Cellular, Tissue, and Gene Therapies Advisory Committee June 29-30, 2022 Meeting Presentation

Emerging Zoonotic Diseases

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US FDA Cellular, Tissue, and Gene Therapies Advisory Committee,
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Zoonotic diseases

Emerged diseases

- AIDS (human immunodeficiency viruses 1 and 2)
- Middle East Respiratory Syndrome (MERS) (MERS-CoV)
- Ebola infection (Ebolavirus)
- COVID-19 (SARS-CoV-2)
- Monkeypox virus infection

Emerging diseases

- Porcine cytomegalovirus/porcine roseolovirus infection

Pig viruses posing risks for xenotransplantation

and how to eliminate them

Pig virome

The pig virome is badly analysed.

Main virus families of the pig virome (Karlsson et al., 2016)

Virus family	Characteri-sation*	Prevalence in healthy pigs (%, n=19)	Prevalence in Swedish diarrhoeic pigs (%, n=10)
Adenoviridae	dsDNA	16	0
Anelloviridae	ssDNA	5	10
Astroviridae	ssRNA	5	0
Caliciviridae	ssRNA	5	0
Circoviridae	ssDNA	42	10
Parvoviridae	ssDNA	11	0
Picornaviridae	ssRNA	53	40
Reoviridae	dsRNA	11	20
* ss, single stranded, ds, double stranded			

Main viruses in the faeces of healthy Chinese pigs (Zhang et al., 2014)

Virus	%	Virus family
Porcine bocavirus	7	Parvoviridae
Porcine epidemic diarrhea virus (PEDV)	7	Coronaviridae
Sapovirus	17	Caliciviridae
Sapelovirus	17	Picornaviridae
Torovirus	7	Coronaviridae
Posavirus-1	17	Picornaviridae
Porcine astrovirus	72	Astroviridae
Porcine enterovirus-9	86	Picornaviridae
Kobuvirus	90	Picornaviridae

Denner J. The porcine virome and xenotransplantation. Virol. J. 2017;14(1):171.

Known zoonotic (inducing disease) viruses

Hepatitis E virus (HEV)

- Pig → human
  - eating undercooked pork and contact
  - human to human: blood transfusion
- Chronic infection in immunocompromised humans
- Disease in individuals with preexisting liver diseases
- Treatment: Ribavirin (inhibitor of RNA synthesis), no vaccine

Denner J. Xenotransplantation and Hepatitis E virus. Xenotransplantation. 2015;22(3):167-73.
Denner J. Hepatitis E virus (HEV)-The Future. Viruses. 2019;11(3):251.

Known zoonotic viruses -2-

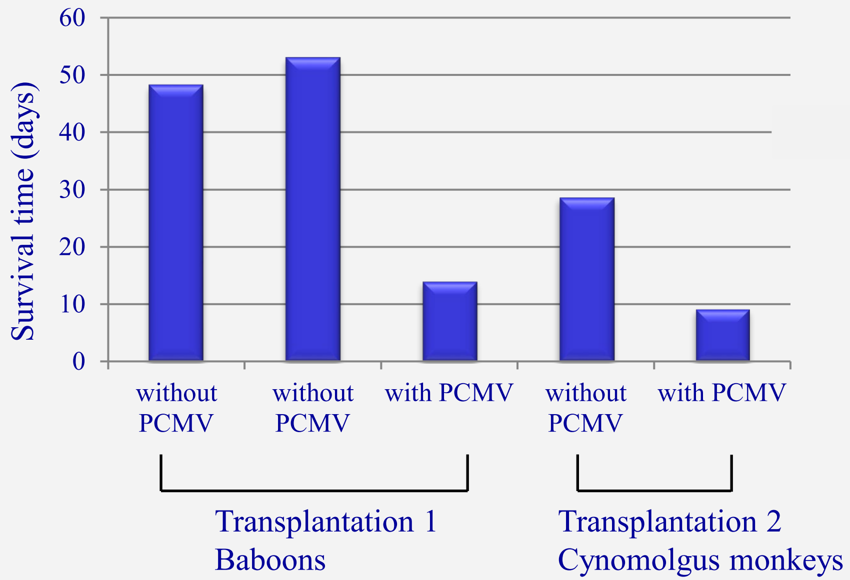
Porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV)

- Roseolovirus: closely related to human herpes virus 6A, 6B and 7. Only distantly related to HCMV (= HHV5) !!
- Contribution to the death of the Baltimore patient
- Significant reduction of transplant survival in non-human primate transplantation
- No treatment, no vaccine

Denner J. Xenotransplantation and porcine cytomegalovirus. Xenotransplantation. 2015;22(5):329-35.
Denner J. Reduction of the survival time of pig xenotransplants by porcine cytomegalovirus. Virol J. 2018;15(1):171.

Effect of PCMV/PRV

Kidney transplantation



Denner J. Xenotransplantation and porcine cytomegalovirus. Xenotransplantation. 2015;22(5):329-35.

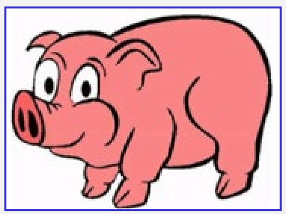
Yamada, K. et al. Porcine cytomegalovirus infection is associated with early rejection of kidney grafts in a pig to baboon xenotransplantation model. Transplantation. 98(4), 411-418 (2014).

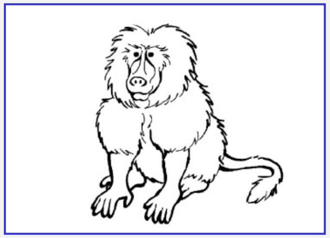
Sekijima, M. et al. Results of life-supporting galactosyltransferase knockout kidneys in cynomolgus monkeys using two different sources of galactosyltransferase knockout Swine. Transplantation. 98(4), 419-426 (2014).

Orthotopic heart transplantation

Genetically modified pigs

Baboons



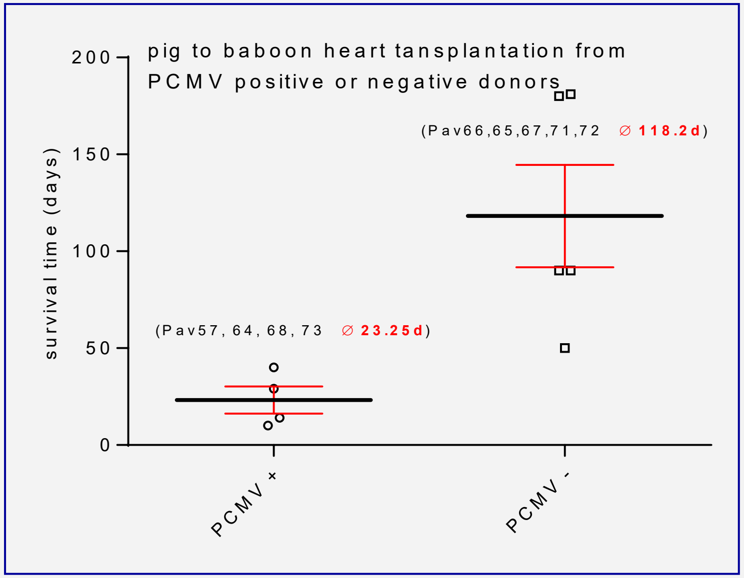


α1,3-galactosyltransferase-knockout (GalT-KO)
human membrane cofactor protein (CD46)
human thrombomodulin (hTM)

Effect of PCMV/PRV

Orthotopic heart transplantation

Pig to baboon



- reduced survival time of the transplant
- IL-6 and TNFα ↑
- tissue-type plasminogen activator (tPA)

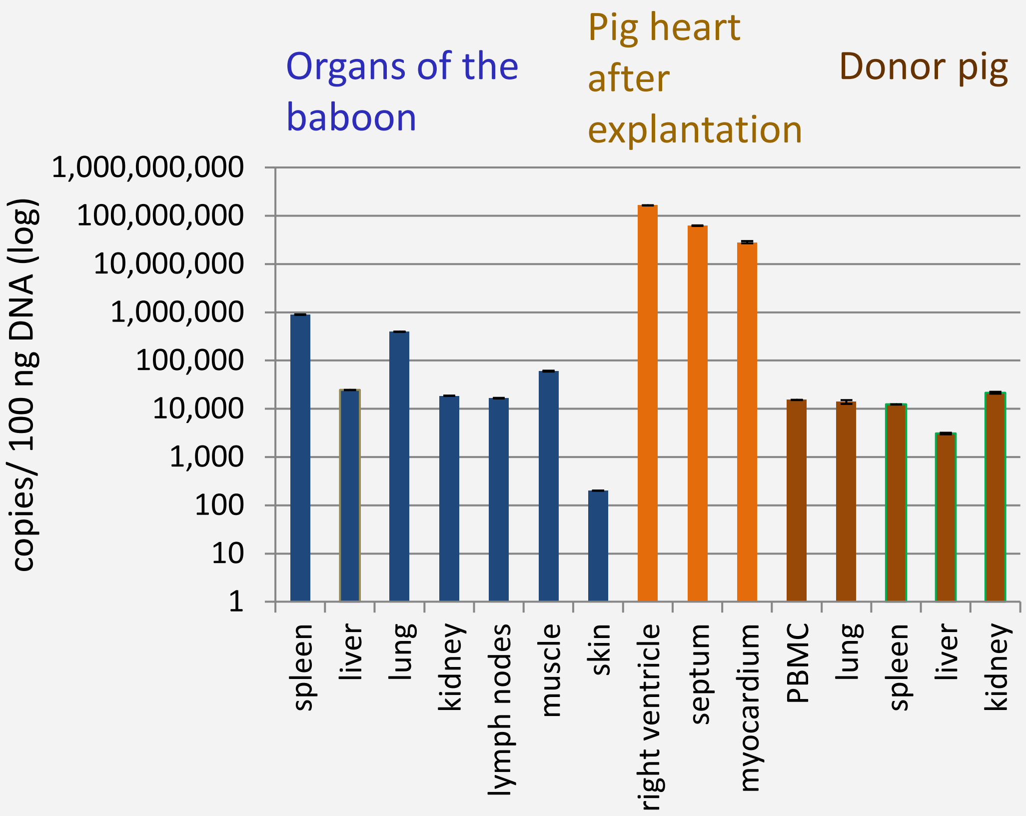
and plasminogen activator inhibitor 1 (PAI-1)

(tPA-PAI-1) complexes ↑

➔complete loss of the pro-fibrinolytic properties

Denner J, Längin M, Reichart B, Krüger L, Fiebig U, Mokelke M, Radan J, Mayr T, Milusev A, Luther F, Sorvillo N, Rieben R, Brenner P, Walz C, Wolf E, Roshani B, Stahl-Hennig C, Abicht JM. Impact of porcine cytomegalovirus on long-term orthotopic cardiac xenotransplant survival. Sci Rep. 2020;10(1):17531.

Transmission of PCMV/PRV



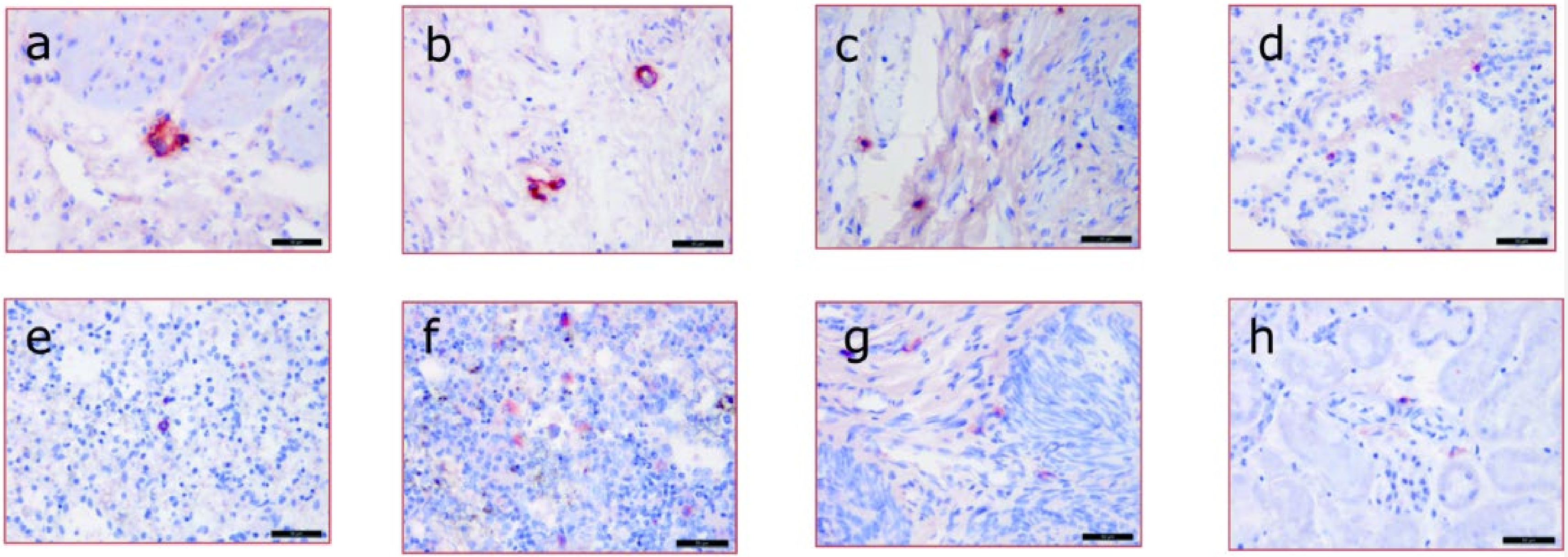
Denner J, Längin M, Reichart B, Krüger L, Fiebig U, Mokelke M, Radan J, Mayr T, Milusev A, Luther F, Sorvillo N, Rieben R, Brenner P, Walz C, Wolf E, Roshani B, Stahl-Hennig C, Abicht JM. Impact of porcine cytomegalovirus on long-term orthotopic cardiac xenotransplant survival. Sci Rep. 2020;10(1):17531.

Transmission of PCMV/PRV -2-

Orthotopic heart transplantation

Pig to baboon ➢ unclear, whether PCMV infects baboon cells

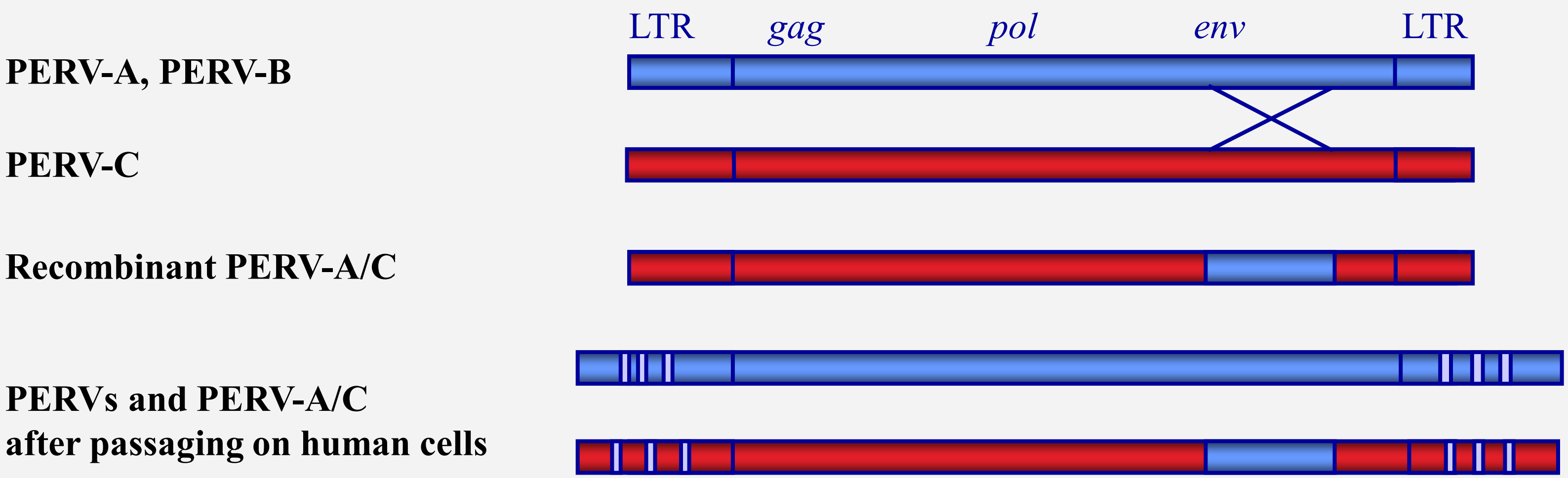
Pig heart | Pig heart | Liver | Lung



Spleen | Lymph node | Testis | Kidney

Fiebig U, Abicht JM, Mayr T, Längin M, Bähr A, Guethoff S, Falkenau A, Wolf E, Reichart B, Shibahara T, Denner J. Distribution of Porcine Cytomegalovirus in Infected Donor Pigs and in Baboon Recipients of Pig Heart Transplantation. Viruses. 2018;10(2):66

Do PERVs pose a risk?



PERV-A/C - increased titres, not integrated in germ line
LTR - multimerisation of NF-Y binding sites in the LTR

Denner J, Tönjes RR. Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. Clin Microbiol Rev. 2012 Apr;25(2):318-43

Denner J, Specke V, Thiesen U, Karlas A, Kurth R. Genetic alterations of the long terminal repeat of an ecotropic porcine endogenous retrovirus during passage in human cells. Virology. 2003;314(1):125-33.

Risk posed by retroviruses

- Tumors, leukemia: FeLV, MuLV, KoRV, HTLV
- Immunodeficiency (AIDS): HIV, SIV, FeLV, MuLV, KoRV, HTLV

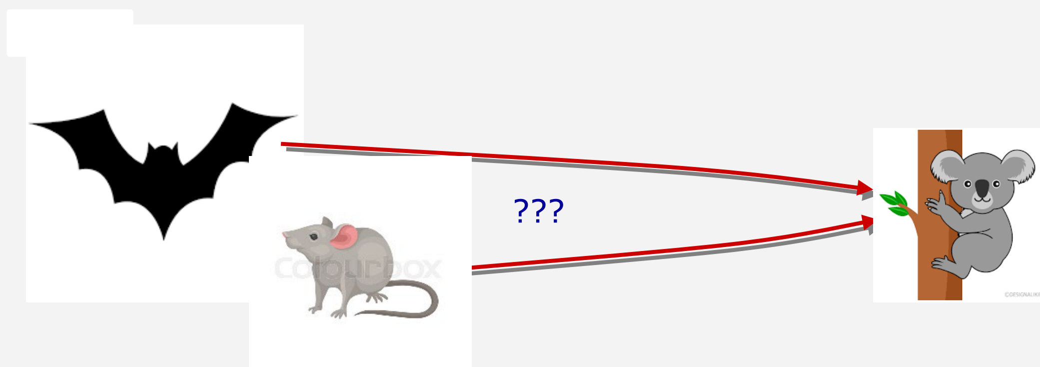
Transspecies transmission of retroviruses is common

Natural host
non pathogenic

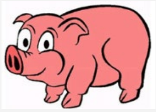
SIVcpz
SIVagm
SIVmnd
SIVsyk
SIVsm
SIVl'hoest
SIVsun
SIVrcm

Heterologic host
AIDS

HIV-1 O
HIV-1 N
HIV-1 M (A-F)
HIV-2 D
HIV-2 A, B, C
HIV-2 E
HIV-2 F
SIVmac, SIVmne



Screening for released human-tropic PERV



Pig blood

↓ PCR

Isolated PBMCs

↓ PCR

Mitogen-stimulation

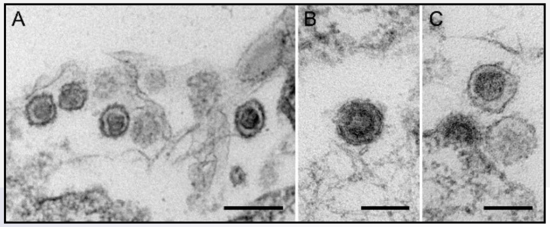
↓ PCR

Co-culture
with human 293 cells

Rare isolation, mostly PERV-A/C

Pig strain	Number of tested animals	Detection of			Infection of 293 cells
		PERV-C	PERV-A/C 1	PERV-A/C 2	
Göttingen minipigs	28	28	5	2	1
German Landrace	19	19	0	0	0
Black Forest minipig	5	5	1	0	0
Aachen minipig	6	6	0	2	0

Halecker S, Krabben L, Kristiansen Y, Krüger L, Möller L, Becher D, Laue M, Kaufer B, Reimer C, Denner J. Rare isolation of human-tropic recombinant porcine endogenous retroviruses PERV-A/C from Göttingen minipigs. Virol J. 2022;19(1):30.



PERV transmission tests

- small animal transplantation and infection experiments
  +/- immunosuppression (rats, mice, mink, guinea pig) ➔ negative

  But: Lack of PERV receptor

- pig to non-human primate transplantation and infection experiments
  ➔ negative

  But: Receptor does not fit well

- first clinical trial with pig islet cells in New Zealand and Argentina
  ➔ negative

  But: No vascularized organ, less severe immunosuppression

Comprehensive testing of pigs for xenotransplantation

Pig breed	Microorganisms including viruses screened for	Viruses detected in addition to PERV	References
Auckland Island pigs	14 viruses, 10 bacteria, toxoplasma	None	Garkavenko, et al., 2004; Wynyard et al., 2011
Large White – Yorkshire x Landrace	28 viruses, 2 bacteria, 11 vaccinations	PRCV, PRRSV, PPV, EMCV, EEEV, VEEV, WEEV	Gazda et al., 2016
Landrace x Large White	31 viruses	PLHV-2, -3 in adults, none in piglets derived by caesarean section	Hartline et al., 2018
CDCD pigs	24 bacteria 3 fungi 6 parasites 21 viruses arthropods	PCV2	Noordergraaf et al., 2018

Abbreviations and references in: Denner J. Sensitive detection systems for infectious agents in xenotransplantation. Xenotransplantation. 2020;18:e12594.

Testing Göttingen Minipigs

Pathogens	Methods
Bacteria (20)	
Brucella abortus, B. microti, B. melitensis, B. pinnipedalis, B. suis, B. canis, B. ovis and B. neotomae	real-time PCR
Burkholderia mallei, pseudomallei	PCR
Chlamydophila felis / Chlamydophila psittaci	real-time PCR
E.coli	real-time PCR
Fusobacterium	real-time PCR
Bacillus anthracis	real-time PCR
Listeria monocytogenes	real-time PCR
M. tuberculosis, M. bovis, M. microti, M. intracellulare, M. avium, M. gastri, M. africanum, M. scrofulaceum, M. ulcerans, M. simiae, M. kansasii, M. chelonae, M. fortuitum, M. marinum, M. genavense, etc.	real-time PCR
*Actinobacillus pleuropneumoniae	real-time PCR
*Bordetella bronchiseptica	real-time PCR
*Brachyspira (Serpulina) pilosicoli	real-time PCR
*Campylobacter, Campylobacter jejuni, C. coli and C. lari	real-time PCR
*Leptospira	real-time PCR
*Pasteullera multocida	real-time PCR
*Salmonella	real-time PCR
*Yersinia enterocolitica	real-time PCR
*Clostridium perfringens	real-time PCR
*Erysipelothrix rhusiopathiae	real-time PCR
*Staphylococcus	real-time PCR
*Streptococcus	real-time PCR
Viruses (20)	
Nipah virus	reverse transcription / real time-PCR
Porcine cytomegalovirus	real-time PCR
Porcine lymphotopic herpesvirus 1 and 2, PLHV-1 and -2	real-time PCR
Rabies virus	reverse transcription / real-time PCR
Hepatitis E virus	reverse transcription / real-time PCR
PERV	reverse transcription / real-time PCR
*Encephalomyocarditis	reverse transcription / real-time PCR
*Rotavirus	reverse transcription / real-time PCR
Influenza virus, H5N1, H5N2, *H1N1, *H2N2, H3N8, H4N6, H7N7, H8N4, H9N2	reverse transcription / real-time PCR
BVDV	reverse transcription / real-time PCR
Swine fever virus	reverse transcription / real-time PCR
PHEV	reverse transcription / real-time PCR
Pseudorabies	real-time PCR
Porcine adenovirus	real-time PCR
Porcine circovirus type 1	real-time PCR
*Porcine circovirus type 2	real-time PCR
Porcine enterovirus	reverse transcription / real-time PCR
PRRSV	reverse transcription / real-time PCR
Swine pox virus	real-time PCR
Swine vesicular disease	reverse transcription / real-time PCR
Vesicular stomatitis virus	reverse transcription / real-time PCR
Nematode/worm (2)	
Taenia solium	real-time PCR
Trichinella spiralis	real-time PCR
Protozoa (3)	
Cryptosporidium	PCR
Trypanosoma cruzi	real-time PCR
*Toxoplasma gondii	real-time PCR
Fungi (4)	
Aspergillus	real-time PCR
Cryptococcus neoformans	real-time PCR
Microsporum	real-time PCR
*Candida albicans	real-time PCR
Total: 49 groups of pathogens (>88 individual microorganisms)	

doi:10.1371/journal.pone.0139893.t001

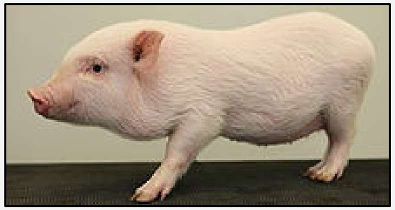
Planned to be used in clinical trials to treat diabetic patients in Germany

Tested negative for 88 individual microorganisms (viruses, bacteria, fungi, parasites, protozoa)

Some animals positive for PCMV (45%), HEV (22%), PLHV-1 (10%), PCV2 (14%)

All positive for PERV-C (risk of PERV-A/C recombination)

Morozov VA, Plotzki E, Rotem A, Barkai U, Denner J. Extended microbiological characterization of Göttingen minipigs: porcine cytomegalovirus and other viruses. Xenotransplantation. 2016;23(6):490-496.



Göttingen minipigs

Results of the first preclincal and clinical xenotransplantations

Pig breed	Preclinical/clinical trials with these animals as donors	Output: Transmission of viruses including PERV	References
Auckland Island pigs	Preclinical trial: Encapsulated islet cells into cynomolgus monkeys. **Clinical trials:** islet cells in New Zealand and Argentina	No transmission	Garkavenko et al., 2008; Wynyard et al., 2014; Morozov et al., 2017
Large White – Yorkshire x Landrace	Preclinical trial: Encapsulated islet cells into diabetic cynomolgus monkeys	No transmission despite PRCV, PRRSV, PPV, EMCV, EEEV, VEEV, WEEV in the donor pigs	Gazda et al., 2016
LEA29Y pigs	Preclinical trial: Islet cells into marmosets	No transmission despite PCMV in the donor pigs	Plotzki et al., 2015

Abbreviations and references in: Denner J. Sensitive detection systems for infectious agents in xenotransplantation. Xenotransplantation. 2020;18:e12594.

Islet cell transplantation

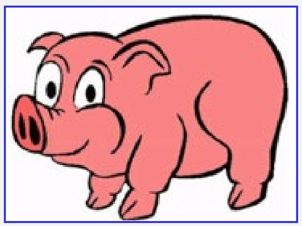
Göttingen minipigs
(Ellegaard)

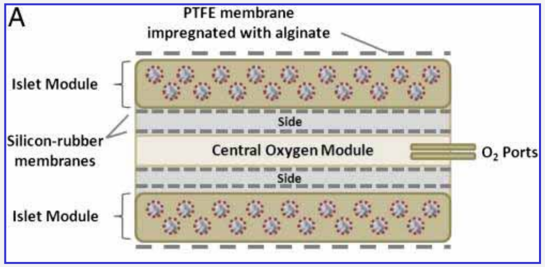
Macroencapsulated

islet cells

12 months

Cynomolgus monkeys





PERV 3/3
PCMV 0/3
HEV 0/3

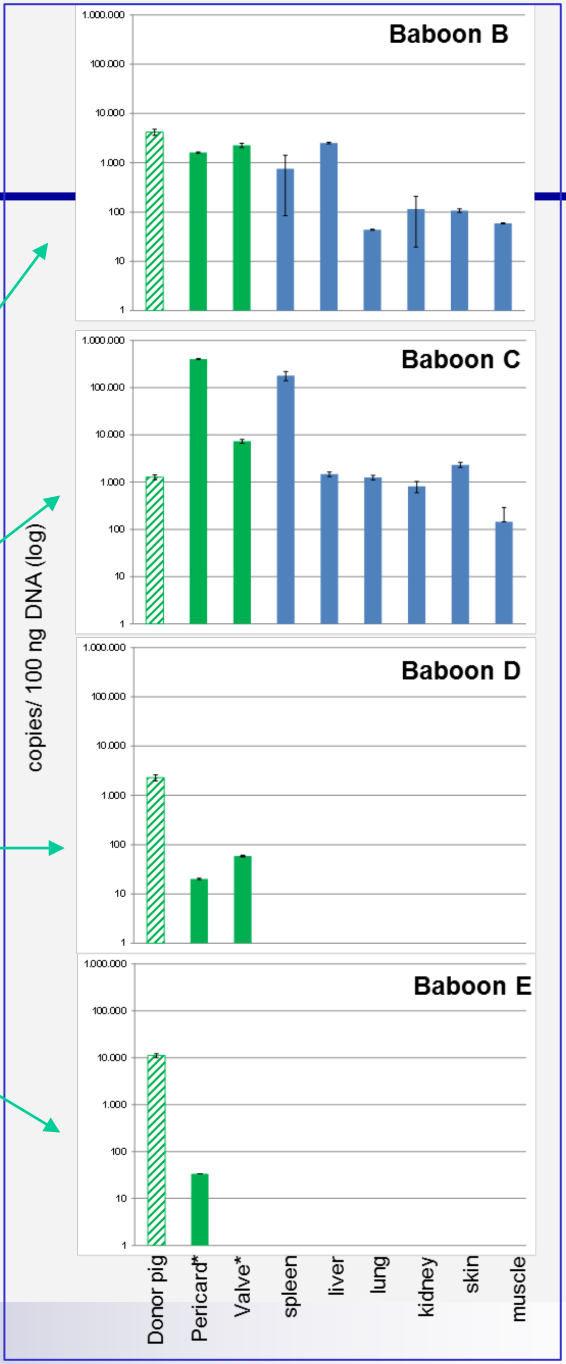
PERV 0/8
PCMV 0/8
HEV 0/8

Morozov VA, Ludwig S, Ludwig B, Rotem A, Barkai U, Bornstein SR, Denner J. Islet cell transplantation from Göttingen minipigs to cynomolgus monkeys: analysis of virus safety. Xenotransplantation. 2016;23(4):320-7

PCV3 transmission

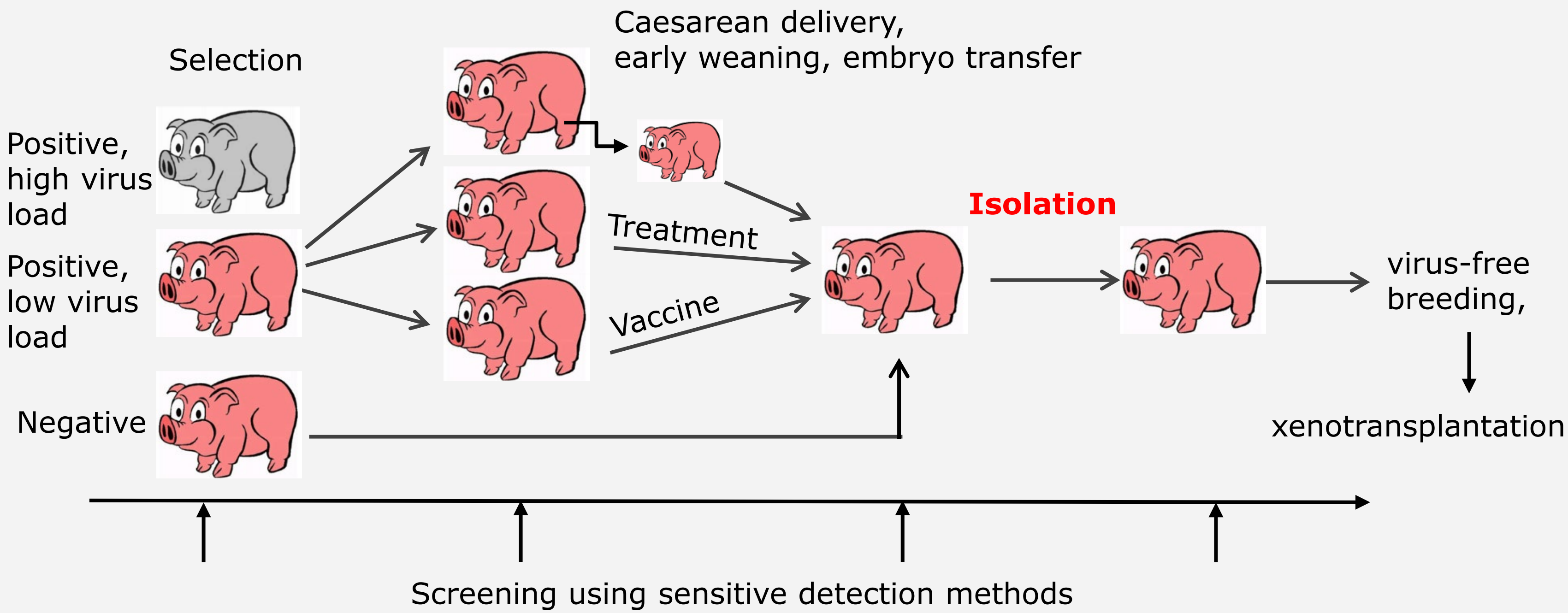
Pig hearts into baboons

Baboon	Transplant survival time (days)	PCV3
A	90	No
B	195	Yes
C	182	Yes
D	15	Yes
E	27	Yes
F	90	No



Krüger L, Längin M, Reichart B, Fiebig U, Kristiansen Y, Prinz C, Kessler B, Egerer S, Wolf E, Abicht JM, Denner J. Transmission of Porcine Circovirus 3 (PCV3) by Xenotransplantation of Pig Hearts into Baboons. Viruses. 2019;11(7):650.

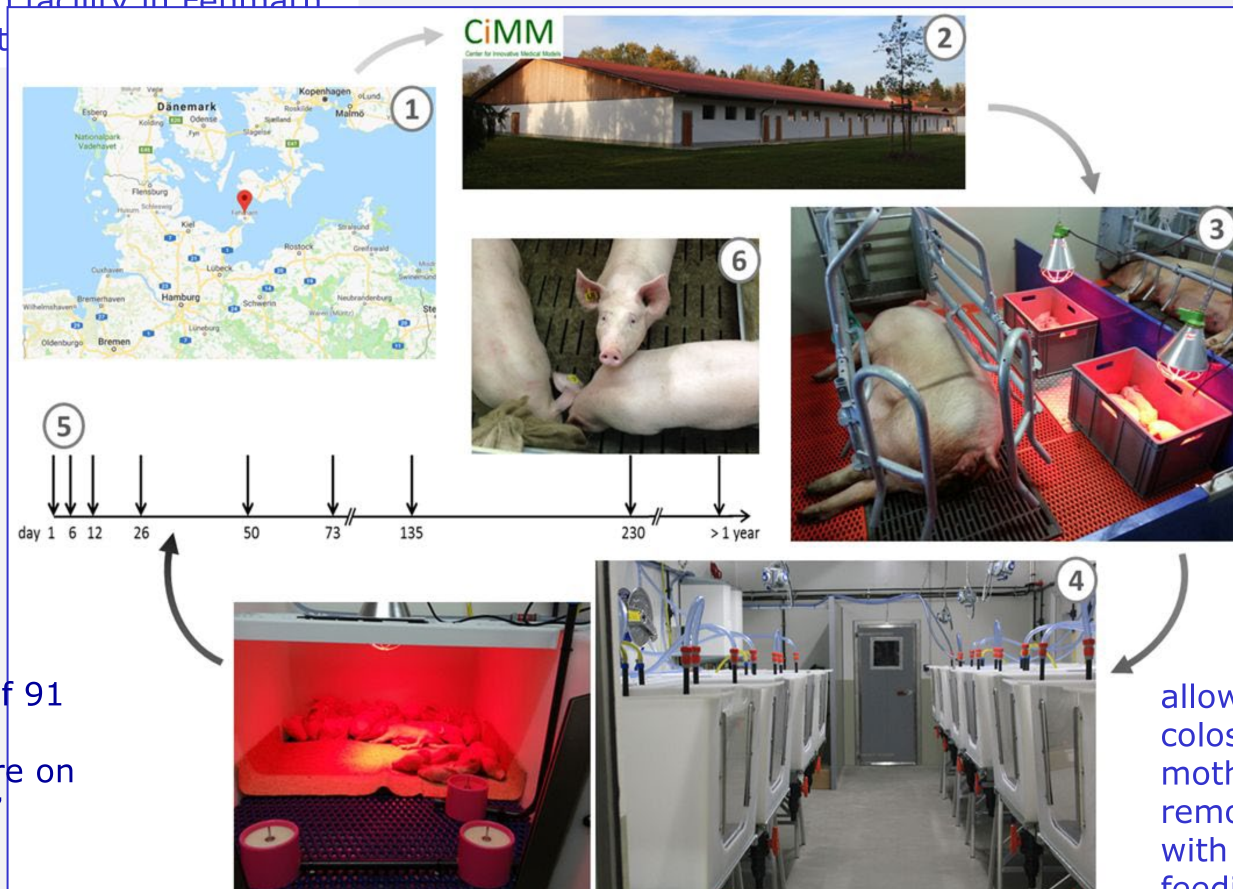
Elimination programs



Denner J. Sensitive detection systems for infectious agents in xenotransplantation. Xenotransplantation. 2020;18:e12594.

Elimination of PCMV/PRV by early weaning

10 sows from facility in Fehmarn
7 PCMV-posit



Screening of 91 piglets
PBMC culture on day 5 and 7
1300 tests

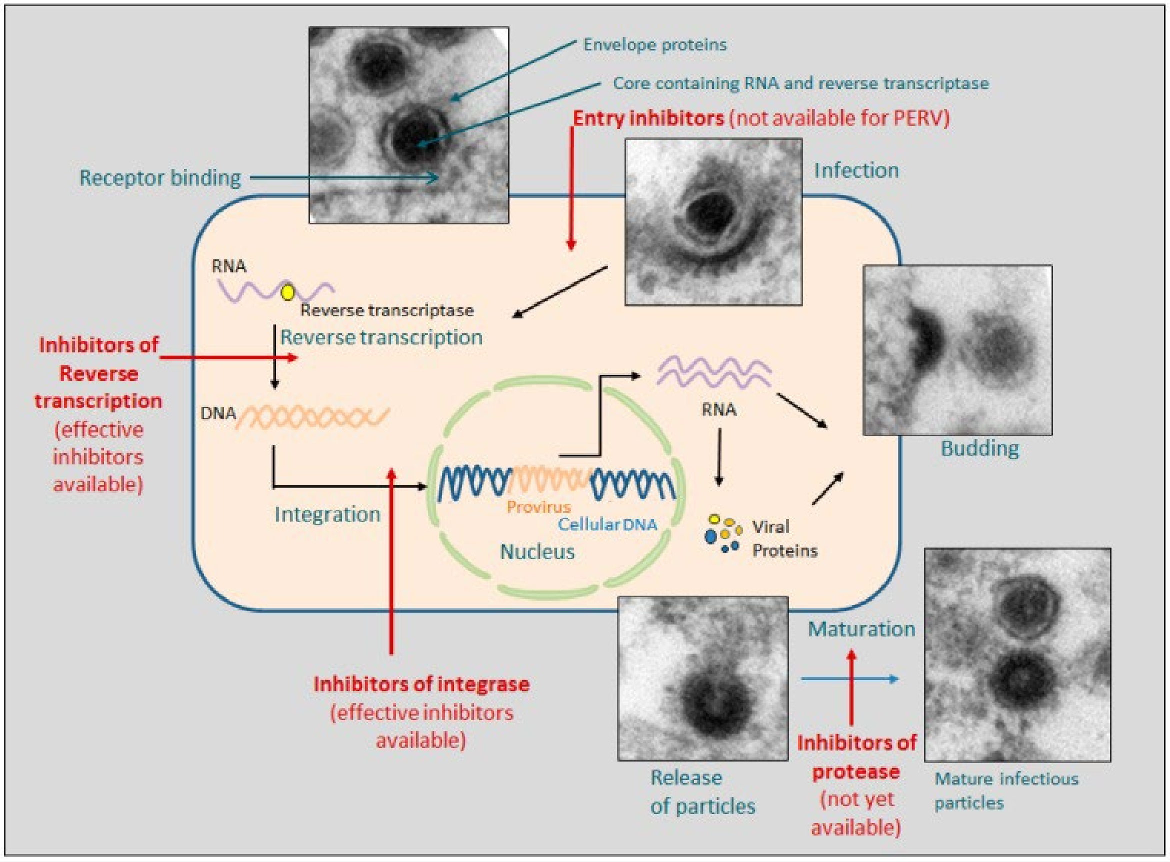
allowed to suckle colostrum, mother sows were removed. Weaned with milk replacer feeding

Egerer S, Fiebig U, Kessler B, Zakhartchenko V, Kurome M, Reichart B, Kupatt C, Klymiuk N, Wolf E, Denner J, Bähr A. Early weaning completely eliminates porcine cytomegalovirus from a newly established pig donor facility for xenotransplantation. Xenotransplantation. 2018 Jul;25(4):e12449.

Attempts to prevent PERV transmission

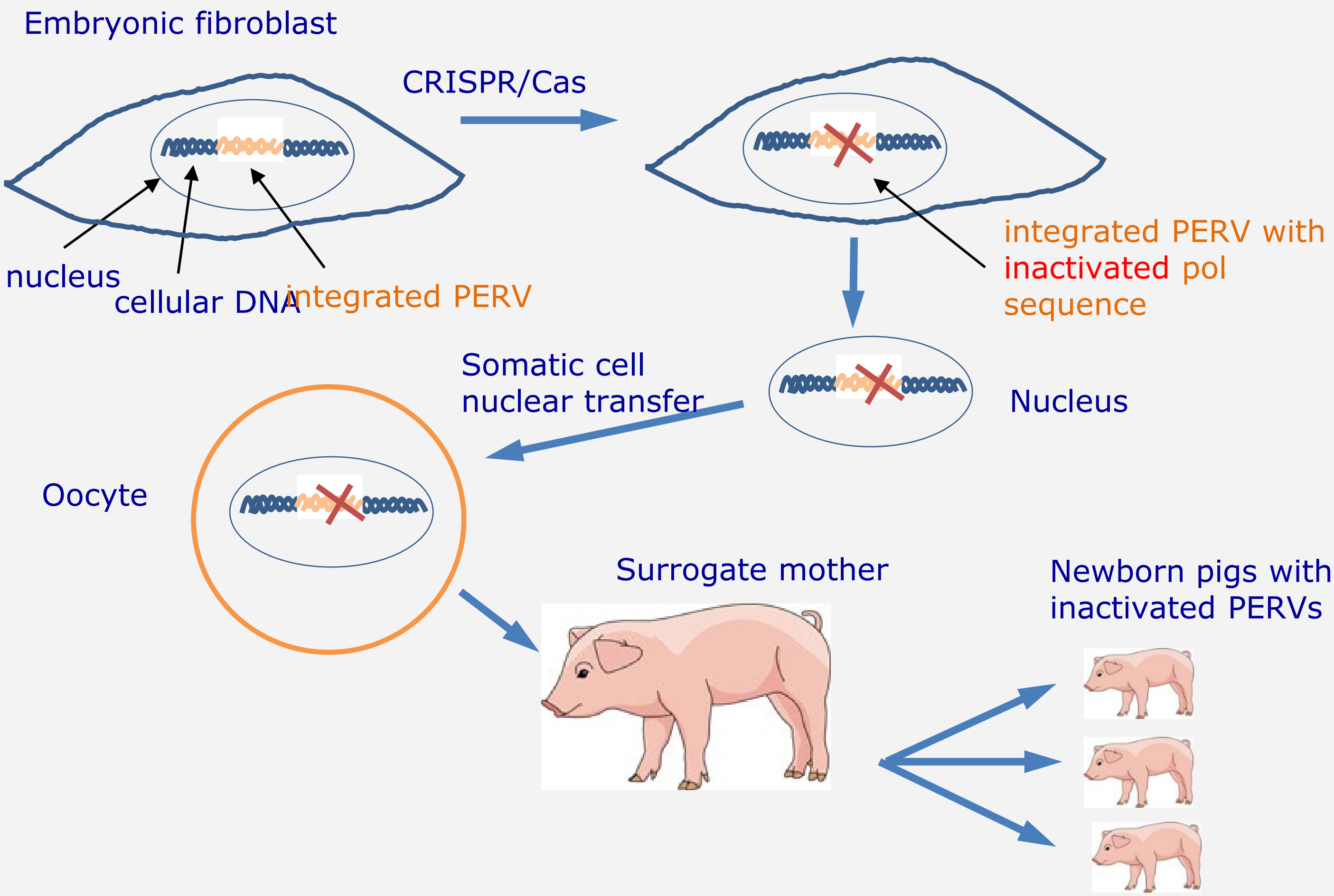
- Vaccine
  - TM and SU envelope protein – neutralizing antibodies
  - No animal model
  - Similar vaccine: FeLV, cats
- Antiretroviral drugs
- siRNA
  - In vitro and in vivo, transgenic pigs
- Genome editing
  - Zink finger nuclease
  - CRISP/Cas

Strategies to prevent PERV transmission: Antiretroviral drugs



Denner J. Can Antiretroviral Drugs Be Used to Treat Porcine Endogenous Retrovirus (PERV) Infection after Xenotransplantation? Viruses. 2017 Aug 8;9(8):213

Inactivation of PERV using CRISPR/Cas



Niu D, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science. 2017;357(6357):1303-1307

Do we need pigs with CRISPR/Cas inactivated PERVs ?

- No transmission of PERV to other animals/human observed

- Off-target effects of CRISPR/Cas
- Risk of inbreeding of CRISPR/Cas-inactivated pigs

Denner J, Scobie L, Schuurman HJ Is it currently possible to evaluate the risk posed by PERVs for clinical xenotransplantation? Xenotransplantation. 2018 Jul;25(4):e12403.

Scobie L, Denner J, Schuurman HJ. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9, editorial commentary. Xenotransplantation. 2017 Nov;24(6).

Denner J. Paving the Path toward Porcine Organs for Transplantation.
Denner J. N Engl J Med. 2017;377(19):1891-1893.

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