Where Do the Pigs Come from?

Henk-Jan Schuurman
Diabetes Institute for Immunology and Transplantation, Minneapolis, MN
Spring Point Project, Minneapolis, MN

Diabetes Meets the New Technology
Breakthrough Series Event
UMN and LifeScience Alley, October 29, 2007
Need for ‘Unlimited’ Donor Supply

Coming from an unlikely source

Why pigs?

• Easy to breed (large litters, 1-yr generation time)
• Size and physiology of organs similar to humans
• Genetic modification possible
• Domesticated for very long times
• No ethical obstacles
Humans Exposed to Pig Tissue from “Head to Toe” Without Disease

- **Pig Meat**
  - Major Food Source
- **Pig Brain Cells**
  - (For Parkinson’s Disease, 2000)
- **Pig Heart Valves**
  - (Over 1,000,000 implants since 1950’s)
- **Pig Liver/Pig Liver Cells**
  - (Hundreds of patients with liver failure since 1960’s)
- **Pig Skin**
  - (Replaced burned skin in over 20,000 patients since 1973)
- **Pig Intestinal Mucosa**
  - (Used for repair since 1990’s)
- **Pig Insulin**
  - (Millions of diabetic patients injected since 1920’s)
- **Pig Islet Transplantation**
  - (1990-1993)

Bioartificial Liver
Risk-Benefit of a Porcine Xeno-product

• Xenotransplantation: any procedure that involves the transplantation, implantation, or infusion into a human recipient of either
  ‣ live cells, tissues, or organs from a non-human animal source
  ‣ human body fluids, cells, tissues, or organs that have had \textit{ex vivo} contact with live non-human animal cells, tissues, or organs

• Benefit: each application requires its own distinct source animal
  ‣ pigs selected or (genetically) modified

• Risk: cross-species infectious pathogen transmission
  ‣ major issue: endogenous retrovirus (PERV)
  ‣ the fear for the ‘unknown’
Stringent Regulations

Guidance for Industry

Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans

FINAL GUIDANCE

Additional copies of this guidance document are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm. For questions on the content of this document contact Eda Bloom, Ph.D. at 1-800-835-4709.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologies Evaluation and Research (CBER)
April 2003
Screening for Infectious Agents

• ...source animals should be maintained in barrier facilities...termed Designated Pathogen Free (DPF). ...

• Infectious agents of concern
  ‣ specific tests based on donor species
  ‣ known pathogens and agents known to infect human cells in vivo or in vitro
  ‣ ability to assay for latent viruses or pathogens

• Collection and analysis of clinical samples
# Exogenous Agents to Test

<table>
<thead>
<tr>
<th>Serology/Culture</th>
<th>Serology/Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus pleuropneumonia.</td>
<td>Leptospira 5 Serovars – Serology</td>
</tr>
<tr>
<td>Bovine Adenovirus – Serology</td>
<td>Mycoplasma sp. – Serology / Culture</td>
</tr>
<tr>
<td>Bovine Viral Diarrhea – Serology / VI</td>
<td>Porcine Parvovirus – Serology</td>
</tr>
<tr>
<td>Bordetella bronchiseptica – Culture</td>
<td>Pasteurella sp. – Culture</td>
</tr>
<tr>
<td>Brucella abortus – Serology</td>
<td>Porcine reproductive and respiratory syndrome</td>
</tr>
<tr>
<td>Campylobacter sp. – Culture</td>
<td>Serology / PCR / VI / IHC</td>
</tr>
<tr>
<td>Chlamydia psittaci sp. – PCR / Culture</td>
<td>Protozoa – Floatation</td>
</tr>
<tr>
<td>Porcine Circovirus – PCR / Histology</td>
<td>Parainfluenza 3 – Serology</td>
</tr>
<tr>
<td>Cryptosporidium parvum – IFA</td>
<td>Pseudorabies Virus – Serology / VI</td>
</tr>
<tr>
<td>Porcine Cytomegalovirus – Histology / PCR</td>
<td>Coronavirus – Serology</td>
</tr>
<tr>
<td>Encephalomyocarditis Virus – Serology, VI</td>
<td>Rotavirus – Serology</td>
</tr>
<tr>
<td>Enterovirus G1-8 – Serology / VI</td>
<td>Salmonella sp. – Culture</td>
</tr>
<tr>
<td>Erysipelothrix rhusiopathiae – Culture</td>
<td>Staphylococcus sp. – Culture</td>
</tr>
<tr>
<td>Giardia – IFA</td>
<td>Streptococcus suis – Culture</td>
</tr>
<tr>
<td>Hemagglutinating Encephalomyelitis Virus Serology / VI</td>
<td>Toxoplasma gondii – Serology</td>
</tr>
<tr>
<td>Haemophilus sp. – Culture</td>
<td>Transmissible Gastroenteritis Virus – Serology</td>
</tr>
<tr>
<td>Hepatitis E – Serology</td>
<td>Trichinella spiralis – Serology</td>
</tr>
<tr>
<td>Infectious Bovine Rhinotracheitis – Serology</td>
<td>Vesicular Stomatitis (NJ &amp; Indiana) – Serology</td>
</tr>
<tr>
<td>Swine Influenza – Serology / VI</td>
<td>Yersinia sp. – Culture</td>
</tr>
<tr>
<td></td>
<td>Virus Screen – EM / VI</td>
</tr>
<tr>
<td></td>
<td>West Nile Virus - VI</td>
</tr>
</tbody>
</table>

Jack Risdahl, The Integra Group, Brooklyn Park, MN
Porcine Endogenous Retrovirus (PERV)

- PERV A/B: can infect human cells in vitro
- PERV C: only infects pig cells in vitro
- PERV is not related to HIV, but has similarity with certain mouse viruses (FeLV, MuLV)
- There is no disease condition ascribed to PERV infection
- No infection in vivo has been demonstrated in humans exposed to living porcine tissue

**In vivo infectivity studies**
- No productive infection in rat, guinea pig, mink, monkey, baboon
- Infection in *Scid* mouse by pig islet inconclusive (pseudotyping)
Assay for *in vitro* PERV Transmission

Stimulated pig cells

Add to target human or pig cells

Culture (up to 2 months) assay for PERV

Human-tropic PERV
PERV A and B

Pig-tropic PERV
PERV A, B, and C
PERV Transmission – Animal Data

• PERV transmission *in vitro* in non-human primates: variable data
  ‣ some groups have documented *in vitro* transmission for NHP species, including PBMC, spleen cells, and cell lines from cynomolgus and rhesus monkeys, baboon, chimpanzee etc.
  ‣ baboon PERV receptor: low efficiency?

• PERV transmission *in vivo*: no evidence
  ‣ NHP studies (limited): no transmission
  ‣ infectivity studies (rat, guinea pig, mink; immunosuppressed pig-tailed monkey, rhesus monkey, baboon): no transmission
  ‣ PERV: no signs of reactivation/disease in miniature swine: Post-Transplant Lymphoproliferative Disease associated with Porcine Lymphotropic Herpes Virus (similar to Epstein-Barr virus in humans)
  ‣ PERV infection in murine models is complicated by pseudotyping

**PERV Transmission in Exposed Patients**

**Imutran's XEN111 study**

Paradis et al., Science 1999; 285:1236-1241

36 patients were under immunosuppression

Serum antibody (>1 lab): negative/inconclusive

PERV RNA in serum (n=160) or saliva (ECSP, n=18): negative

No signs for PERV infection

Remarkably long microchimerism in patients after extracorporeal spleen perfusion
PERV: Conclusions

• Experts in swine infectious disease: PERV is a non-issue (!?)
• Regulatory authorities: PERV is an issue!
  ▸ public health aspects
  ▸ but, no clinical trials are on hold because of PERV safety risk
  ▸ guidance: regular monitoring, archive donor/recipient tissue for 50 years

Present results show a diminished (manageable) safety risk for clinical xenotransplantation

• Approach: carefully controlled clinical trials, initially in small numbers of patients
  ▸ monitor pig-to-patient transmission
  ▸ sequential enrollment of patients, regular report to regulatory authorities
  ▸ archive materials from donor and recipient
Where do We Meet Regulatory Aspects?  
The Pig Donor

• Designated pathogen-free status (closed herd)
  ‣ pig-to-pig disease control: herd management
  ‣ pig-to-human pathogen transmission
    • Transmission: *Erysipelotrix rhusiopathiae*, *Leptospira* sp, *Brucella* sp, *Salmonella* sp, *Streptococcus suis*, Influenza
    • Disease: Nipah virus encephalitis, swine influenza

• 2nd generation in barrier unit
  ‣ experimental laboratory animal conditions

• No rendered or recycled mammalian material in feed (other than pasteurized milk) for 2 generations
  ‣ reason: prion disease (rare in pigs)
  ‣ only unproven suspicions for pig-to-human transmission
Where do we meet Regulatory Aspects? The Patient

• Informed Consent (also here: Public Health Aspect)

• Pig product monitoring
  ‣ type of tissues/cells and contaminants
  ‣ genetic modification (GMO)
  ‣ retransplantation: sensitization
  ‣ fate of transplant and contaminants

• Immunosuppression and other medication
  ‣ each component should be proven
  ‣ combination treatment: safety evaluation
  ‣ no guidance for use of antivirals

• Archiving of donor and recipient material
The Diabetes Research & Wellness Islet Resource Facility
Home of Spring Point Project

- Operations started 1Q2007
- Young pigs, healthy and pathogen free
- Design and operations got favorable response from FDA
- USDA inspected and approved
- AAALAC certification pending