaccination**, under **appropriate management** **&** **large-scale adoption** of gene editing

Caution: 

The **proof of concept** model ignores demographic effects affecting PRRSV transmission & many technical, practical, societal, ethical and political issues around gene editing

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Petersen et al., PNAS 2022

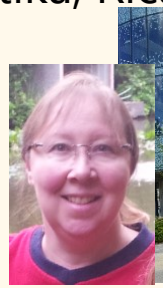
Conclusions

- There is overwhelming evidence for substantial natural genetic variation in animals' response to infection
  - Full scope for disease control yet to be realized
- Genome editing provides promising innovative solutions
  - Unlikely a silver bullet for all diseases, but we can't afford to dismiss it
- Genetic disease control will not replace, but complement biosecurity, vaccination and other control strategies
  - Requires unified approaches and mathematical prediction models to help identify the optimal strategy

Acknowledgements

Roslin students / post-docs & colleagues

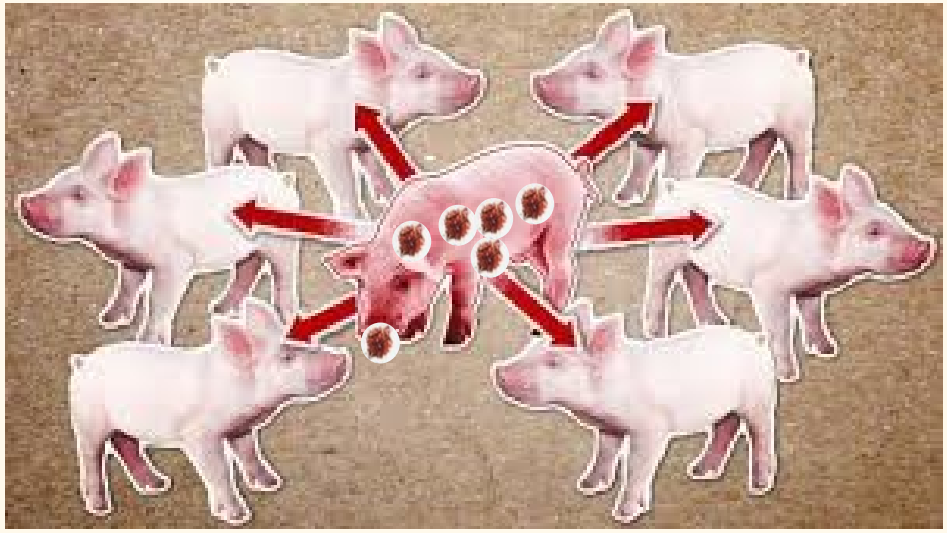
- Zeenath Islam, Graham Lough, Natacha Go, Chris Orrett, Osvaldo Anacleto, **Margo Chase-Topping**, Ozzie Matika, Ricardo Pong-Wong, Chris Pooley, Jamie Prentice
- Tanja Opriessnig, Steve Bishop, Sam Lycett, Alan Archibald



External

- **G. Plastow** (UAlberta), B. Kemp (PigGen), John Harding (U Sask.)
- **G. Petersen**, Fiona Hely, Tim Byrne (Abacus Bio, UK / NZ)
- Jack Dekkers, A. Hess (Iowa State University)
- Pieter Knap, Craig Lewis (PIC)
- J. Lunney (USDA), B. Rowland (KSU) & PHGC
- H. Mulder, H. Rashidi (WUR, NL)
- S. Touzeau, C. Belloc (INRIA / INRA, France)
- J. Dunkelberger, E. Knoll, P. Mathur (Topigs Norsvin, NL & USA)

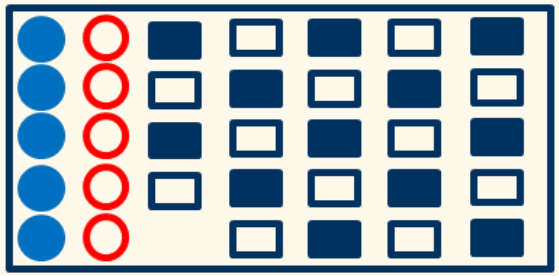
Does selection for resistance reduce PRRSV transmission?



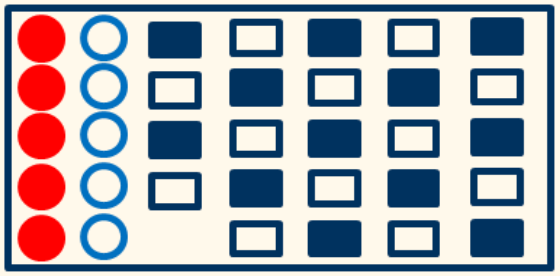
Are genetically more resistant / resilient pigs also less **susceptible** to PRRSV infection and less **infectious** under natural conditions**?**

- Focus on GBP5

PRRS transmission experiment to assess genotype effects on susceptibility & infectivity



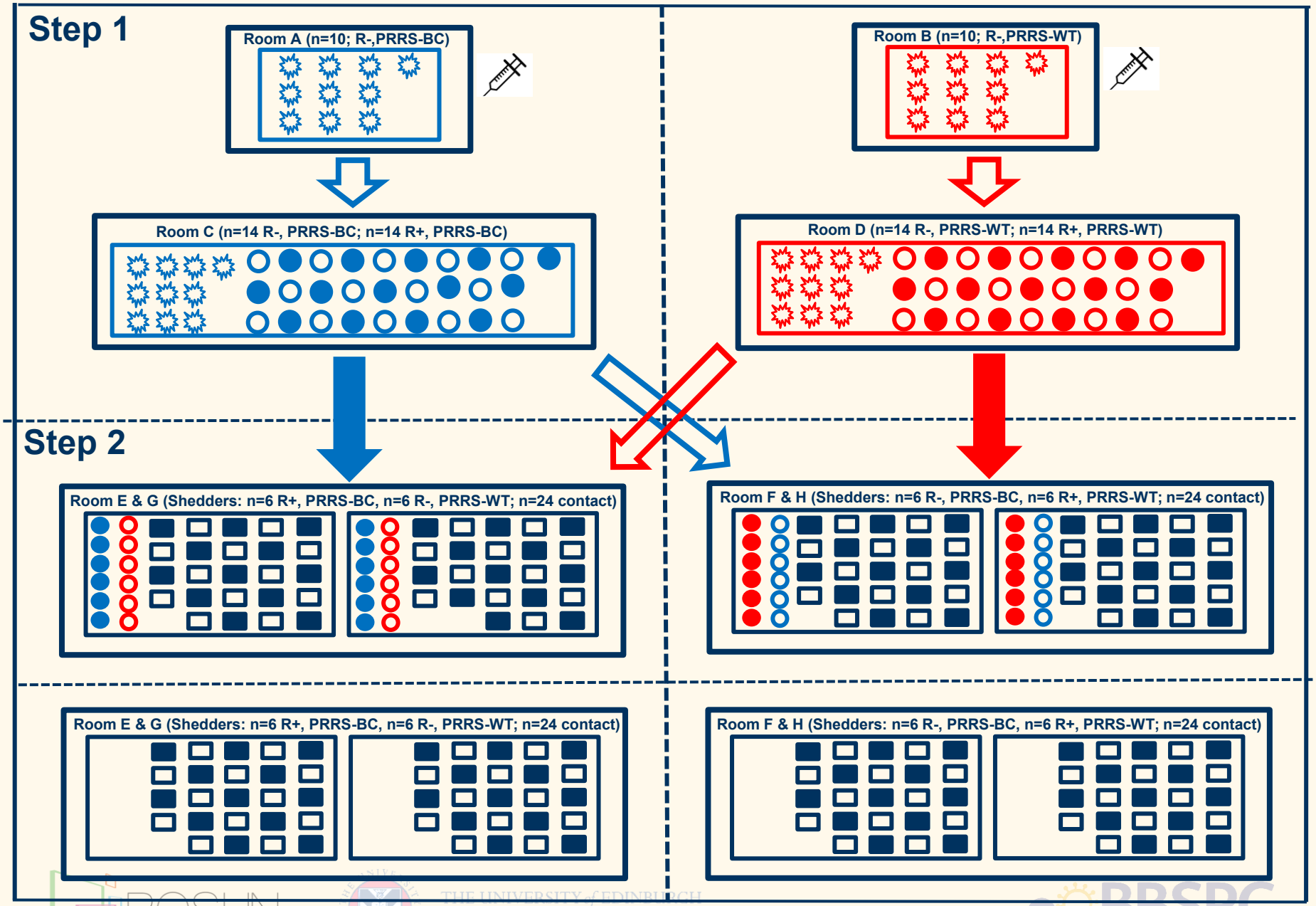
R+ Shedder: infected with **V2**
R- Shedder, infected with **V1**
R- Contact pig
R+ Contact pig



R - Shedder, infected with **V2**
R+ Shedder, infected with **V1**
R- Contact pig
R+ Contact pig

x2 experimental replicates

- Transmission experiment with ~400 pigs from ~70 full-sib families (>4 pigs per family)
- Half of the pigs from each family carry the resistance allele (R+), half don't (R-)
- Full-sibs distributed equally across groups (each group contains 1 R+/R- pig per family)
- Generate 2 barcoded PRRS virus strains (**V1**/ **V2**) that are otherwise identical
- Naturally infected shedder pigs with either V1 or V2
  - Within a group, shedder pigs with same genotype have the same barcoded PRRSV
- Monitor infection status of the contact pigs over time (3 sampling times)
  - Barcoding provides information of whether infection comes from an R- or R+ shedder
- This allows assessment of genotype effect on the susceptibility of contact pigs and the infectivity of shedder pigs



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GBP5 gene does not appear to affect transmission

Contact pig genotype	Replicate 1				Replicate 2				Replicate 3				Replicate 4			
contact Genotype	Animal ID	Day 9	Day 14	Day 17	Animal ID	Day 9	Day 14	Day 17	Animal ID	Day 9	Day 14	Day 17	Animal ID	Day 9	Day 14	Day 17
R-	348				366	2.32	4.89	5.09	368		5.11	5.00	359		4.15	3.94
	355		4.50	3.76	374		4.88	4.83	372		4.28	5.12	367		4.54	4.43
	385		3.62	5.54	375	2.13	5.06	4.96	378	1.83	4.94	4.40	371		4.70	5.53
	395	1.44	3.75	4.49	381		3.24	4.39	382		5.14	5.13	373		4.51	4.36
	424		4.66	4.99	398			3.96	418		3.55	3.74	407		3.69	3.92
	466		3.77	4.66	408		5.10	5.70	422		4.30	5.07	414		4.00	3.61
	470		4.34	4.24	412		4.24	4.91	431			3.63	423		4.84	4.63
	481		5.16	4.07	440		2.58/4.07	4.57	447		4.01	5.34	467		3.53	4.07
	483		3.90	4.38	455		4.37	4.95	449		4.91	5.31	471		4.48	4.80
	484		4.34	4.69	465		3.43	4.50	460	3.39	5.28	5.20	478		2.95	3.87
	488			2.49	486	2.42	4.95	4.80	493		4.20	3.98	502		3.86	4.69
	504		4.75	5.10	503		3.05	3.80	495		3.12	3.87	516		3.71	5.03
R+	353		3.03	3.95	392		3.74	3.45	352		3.49/2.1	3.87/3.7	354		5.79	5.13
	365		5.44	5.15	401		3.81	4.15	357		4.34	3.96	370		4.18	4.08
	380		5.44	5.36	405		3.87	5.19	360		5.11	5.16	384		4.12	4.61
	397	2.30	4.09	4.62	436		3.03	3.84	369		5.64	5.56	391		3.47	4.06
	416		2.45	3.62	437		4.06	4.96	376	2.53	3.58	3.73	393		4.42	3.59
	426		4.47	5.07	451		4.13	4.28	394		3.21	3.77	444	2.69	4.99	4.68
	427		4.40	4.47	462		4.28	4.34	403	2.12	5.50	5.36	458		4.43	4.72
	450		5.29	5.20	464	1.79	5.18	5.07	410		4.42	3.72	469		4.88	5.31
	453		5.50	5.68	479		4.45	5.36	448		4.01	5.28	476		4.68	4.71
	468		2.48	3.53	500		2.51/3.92	3.72	452		4.04	3.95	498		5.09	5.69
	501		4.58	4.94	506		4.71	4.44	457		3.92	3.72	514		4.30	4.44
	508		3.26	3.63	510		5.40	5.18	494		5.03	5.27	515		5.72	5.56

Contact pig

- infected by R- shedder
- infected by R+ shedder

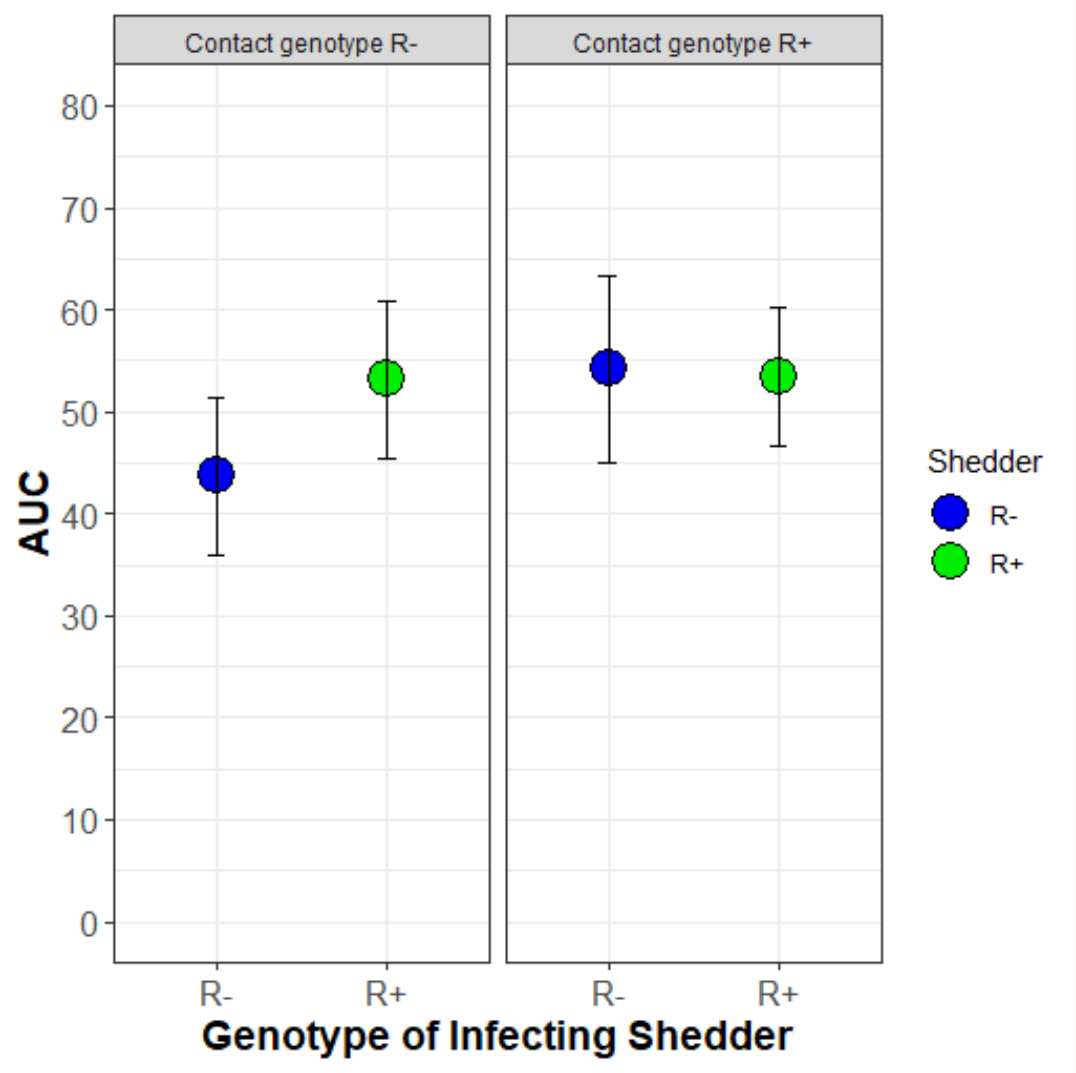
Equal distribution of blue and red cells in above table indicates

- No evidence for genotype effects on contact pig susceptibility
- No evidence for genotype effects on shedder pig infectivity

Results confirmed with statistical analyses

Conclusion: No evidence that the GBP5 gene reduces PRRSV transmission

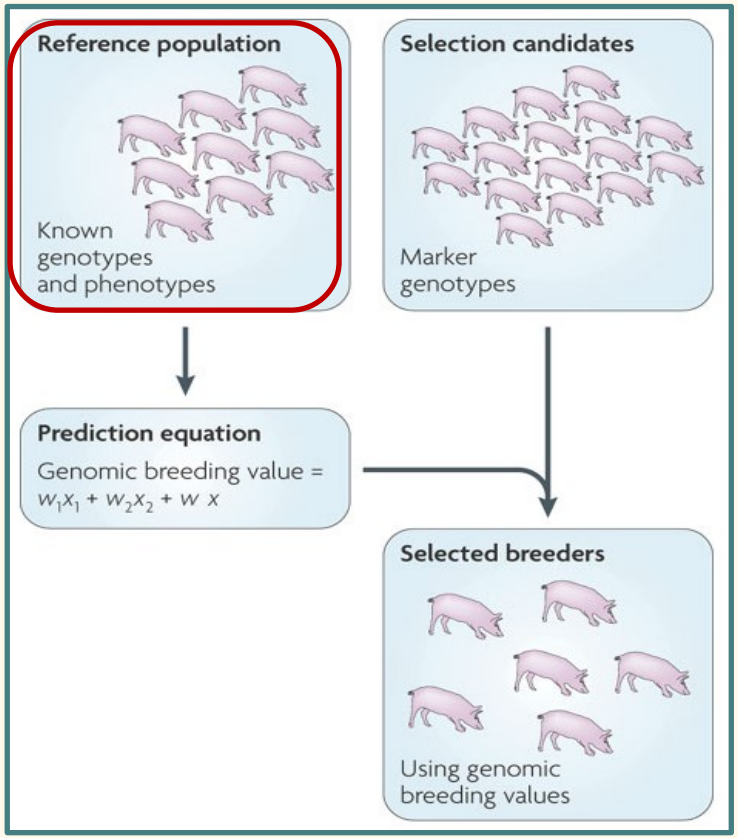
GLMM results for Contact pig model of serum Area Under the Curve (AUC) representing the level of infection for Contact pigs. Results are displayed with respect to the genotype of the shedder pig (R-, R+) they were infected by and their genotype (R-, R+). Error bars are 95% confidence intervals.



Future research

- Validate results for GBP5 for different pig populations / PRRSV strains
- Investigate the effects of different resistance / resilience markers (GBV groups) on PRRSV transmission
- Incorporate vaccination effects

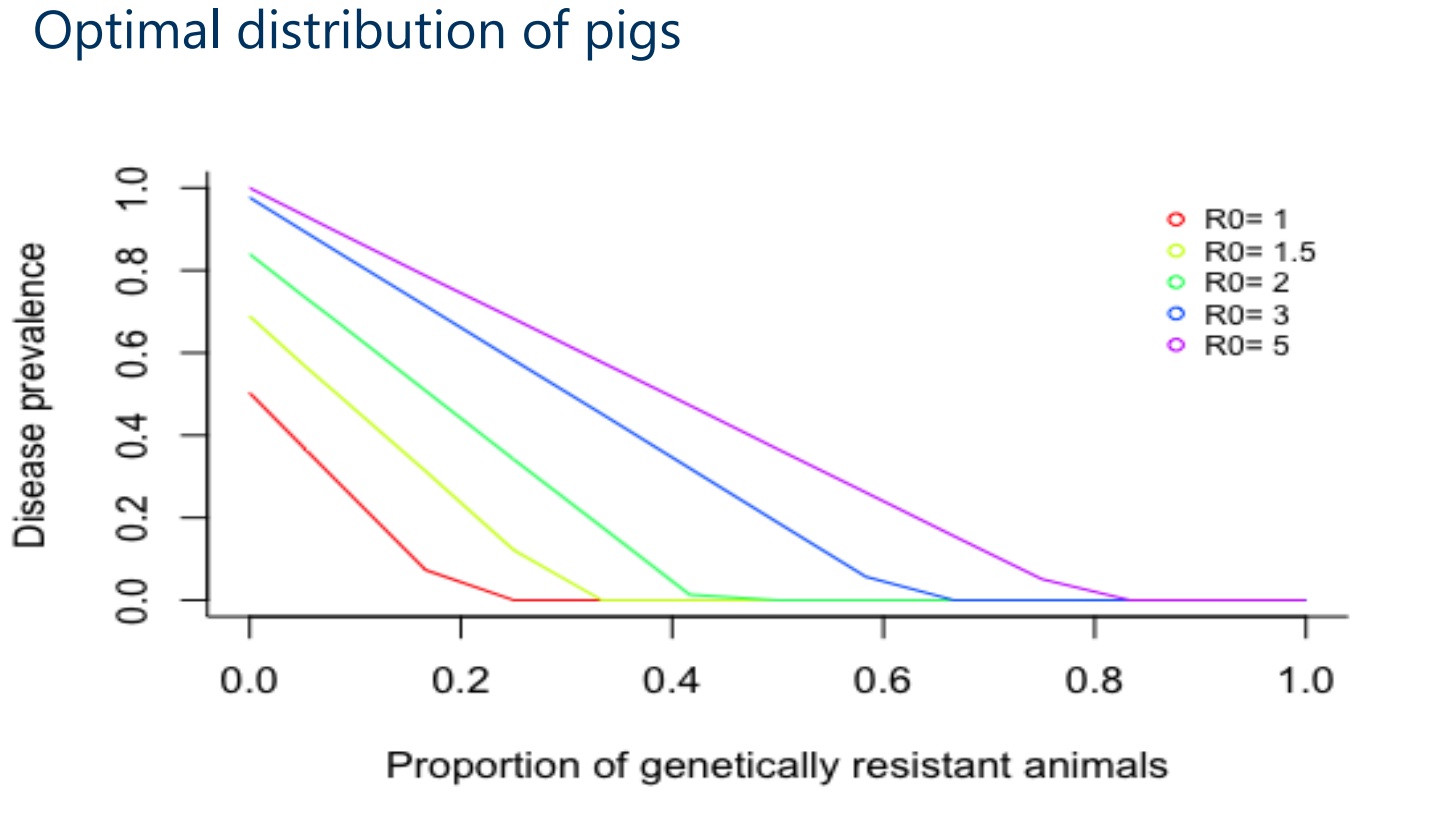
II. Exploiting natural genetic variation in disease resistance



Genomic selection for disease resistance / tolerance / ...:

- Applicable to any genetic architecture
- Requires genotyping of many animals & reliable resistance phenotypes (in the reference population only)

Expected reduction in PRRS prevalence through gene editing alone



- Genome editing can significantly reduce PRRS prevalence

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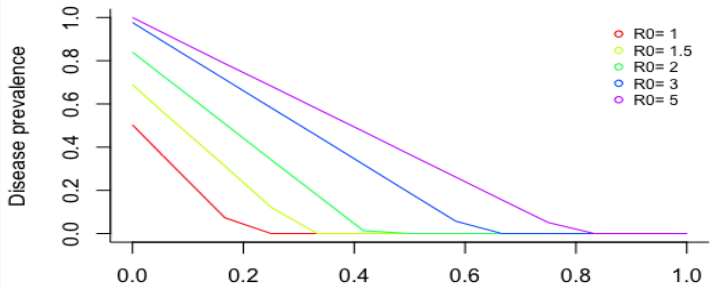
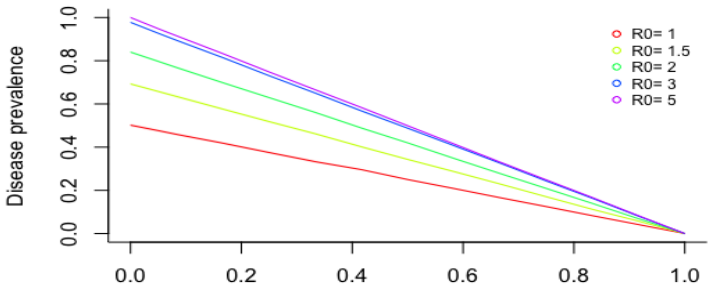
Expected reduction in PRRS prevalence through gene editing alone

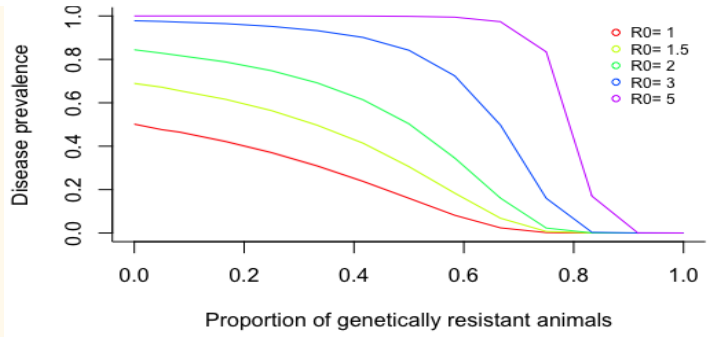
1. Optimal distribution



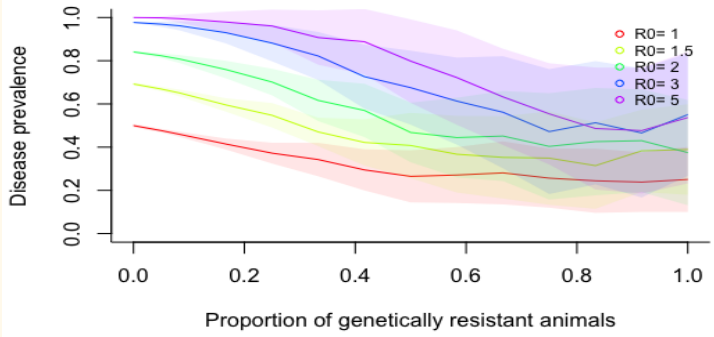
2. Concentrated: Distribution regulated by breeding companies



3. Comprehensive: National distribution scheme



4. Unregulated: voluntary uptake



- Genome editing can significantly reduce PRRS prevalence
- Distribution of these pigs across herds matters
- Little benefits if distribution is not regulated