

Report of the Xenotransplantation Surveillance Workshop

Infection Control Database and Sample Archiving

***Our mission is to help the people of Canada
maintain and improve their health.***

Health Canada

This publication was produced by the Scientific Publication and Multi-media Services Section of the Management Planning and Operations Directorate, Health Canada.

To obtain additional copies or subscribe to the Canada Communicable Disease Report, please contact the Member Service Centre, Canadian Medical Association, 1867 Alta Vista Drive, Ottawa, ON, Canada K1G 3Y6, Tel.: 888-855-2555 or by Fax: (613) 236-8864.

This publication can also be accessed electronically via Internet using a Web browser at <http://hc-sc.gc.ca/hpb/lcdc>



Report of the Xenotransplantation Surveillance Workshop

*Infection Control Database and
Sample Archiving*

Ottawa, Ontario
March 31, 2000

This report was compiled by Dr. Marian Laderoute from the results of a written survey completed by participants at a Health Canada sponsored Xenotransplantation Surveillance Workshop, March 2000, and from her analysis of the current literature on xenotransplantation. The issue of xenotransplantation is characterized by many different views. Health Canada supported this workshop in order to assist in promoting discussion; however the opinions expressed by Dr. Laderoute and the participants do not reflect a Health Canada policy on the issue.

Dr. Laderoute is the Research Manager, Blood Zoonotics Unit, Bloodborne Pathogens Division. All correspondence should be addressed to Dr. Laderoute at the following address:

Dr. Marian Laderoute
Research Manager, Blood Zoonotics Unit
Bloodborne Pathogens Division
Bureau of Infectious Diseases
Centre for Infectious Disease Prevention and Control
Health Canada, LCDC Building #6, Room 1420
Tunney's Pasture AL 060 1E2
Ottawa, Ontario K1A 0L2

tel: (613) 941-6087

fax: (613) 952-6668

e-mail: marian_laderoute@hc-sc.gc.ca

Table of Contents

OVERVIEW	1
▪ Why an Enhanced Surveillance System for Xenotransplantation in Canada?	
I: INTRODUCTION	3
▪ Background to the Current Regulatory Framework for Xenotransplantation in Canada	3
▪ Adverse Event Reporting in Canada	3
▪ Potential Attributes of an Enhanced Surveillance Scheme	5
▪ Enhanced Xenotransplantation Surveillance in Other Nations	5
▪ The Issue of Endemic Viruses	6
▪ The Issue of Endogenous Viruses	7
▪ Published Adverse Events in Xenograft Recipients	9
▪ Infectious Disease Risks of Endemic and Endogenous Porcine Viruses	10
II: SURVEY RESULTS AND DISCUSSION	11
▪ Patient Registry and Adverse Event Reporting Database	11
▪ Definition of Adverse Event for Xenotransplantation	12
▪ Linking Adverse Events to Xenozoonosis	14
▪ Criteria for Follow-Up Investigation of Potential Zoonosis	14
▪ Time Lines for Reporting Data and Adverse Events to Registry	15
▪ Sample Archiving	15
▪ Schedule for Recipient Archiving/Testing	17
▪ Pre-Clinical Work-Up on the Source Herd	18
▪ Surrogate Markers	19
▪ Testing for Unknown Pig Infectious Agents	19
▪ Xenozoonoses Outbreak Responses	19
III: CONCLUSIONS AND SUMMARY	21
IV: REFERENCES	22
V: APPENDICES	24

OVERVIEW

Why an Enhanced Surveillance System for Xenotransplantation in Canada?

The potential for a novel, zoonotic agent to gain hold in the human population through xenotransplantation protocols is internationally recognized as a grave concern for public health. Although not all zoonotic agents can or will lead to pandemics or epidemics, the examples of HIV and “mad cow disease” (new variant Creutzfeldt-Jakob Disease [vCJD]) are recent prototypes of serious, latent infections that humans have probably acquired from animals. HIV has widely decimated lives and has threatened blood safety around the world, requiring testing for the agent to reduce risk. As yet, the transmission of prion mediated disease or vCJD has not been demonstrated to occur through the use of blood or blood products. Nevertheless, precautions have been undertaken on the basis of a theoretical risk. For example, the United Kingdom (U.K.), the country most affected by vCJD, now imports plasma from countries that have not reported cases of vCJD to make pooled blood products. Canada does not accept blood donors who have lived for more than 6 months (cumulative) in the U.K. and, more recently, in France. Unlike the case with HIV, there is no effective screening tool to reduce the risk of transmission of vCJD. The containment and control of zoonotic agents and the negative impact on blood availability or safety are problematic and costly for public health. Prevention of new epidemics, if possible, would be the preferred option.

The infectious agents of most concern to xenotransplantation and blood safety are those producing silent infections that are latent for many years and that result in incurable and devastating disease. In Canada, no clinical trial applications involving xenografts have been received or authorized to date. Thus, in accordance with the recommendations of the National Forum on Xenotransplantation, held in November 1997⁽¹⁾, Canada has a unique opportunity to put into place precautionary measures that will allow us to more carefully assess and at the same time minimize the infectious disease risks associated with xenotransplantation clinical trials. An enhanced surveillance system for infectious agents associated with xenotransplantation is essential for this purpose.

On March 31, 2000, the Xenotransplantation Surveillance Workshop was hosted in Ottawa by the Bloodborne Pathogens Division of the Bureau of Infectious Diseases,*

* Now within the Centre for Infectious Disease Prevention and Control, Population and Public Health Branch.

Laboratory Centre for Disease Control, Health Canada, to obtain comments from experts on an enhanced xenotransplantation surveillance system that would best meet the needs of Canadians. The workshop was not intended to be a consensus conference but, rather, to examine the issues and to serve as a starting point for future deliberations on xenotransplantation surveillance, including public debate.

Twenty-six infectious disease and xenotransplantation experts together with two observers attended the workshop (see Appendix A). The morning plenary session covered the goals and objectives of setting up enhanced xenotransplantation surveillance and summarized the recommendations of the 1997 National Forum on Xenotransplantation. This was supplemented by a brief discussion of the draft Proposed Canadian Standard for Xenotransplantation. The international surveillance issues that were covered included a report by the U.S. Centers for Disease Control and Prevention. The morning session concluded with an overview of the U.S. pilot xenotransplantation database. During the afternoon session, a discussion on databases and sample archiving was conducted, guided by specific survey questions (see Appendix B).

This report provides a background to the current framework for xenotransplantation regulation in Canada and addresses the main xenotransplantation surveillance concerns. The survey results are compiled, reviewed and interpreted in the context of this background, which includes a discussion of the Proposed Canadian Standard for Xenotransplantation.

I: INTRODUCTION

Background to the Current Regulatory Framework for Xenotransplantation in Canada

Xenografting is legally permissible in Canada only upon authorization of a clinical trial application, such as an Investigative New Drug (IND) application or an Application for Investigational Testing - Medical Devices, to the Therapeutic Products Programme* of Health Canada (HC)^(2,3). No special access program for xenografts is allowed in Canada because of the complexity of establishing risks and safety to third parties⁽³⁾. Some xenografts, such as extracorporeal perfusion devices, may more resemble medical devices than drugs, whereas others fall more under the description of a drug (biological drugs) or cellular transplantation^(2,3). Accordingly, both sets of regulations have to be taken into account when surveillance for xenotransplantation clinical trials is considered. In Canada, fixed pig valves are not considered xenografts as they are not viable⁽⁴⁾.

Adverse Event Reporting in Canada

When clinical trials on new therapeutic products are conducted to address safety and efficacy, annual reporting on patient outcomes and immediate reporting of adverse events (AEs) to HC are mandatory. For fatal and life-threatening *unexpected* adverse drug reactions (ADRs), the initial report must be sent to HC within 7 calendar days. All other serious and unexpected ADRs must be sent within 15 calendar days⁽⁵⁾. For serious AEs occurring during clinical trials of medical devices, HC must be informed within 72 hours⁽⁶⁾. Generically, once approval to market has been obtained, the reporting of serious drug-related AEs is voluntary, whereas for medical devices AEs must be reported within 10 days⁽⁷⁾.

In the draft Proposed Canadian Standard for Xenotransplantation (PCSX)⁽⁸⁾, it is recommended that registration of the xenograft recipient occur within 72 hours and that all serious AEs be reported immediately (although time lines are not made explicit). Although there are, as yet, no regulations referring to the proposed standard, an interim guidance document could be issued for xenotransplantation clinical trials.

* The Therapeutic Products Programme has now been separated into the Therapeutic Products Directorate and the Biologic and Genetic Therapies Directorate, Health Products and Food Branch.

According to the proposed standard, there would have to be lifelong monitoring of recipients, and this would exceed the time lines of clinical trials. Therefore, it is clear the existing regulations, at least for drugs, are not appropriate for xenotransplantation surveillance. In the U.S., the Public Health Services (PHS) Guideline on Infectious Disease Issues in Xenotransplantation⁽⁹⁾ was recently revised⁽¹⁰⁾. It now states that records and specimens on source animals and recipients should be kept for 50 years rather than for life⁽¹⁰⁾. This information and sample archive requirement were subject to public comment⁽¹¹⁾. Given the lengthy time of the recommended U.S. follow-up, further clarification on the duration of follow-up in Canada is clearly needed. This is especially true if the xenograft protocol under consideration is reviewed as a biological drug, for which (unlike medical devices) post-market reporting of adverse events is voluntary.

In Canada, precedents have already been set for enhanced surveillance schemes. For example, in February 2000, new guidelines for reporting AEs associated with vaccine biological products were released⁽¹²⁾. The Division of Immunization, Bureau of Infectious Diseases, collaborates with the Vaccines Division of the Bureau of Biologics and Radiopharmaceuticals. The Division of Immunization retains the responsibility for conducting post-marketing surveillance activities and maintains the Vaccine Associated Adverse Event Surveillance System (VAAESS). Not only was the definition of vaccine-associated adverse event (VAAE) expanded and more clearly defined in the February guideline but, as well, an Advisory Committee on Causality Assessment (ACCA) was set up in order to determine whether a particular AE was related to use of the vaccine and whether it merited further investigation⁽¹²⁾. Reporting of VAAEs from health care practitioners is voluntary (except in Ontario, where it is mandatory) and is usually channeled through provincial health authorities. However, serious VAAEs reported to manufacturers must be submitted to HC within 15 days and, for less serious AEs, within 30 days (i.e. reporting to HC is mandatory for manufacturers).

The Canadian policy on INDs (i.e. the drug regulations) states that the serious reportable AEs must be unexpected⁽⁵⁾. For xenotransplantation clinical trials, however, probably all deaths and serious changes in health should be reported immediately irrespective of cause. This would be more in line with the enhanced surveillance system in place for vaccine products⁽¹²⁾. As well, it would be reasonable to expand the definition of immediately reportable xenotransplantation-associated adverse events to include graft failure, cancer, and other disabilities related to immunosuppression, since these would be related to the xenograft protocol, though not necessarily to infectious diseases. The PCSX captures this broader definition of adverse event⁽⁸⁾.

The definition of reportable AEs for xenotransplantation is very important. Not surprisingly, it is still under heavy debate internationally, as is the definition of xenograft. In terms of the latter, the U.S. PHS recently amended its definition of xenograft to include not only live cells, tissues, or organs from a nonhuman source but also human body fluids, cells, tissues, or organs that have had *ex vivo* contact with them⁽¹⁰⁾. Thus, this new definition covers skin substitutes — i.e. human skin that is grown on nonhuman fibroblasts — and applies to several hundred patients treated in the U.S. Whether fixed porcine valves should be considered to be xenografts has been debated by the

Council of Europe (Dr. Larry Whitehouse, personal communication), but the Council's definition⁽¹³⁾ does not currently include them. Nevertheless, it is important to determine the level of residual risk, if any, derived from the implantation of fixed porcine valves as part of the pre-clinical risk assessment process, despite the fact that no AEs have been reported so far⁽¹³⁾.

Potential Attributes of an Enhanced Surveillance Scheme

The xenotransplantation workshop itself was not set up to determine whether enhanced surveillance was necessary but, rather, what it would comprise, given the necessary resources. A national enhanced surveillance scheme for xenotransplantation might involve some of the following:

- national patient registry and adverse event reporting database;
- national specimen archiving site with a database for animal source and recipient materials and including specimens from any investigations of close contacts or health care workers;
- national testing and sample monitoring laboratory facilities (linked to the specimen archiving database) for active surveillance and for investigations/follow-up;
- minimal exclusion and inclusion criteria to reduce infectious disease risks to recipients, their offspring, and third parties; and to also enhance the likelihood of lifelong compliance with monitoring, personal infection control, and safe sex practices;
- national committee of experts to oversee xenotransplantation surveillance and testing (this may or may not be separate from the existing Expert Advisory Committee on Xenotransplantation Regulation, which deals with problems encountered during the approval process for xenotransplantation clinical trial applications);
- national emergency preparedness procedures and response team for outbreaks in collaboration with provincial, territorial, and local health authorities;
- national inspection team (before and after clinical trial approval or for specific investigations);
- careful and precise definitions of “xenograft”, “serious adverse events”, “other adverse events”, and “suspected zoonosis”;
- clear time lines for reporting to HC and clarification of duration of follow-up if “lifelong” is too broad, as may be detailed in an interim guidance document.

Enhanced Xenotransplantation Surveillance in Other Nations

In the U.S., the PHS has continued the development of a national xenotransplantation database that will monitor xenotransplantation patients on a lifelong basis and is exploring an option to develop a central biologic specimen archive. For now, the revised PHS Guideline recommends that sponsors should archive materials designated for use by the PHS. Source tissues will be kept in archives for at least 50 years for future reference. In addition, the U.S. Department of Health and Human Services has

established a 15-member Secretary's Advisory Committee on Xenotransplantation, which will keep the public informed, serve as a public sounding board, advise the Department on the current state of knowledge regarding xenotransplantation, and review current and proposed xenotransplantation clinical trials. To date, the U.S. Food and Drugs Administration (FDA) has not announced an enhanced xenotransplantation surveillance scheme over and above what is required for biological drug clinical trials in general. Nevertheless, in 1997 it did place all porcine clinical trials on hold until sponsors could address certain issues, such as the testing of all recipients for porcine endogenous retroviruses (PERVs). As well, source animal facilities and the clinical animal sites do require accreditation. Further guidance from the U.S. FDA on requirements for xenotransplantation clinical trials can be found at <http://www.fda.gov/cber/gdlns/clinxeno0201.pdf>.

The U.K. situation is different, in that no clinical trial applications have yet been approved. The United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) has released a draft document for consultation called the Draft Report of the Infection Surveillance Steering Group of the UKXIRA⁽¹⁴⁾. Three clinical trial applications have been submitted to the UKXIRA, of which one was subsequently withdrawn; the other two were not accepted because of lack of supporting data.

The Issue of Endemic Viruses

The issue of testing and exclusion of pig herds bearing endemic viruses such as porcine cytomegalovirus (pCMV) requires further clarification. WHO recommendations⁽¹⁵⁾ state that herds should be free of certain viruses, including even those not established to be zoonotic, such as pCMV, as their transmission (or pathogenicity) may be enhanced under xenotransplantation protocols. While this is intended to mean that exclusion criteria should address pig infectious agents not known to be transmitted to humans but for which the risk of transmission may be facilitated through xenotransplantation⁽¹⁵⁾ (Clara Witt, personal communication), it would not necessarily preclude xenotransplantation. However, all herds apparently harbour pCMV and other endemic viruses, such as other herpesviruses, hepatitis E virus, circoviruses, parvoviruses, and papillomaviruses, which generally do not cause significant disease in their natural hosts⁽¹⁶⁾. Whether some or any of these endemic viruses can cross the species barrier under xenotransplantation conditions remains unknown. Moreover, if transgenic organs, tissues, or cells are used or if recipients are immunosuppressed, this is likely to significantly increase the risk of transmission or pathogenicity respectively.

An initial risk assessment may be possible by examining samples from occupational exposures to pig blood and medical exposures of biologic drugs or medical devices derived from pigs. However, this may not provide sufficient data to predict risk when the recipient of a xenograft is immunosuppressed or the material is derived from a transgenic animal. Such an assessment is likely to underestimate rather than overestimate risk, but would be valuable as a starting point.

A good example of variable risk dependent on the immunosuppression status of the host is hepatitis E. Although human hepatitis E virus usually involves only a temporary hepatitis, which does not lead to a chronic carrier state, an unusually high mortality rate (about 20%) occurs in third trimester pregnancy in humans, associated with vertical transmission and fetal demise⁽¹⁷⁾. Swine hepatitis E virus has 97% homology to some strains of human hepatitis E virus isolated in non-endemic countries such as the U.S. It remains to be determined whether swine hepatitis E virus, which is likely zoonotic to humans, results in an increased risk of mortality associated with pregnancy in humans. Its potential pathogenicity in immunosuppressed xenograft recipients would also need careful evaluation.

The revised U.S. PHS guideline recommends that for any infectious agent known or suspected to be in the source herd or animal, *active* monitoring of the recipient be done, especially in the immediate post-xenograft period⁽¹⁰⁾. Thus, while the guideline has not specifically identified which endemic porcine viruses need to be excluded from the herd, it does suggest that prospective “active” risk assessment should be done to determine whether an endemic infectious agent might need to be excluded in the future.

The Issue of Endogenous Viruses

The unknown risk of endogenous viruses, such as PERVs, has received far more attention and scrutiny than endemic pig viruses. This may be in part because HIV-1, a retrovirus, became established as a zoonotic pathogen when it jumped the species barrier, probably from chimpanzees to humans⁽¹⁸⁾. In this case it is the relatively high rate of sexual transmission that contributed most to the pandemic⁽¹⁸⁾.

In March of 1997, Patience et al. reported on the existence of PERVs that can infect human cells *in vitro*⁽¹⁹⁾. The following year they reported that once the endogenous retrovirus gained entry to human cells and replicated, the enveloped virions then produced were no longer susceptible to complement-mediated lysis by human serum, and infectivity rates increased with this change⁽²⁰⁾. Human serum contains naturally occurring antigalactose antibodies, which react with terminal galactose residues on viral proteins when the virus replicates in non-human cells. This suggests that a higher rate of transmission of PERVs and probably other enveloped viruses may occur if transgenic sources of animal organs, cells, or tissues are used for xenotransplantation. Denner et al., in 1998, reported that peptides in PERVs, which are at a site in the envelope protein known to be immunosuppressive in general for retroviruses, were found to be immunosuppressive *in vitro*⁽²¹⁾. Thus, PERV infection in humans has the potential to lead to immunosuppression *in vivo*. Given the difficulty in excluding PERVs from pig source animals, since the retrovirus is contained within the pig genome (i.e. is endogenously present), the question is, do pig cells, tissues, or organs release functional “infective” virions? If they do, what is the risk for cross-species transmission to humans? Finally, will PERVs exhibit significant rates of human-to-human transmission through sexual contact, the blood supply, or other means?

In many instances, endogenous retroviruses are inactive (as a result of genetic lesions), they are expressed but incapable of producing infective virions, or they are latent until activated by a number of agents, such as ionizing radiation, stress, inflammation, or potent stimulators of proliferation and/or activation (mitogens), etc. Thus, the findings by Martin et al. that infectious PERVs are released from porcine aortic endothelial cells without mitogenic or other stimulation²² and, more recently, by Van der Laan et al.⁽²³⁾, that they are released from pig islet cells when xenotransplanted into NOD/SCID (diabetic/immunosuppressed) mice suggest that the risk of retrovirus transfer to humans after xenotransplantation is at least theoretically possible. They also suggest that the screening of serum, biopsies, or preferably tissue samples of pig herds with sensitive techniques will detect PERV virions, making it difficult to obtain pig source animals that do not excrete PERV virions. Indeed, others have found that there is variability in the production of PERVs in blood cells from different pig strains⁽²⁴⁾, and that in Specific Pathogen Free (SPF) herds there is variability of viral mRNA load with tissue sources (kidney being the highest producer, then liver, lung, and heart)⁽²⁵⁾. Most notably, the SPF herds expressed PERV mRNA at equivalent or higher levels than conventional herds. This indicates, as expected, that the use of SPF herds does not reduce the risk of PERV transmission to humans.

The finding of the presence of an infectious agent in all or most potential source animals is necessary but not sufficient to indicate the level of risk of a) transmission to humans, b) disease causation, or c) the potential for an epidemic. Thus, while there is an apparent risk of transmission of PERVs to humans with xenotransplantation protocols, the question is, how often does it happen? A number of investigators have tried to address this issue⁽²⁶⁻³¹⁾. So far there is no unequivocal evidence that PERVs have infected humans or replicated in human hosts *in vivo*⁽²⁶⁻³¹⁾. However, in splenic perfusion models, 23% of the recipients followed up had circulating pig cells (referred to as a microchimerism) containing latent PERVs⁽²⁶⁾. The long-term consequences of microchimerism are unclear, although some individuals had had their xenograft exposure 8 years before⁽²⁶⁾. In another study, involving 10 patients with implanted fetal pig islet cells, five had microchimerism at 6 months or longer⁽³¹⁾. In neither study were antibodies to PERVs detected, suggesting that no release of infectious particles took place; however, it is possible, though not usual, to have an infection without antibody production, such as early in the course of infection during the window period, or as a result of tolerance induction. Nevertheless, there is no clear evidence for PERV replication or infection in human hosts even when microchimerism might exist^(26,31), although there are caveats to this interpretation, since transgenic xenograft sources were not evaluated. The report of active PERV infection in a murine model⁽²³⁾ gives great concern about a transpecies infection in humans, however.

If prospective testing of appropriate tissue samples, such as kidney or epithelial cells, were carried out instead of retrospective testing of serum or blood samples, positive results might be found under certain conditions, such as in immunosuppressed human xenograft recipients or in those receiving transgenic xenografts. Recently, it was reported that 68% (17 of 25) NOD/SCID mice xenotransplanted with pig islet cells had microchimerism, and all 8 mice that could be evaluated had evidence of PERV

infection, albeit only in the tissues where microchimerism could be demonstrated⁽²³⁾. The authors suggested that pig islet xenotransplantation to humans may result in long-term exposure to replication-competent endogenous retrovirus. However, since these investigators did not exclude the possibility of pseudotyping of PERV with mouse endogenous retrovirus (which may have increased the risk of transmission), it is currently unclear whether this increased risk of transmission in mice can be extrapolated to humans.

Published Adverse Events in Xenograft Recipients

The only published autopsy report involving the death of a xenograft recipient was at 7.5 months after implantation of porcine fetal neurons into a patient with Parkinson's disease in the U.S.⁽³²⁾. The cause of death was a pulmonary embolism deemed to be unrelated to the xenotransplantation protocol⁽³²⁾. However, this patient was immunosuppressed with cyclosporin, and there was an associated higher risk of infection. Additionally, infectious agents can be associated with pulmonary emboli. There was no indication that a follow-up investigation was done for porcine infectious agents, so it is unclear whether endogenous or endemic porcine infectious agents contributed to the patient's demise. A complete analysis of autopsy samples for endogenous and endemic infectious agents of pigs (along with source animal testing) would have been critical to address the general issue of transmission of these known viral agents to humans. An argument could be made that deaths in xenotransplantation clinical trials should be reported immediately and autopsy tissue samples fully investigated by health authorities, irrespective of perceived cause of death.

In a study of 10 Swedish patients who received transplanted fetal pig islet cells with immunosuppression, the follow-up was 4.5 years or more⁽³¹⁾. During this time two patients died of myocardial infarction, and one patient lost a renal allograft (at 2.5, 5 and 6 years respectively). No lymphoproliferative or neurologic disease was reported. It is unknown whether any of these serious adverse events were associated with xenozoonosis. Although no evidence was found from blood testing to suggest PERV infection⁽³¹⁾, the report did not indicate whether autopsy or biopsy samples were analyzed, particularly with respect to endemic and endogenous porcine viruses. Interestingly, in the same study the majority of pigs tested (9 of 12, 75%) exhibited PERV RNA in the serum correlated with demonstrable reverse transcriptase (RT) activity⁽³¹⁾. Since the patients did not show PERV RNA or RT activity in serum samples but most pigs did, this offers a measure of confidence that the patients, half of whom exhibited microchimerism, did not produce replication-competent PERVs. On the other hand, this finding in pigs suggests that the infectious disease risks related to PERVs are likely to be there irrespective of the types of organs, tissues, or cells used as xenografts. This may be a problem if some individuals already express endogenous or contract exogenous retroviruses that might contribute to pseudotyping of PERV. This is of concern as it may subsequently allow for generation of replication-competent PERVs. The possibility of endogenous retrovirus likely contaminating most or all xenografts is cause to take extra precautionary measures. If these endogenous viruses could be genetically or

functionally deleted from the pig genome (of which many scientists are not hopeful), the risk of infectious disease associated with xenotransplantation is likely to be greatly reduced.

Infectious Disease Risks of Endemic and Endogenous Porcine Viruses

In summary, the risks of infection to human populations by pig endogenous and endemic viruses remain largely unknown, although there is suggestive evidence that swine hepatitis E may be transmissible to humans. Active monitoring of common pig infectious agents after xenotransplantation will be necessary to provide a risk assessment for endogenous and endemic viruses. In particular, the immediate post-xenotransplantation period (i.e. before antibodies clear the virus from patient blood), which can be anywhere from 2 to 8 weeks, would be the optimal time to investigate for viral replication by, for example, polymerase chain reaction (PCR). Close examination of the viruses that are replicating in the early post-transplantation period would at least answer the question of what the infectivity risk is of the various pig infectious agents for humans. This would indicate which viruses need to be closely monitored over the longer term in order to control and contain a potential epidemic.

As is well known, absence of data does not substantiate absence of risk. The finding of cross-species transfer of PERVs⁽²³⁾ to immunosuppressed mice in association with microchimerism raises the question of whether immunosuppressed humans receiving transgenic organs, tissues, or cells or, alternatively, recipients with microchimerism will also produce replication-competent PERVs. Furthermore, as will be discussed later, any adverse event could be linked to infection with a zoonotic agent. Without active screening, it will be difficult to determine whether the symptom relates to a potential xenozoonosis or not. Initial trials should be closely and carefully monitored⁽¹⁾. The revised draft U.S. PHS guideline also now calls for active monitoring after xenotransplantation of any pig infectious agents known or suspected to be in the source herd, including endogenous and endemic pig viruses.

II. SURVEY RESULTS AND DISCUSSION

The main goal of the workshop was to capture the views of the various participants on certain issues and not to mould opinion or reach any kind of consensus. Generally, most issues elicited a wide range of views and comments. These responses are instructive because they help identify where problems lie and what issues need to be addressed in future deliberations and, presumably, in guidance documents.

Patient Registry and Adverse Event Reporting Database

Participants noted that the design of a xenotransplantation surveillance database will depend greatly on what purpose(s) it is to serve. Surprisingly, some participants considered enhanced surveillance measures by HC to be a research project on safety assessment. However, since xenotransplantation carries an as yet undefined risk to the public in the way of a new epidemic, the assessment and containment of this risk at the clinical trial level is essential for the health protection of Canadians. Carefully designed and closely monitored clinical trials are viewed as the only means to adequately assess the risk of xenotransplantation procedures to public health and safety⁽¹⁾. Furthermore, clinical trials should not be allowed to go ahead without public consultation, oversight by a national body, and precautionary measures to assess and contain the risk of infectious diseases to third parties⁽¹⁾.

One could envision the initial registry of the small number of recipients being merely paper-based; however, once several trials are approved and under way, it will become necessary to develop an electronic version capable of linking various data sets. Discussion on the registry at the workshop identified two functional types of data elements, those contained within the national registry and those linkable or accessible through common identifiers to other databases, such as an archiving database or the medical records of the recipient at the local site. Most participants felt there would not be a need to have the full medical history or patient file at the national registry, since the relevant information could be ascertained upon inspection, investigation, or linkage. It was felt that if the registry database became too detailed or complex, the ability might be lost to respond to an early sign or trend indicating transmission of an infectious agent and that the costs and potential for information overload would be greater.

Most respondents indicated that the demographics of the source animals and tissues should be accessible or linkable, but not necessarily held in the national registry. On the other hand, there was general agreement that the demographics of the recipient

(age, sex, hospital site of xenograft, type of xenograft) *should* be included. The Proposed Canadian Standard for Xenotransplantation calls for the inclusion of the following data elements: xenotransplant program identification number, recipient coded identification, race, sex, age and date of birth, SIN number, ABO blood grouping, organs received and cause of organ failure, date and time of transplantation, unique source animal identification number and all data relevant to source animal parentage, husbandry, and testing for transmissible (or potential) pathogens⁽⁸⁾. Although the proposed standard recommends that source animal information be contained within the national registry, many participants thought that linkability or accessibility only was needed. They did not feel that other data elements, such as whether the patient was immunosuppressed, type of immunosuppression used, the patient's transfusion or transplantation history, and history of human infectious diseases or cancer, were important enough at this time to be in the national registry.

All responding participants indicated the critical importance of recording serious adverse events in the registry, and most felt that other AEs should also be included. However, the types of AEs to be submitted to the patient registry will require further clarification and definition (see also below).

In conclusion, participants agreed with the minimal data elements to be submitted to the national registry as outlined in the Proposed Canadian Standard for Xenotransplantation⁽⁸⁾, with the potential exception of the xenograft source demographics.

Definition of Adverse Event for Xenotransplantation

The definition of an AE received much debate. In the Proposed Canadian Standard for Xenotransplantation an AE is defined as “an undesirable outcome directly or indirectly related to the allo/xeno graft (e.g. infection, disease transmission, graft failure)”⁽⁸⁾. It is assumed that death, though not listed, would be considered “undesirable”. As an alternative, the following definition was suggested in the survey (see the enclosed survey template in Appendix B): “any notable change in the recipient's health or well-being, reversible or not, requiring medical attention or intervention, inclusive of death, disability, disease transmission, infection or hospitalization whether directly or indirectly related to the xenograft protocol”. Many participants chose one of the two similar definitions provided but listed caveats or additions, such as graft failure or the diagnosis of cancer.

One suggestion during the workshop discussion was to adopt the WHO or other internationally agreed upon definition; however, at present the WHO has not released its definition of “adverse event”, although international discussions are under way. Some participants preferred that the sponsor/clinicians define for themselves what is reportable or decide whether the AE was related to xenotransplantation before reporting. For AE reporting in the enhanced surveillance of vaccines, there is a separate panel of experts that reviews the AEs reported to decide whether they are related to the vaccination process⁽¹²⁾. If a serious AE is rare or unusual, this decision may be very difficult. Some workshop participants suggested that all serious AEs should be reported,

closely monitored, and/or investigated, since xenotransplantation is new, and it may be difficult to recognize when the AE relates to the procedure and/or xenozoonosis. Some felt that any abnormal laboratory test results or any persistent AE, such as fever, should be reported, whereas others cautioned against flooding the system. The challenge is to ascertain the true AE related to xenotransplantation as opposed to background noise. Some participants also felt that if family members suddenly experience an unexplained illness this should also be reported.

The UKXIRA guidelines had defined “untoward event” as a suspected xenozoonosis “and” confirmed xenozoonosis⁽¹⁴⁾. The UKXIRA has recently changed this to “and/or”, as most xenozoonosis would not be confirmed before an investigation is conducted. However, restricting AE reporting to xenozoonoses may be counterproductive, as there are no uniform clinical criteria for suspecting a xenozoonosis, especially with regard to unknown or latent viruses. There is no easy way to discriminate human from zoonotic infections based on the clinical picture. Under these circumstances, either all AEs or, what is more likely, few AEs would be reported and investigated. Without further clarification, the UK definition of an adverse event seems inadequate, as even deaths may go unreported. Certainly, analysis of autopsy tissues from xenograft recipients would provide essential sources of information about transmission of endogenous and endemic pig viruses to humans and would be key to the assessment of the infectious disease risks of xenotransplantation. At the very least the UKXIRA would need to reconsider whether deaths should or should not be reported and infectious agents investigated on autopsy samples, regardless of causality.

For AE reporting, the Proposed Canadian Standard for Xenotransplantation states that “the following adverse events shall be immediately reported (i.e. reporting is mandatory and immediate): potential or confirmed xenozoonosis in the recipient or positive test for infectious agent(s) in the source animal; appearance in the recipient of a reportable infection such as HIV, hepatitis B or C, tuberculosis, or any other infection(s) including zoonoses; appearance in the recipient of a new cancer (excluding basal cell or squamous cell skin carcinomas); primary non function of the transplanted xeno-organ; and death of the recipient”⁽⁸⁾. The definition of adverse event for xenotransplantation and the mandatory reporting differ considerably from the Adverse Drug Reaction definition for INDs, in which the reporting of serious AEs within clinical trials contains the element of “unexpected” and suggests that “a causal relationship is at least a reasonable possibility”⁽⁵⁾. For medical devices, the report is mandatory when it relates to the failure of the device or its effectiveness, *and* has led to the death or serious deterioration in the state of health of a patient⁽⁷⁾. Clearly, the scope, reporting, and testing requirements relating to AEs in xenotransplantation protocols need further definition and clarification in a guidance document before xenotransplantation clinical trials are considered in Canada. Given the likelihood that most pig herds are positive for PERVs (blood and/or tissues) and probably other endemic viruses, such as pCMV, it remains to be decided whether the presence of these viruses (in the source animal or the herd) should or should not preclude xenotransplantation.

Linking Adverse Events to Xenozoonosis

Most workshop participants thought that death, cancer, autoimmunity, disabilities such as paralysis, graft rejection, rashes, and fevers might be causally linked to an infectious agent (human or porcine). The notion that viruses may be implicated in a number of autoimmune conditions and chronic inflammatory diseases is not new. Several human endogenous and exogenous retroviruses have now been implicated, including a new exogenous virus called human retrovirus five (HRV-5), which is implicated in arthritis and was recently described by Weiss et al.⁽¹⁸⁾. The concern has been raised before about PERV pseudotyping by other human retroviruses present in the host and the subsequent release of replication-competent PERVs. Furthermore, retroviruses can be oncogenic (cancer-causing). Patients with autoimmune, chronic inflammatory processes or cancer may need to be excluded from or (if included) more closely monitored in xenotransplantation clinical trials until such time as PERVs can be excluded from source herds and xenografts.

Other AEs potentially linked to xenozoonosis include a range of illnesses and syndromes (in recipients and close contacts) and could include any abnormal laboratory value, particularly if there is a positive test result for pig infectious agents (specific or non-specific). One participant went so far as to say that any AE might be causally linked to an infectious agent. The Proposed Canadian Standard requires mandatory creation of Standard Operating Procedures (SOPs) to be used for the local investigation and follow-up of suspected infections and communicable diseases⁽⁸⁾. If local xenotransplant teams are free to develop their own standards, a non-uniform set of reporting and investigative processes will likely result. A national investigative team may be better suited to perform the investigation and determine, in an unbiased way, whether a porcine infectious agent is involved or is potentially linked to the AE. Analysis in an aggregate fashion by a national authority may improve consistency and facilitate the earlier detection of trends. Nevertheless, it is expected that there will be general difficulty in confirming whether an adverse event relates to an unknown xenozoonosis. Accordingly, there seems to be little value in limiting the definition of an adverse event to a xenozoonosis.

Criteria for Follow-up Investigation of Potential Zoonosis

Essentially any unexplained AE could be followed up for a potential zoonotic agent. Some participants thought that only if there is some evidence for a zoonotic infection (such as that established through screening methods) should there be follow-up. Several participants believed the sponsor or local expert team of investigators should decide. In keeping with the recommendations of the National Forum⁽¹⁾, many participants felt that when a death occurs, an investigation for pig infectious agents should be performed on autopsy samples. Some suggested that a diagnosis of cancer or autoimmunity may also warrant such an investigation. Thus, the criteria for a follow-up investigation will need further debate and resolution. As well, efforts should be made to harmonize these internationally⁽¹⁾. Whatever criteria are developed, they should be established in advance of clinical trials and made available through an interim policy

released by HC on the conduct of clinical trials involving xenotransplantation. Although it may be relatively straightforward to investigate known agents with defined sequences, unknown pig infectious agents may prove more difficult to confirm.

Time Lines for Reporting Data and Adverse Events to Registry

In general, there was a consensus that serious adverse events should be reported immediately, in agreement with the Proposed Canadian Standard for Xenotransplantation⁽⁸⁾, but the definition of “immediately” was somewhat murky. During the open discussion most participants agreed that 24 hours should be the definition of “immediately”, whereas in the survey report there was a range from 12 hours upon receiving confirmation to 72 hours. With regard to reporting by 24 hours, one participant questioned whether anyone at HC would be available on the weekends. If the criteria for follow-up investigation are clarified in the interim guidance document and it is stipulated what enhanced surveillance is mandatory (including required samples, where to send the samples, when a follow-up investigation is needed), then perhaps a 72-hour maximal time line would be acceptable. Again, whatever minimal requirements are needed, these should be established before the authorization of clinical trials in Canada.

There was a lot of variability in participants’ responses about serious versus not-so-serious AEs. Many participants felt that quarterly instead of annual reporting should be required for xenotransplantation clinical trials, because some less serious AEs, such as a flu-like condition, when analyzed in an aggregate fashion, may provide the earliest indication of a xenozyoonosis epidemic.

Sample Archiving

Many participants preferred the word “archiving” in the survey questionnaire to “monitoring”, since they felt that routine or active testing by a national body was not warranted. As well, many felt that there is no need to duplicate archiving at a national and local site. Instead, samples could be made available in the event of an investigation, or duplicate samples for a national body could be held locally. The latter is consistent with the new U.S. PHS guideline but not with the recommendations of the National Forum on Xenotransplantation⁽¹⁾, which greatly favours separate retention of locally and nationally archived duplicate samples. The Proposed Canadian Standard for Xenotransplantation⁽⁸⁾ states that “in the absence of a central facility, designated public health biologic specimens (serum, plasma, leukocytes, and tissues of the recipient, if available) should be archived with appropriate safeguards to ensure long-term storage and an efficient document system for the prompt retrieval and linkage of data to medical records of recipients and source animals.”

For recipient samples, most participants agreed that baseline, routine, and investigative samples would need to be archived on all patients and that serum (5 aliquots of 0.5 mL for both the local/national sites), plasma (same quantity as for serum), and peripheral blood mononuclear cells (PBMC, at least 3 aliquots of 1 million cells for local and

national investigators) should be stored. Other suggestions for archiving included biopsy and autopsy specimens if appropriate/available, and RNA and DNA samples; few felt that urine samples would be useful. Some suggested that there is no need for a national archive, whereas others maintained that there should be a national archive and all testing be done by HC. The ability to obtain blood should be considered, and the volumes collected need to be practical. Indeed, one participant suggested that the sponsor should determine amounts of samples to be collected. It was also proposed that expert committees should determine which tests should be performed under what circumstances, since the total sample volume would be limited, and certain samples may be critical for the investigation. Since not all tests would be available in a validated and standardized format, it may be preferable that an independent approval committee decide what tests are to be performed.

Like the Proposed Canadian Standard, the UKXIRA guidelines propose lifelong monitoring. If the storage of samples is recommended for at least a minimum of 50 years, as per the new U.S. PHS guideline⁽¹⁰⁾, the question arises of whether age restrictions on xenograft recipients should be required (favouring elderly patients), or whether initial trials should be initially limited to terminal patients. As well, a mechanism is needed to ensure the availability of specimens for this length of time, probably through storage at a national archive site.

In terms of the source animal samples to be archived, many respondents felt the samples should be held at the local site or by the sponsor, and HC be allowed access in the event of an investigation. Assuming the animal is sacrificed at the time of cell, tissue, or organ harvest, as was recommended at the National Forum⁽¹⁾, then tissues such as lymph node, spleen, bone marrow, and any other tissue relevant to the type of xenograft used could also be archived, along with plasma (5 aliquots of 0.5 mL), serum (5 aliquots of 0.5 mL) and PBMC (5 aliquots of 10 million cells). Because of the concern that hepatitis E is endemic in herds and transmissible to humans, some participants suggested collecting fecal samples from the source animal for PCR tests, since this is the most sensitive and reliable method for early detection⁽¹⁷⁾.

For close contacts, baseline and investigative samples should be taken (as with recipients), but only a few respondents felt that routine annual samples were necessary. Most suggested that local storage of close contact materials would be sufficient.

Sample archiving for health care workers was judged to be similar to that for close contacts, with additional samples taken in the event of needlestick injury. Many thought those at highest risk might be monitored (archived and/or tested) more frequently. The U.K. guidelines suggest that, aside from baseline blood samples, samples on health care workers should be archived and tested only if there is significant exposure to bodily fluids of xenograft recipients (such as for surgeons) or if there is any untoward event⁽¹⁴⁾.

Schedule for Recipient Archiving/Testing

Aside from the need for a baseline sample on all recipients and for investigative samples for an AE, the Proposed Canadian Standard for Xenotransplantation⁽⁸⁾ does not detail a minimally acceptable schedule for the archiving or testing of recipient samples. Workshop respondents were given a choice of two schedule types for sampling patients, or they could develop their own schedule (see attached survey template in Appendix B). In the initial trials, the schedule is expected to be more intense in order to collect rudimentary information as to which samples are the most informative. Once the initial clinical risk assessment is achieved, it may not be necessary to collect samples as frequently in the immediate post-xenograft period. Many participants agreed on weekly or biweekly sampling in the first 3 months, then monthly for the next 3 to 9 months, and then annually. Most felt that at least a sample at 1 month and at least twice in the first year would be necessary.

The U.K. guidelines recommend archiving only for baseline, month 1, month 6, and then annually; this is based on the 1996 U.S. PHS guideline⁽⁹⁾. On the other hand, the U.K. guidelines call for testing of samples at 0, 1-2 days, 2, 4, and 6 weeks, 3 and 6 months, and annually thereafter. The rationale for testing but not archiving of some samples is unclear in the UKXIRA guidelines. The revised 2000 U.S. PHS guideline⁽¹⁰⁾ (in section 4.1.2) now states that for archiving there should be two separate baseline samples (preferably separated by a month and where one sample is time 0), and that samples in the immediate post-transplant period should be archived along with those from 1 and 6 months, 12 months, 24 months, 60 months, 120 months etc.

With regard to testing, the revised PHS guideline recommends that if xenogeneic infectious agents are known or suspected to be present in the xenotransplantation product, then active screening of the recipient should be performed frequently in the immediate post-xenotransplantation period. They cite, as examples, the 2, 4, and 6 week specimens and suggest screening could include endogenous retroviruses, herpesviruses, and papillomaviruses. Since all pig herds harbour PERVs and many, if not all, harbour endemic viruses of one kind or another⁽¹⁷⁾, then as a standard (for the U.S., U.K., and probably Canada), archiving and active testing for endogenous and endemic pig infectious agents may need to be performed minimally at baseline, 2 weeks, 4 weeks and 6 weeks, 6 months, annually (for 2 years), and then every 5 years. This is one possible interpretation of the revised U.S. guideline.

The word “monitoring” in our survey template was meant to include testing and archiving. However, many participants felt that “monitoring” should be changed to “archiving”, and this would eliminate the need for active testing. However, as mentioned, the U.S. guideline (referred to by the UKXIRA⁽¹⁴⁾) makes it very clear that active testing and archiving are now recommended as part of the monitoring. This is particularly important in the immediate post-xenograft period, and especially since most herds probably harbour known endogenous^(19-25,31) and endemic^(16,17) viruses.

Most participants felt that close contacts need baseline and annual sample archiving, and testing and archiving in the investigation of an AE. Some, however, thought quarterly

or monthly sampling for the first 3 months could be considered, presumably to allow for the initial assessment of risk of sexual or household transmission. The U.K. guidelines recommend that a baseline and a sample at 1 year should be archived; testing and further investigational archiving would be necessary only in the event of an AE potentially related to xenozoonosis⁽¹⁴⁾. The U.S. PHS guideline suggests that only a baseline sample is required on health care workers, and testing and further archiving is only necessary in the case of an AE⁽¹⁰⁾. The U.S. PHS guideline does not cover close contacts.

Pre-Clinical Work-Up on the Source Herd

When referring to the xenograft animal materials or animals, most participants preferred the use of the term “source” to “donor”. Many felt that a sentinel health surveillance program is required, possibly with some sentinels being temporarily immunosuppressed (i.e. for up to 2 months) and observed; others did not think this was feasible. The Proposed Canadian Standard for Xenotransplantation suggests, among other measures to isolate the source herd from infectious agents, that a sentinel surveillance program should be encouraged as a standard⁽⁸⁾. Some participants proposed that the sentinel program be instigated on the biosecure herd and not on each litter, since conclusions cannot be drawn for about 15 years (the life span of a pig), and it would be best to have this assurance of safety long before, or as soon as possible after, xenotransplantation.

Many respondents did not favour the inoculation of nonhuman primates (NHPs) with animal source blood, cells, and/or tissues (whatever tissue source may be relevant to the proposed human clinical trials) and then monitoring the NHPs for 2 years, with or without immunosuppression. One reason was that the significant number of infectious agents harboured by NHPs would result in a high background level, clouding the interpretation. This would be particularly problematic for nonspecific tests for unknown agents. As well, there is some evidence that nonhuman primates may not be appropriate models for PERV transmission⁽²⁰⁾. The proposed standard does not address the issue of preclinical testing in animal models⁽⁸⁾, although, in general, as a prerequisite for clinical trials it is required. Given the issue of the anti-gal naturally occurring antibodies limited to humans and old world primates, studies in NHPs would be required as relevant animal models for interpretation of infectious disease risks as part of the “pre-clinical testing”. In some respects, since xenotransplantation clinical trials have been formally ongoing in the U.S. since at least 1995 and may involve 50 to 100 recipients, it is not clear whether only short-term studies (days or weeks) with NHPs or follow-up over a number of years would be needed.

Most participants agreed that relevant source animal samples should be co-cultured with various relevant human indicator cell types (including PBMC and activated PBMC), tested for retroviruses and herpesviruses, examined by electron microscopy (EM), and examined for nonspecific cytopathic effects. Other means of identifying unknown pathogens (whatever is state of the art) should also be employed. Other tests include routine surveillance for infectious agents to be excluded from the herd as well

as routine histopathology, hematology, and biochemistry. With regard to screening for endogenous and endemic viruses, the majority of respondents felt it was necessary to screen the herd and presumably the intended source animal(s). Many participants had trouble with the use of EM for the visual detection of virions in source animals or the herd, primarily because EM is labour intensive and relatively insensitive, in that many sections of many tissues would have to be examined in order to rule out a false negative result (sampling error). Instead, it was suggested that EM could be used in an investigation and/or on the source tissue intended for xenotransplantation, either on a biopsy specimen or an unused portion. For example, if a kidney is to be xenografted, the remaining kidney could be examined by EM.

Surrogate Markers

Several participants suggested that the following markers could be investigated as a potential surrogate for infectious disease risk: erythrocyte sedimentation rate (ESR), the PCR Enhanced Reverse Transcriptase (PERT) assay on serum, which would detect reverse transcriptase enzymatic activity characteristic of retroviruses, liver function tests, perhaps C-reactive protein, and the culture of patient materials with indicator cells (pre- and post-xenotransplantation). Some questioned whether the development of chronic fatigue or insomnia would be a useful clinical surrogate marker; others suggested that we need to develop a compendium of markers potentially related to post-xenograft complications and to establish the correlation of these to confirmed xenozoonoses. Almost no participants felt that an early cancer blood test, testing for RNase L activity in the urine, or examination of immunosuppression of PBMC (in non-immunosuppressed recipients) would be of value as surrogate markers for xenozoonosis at this time.

Testing for Unknown Pig Infectious Agents

Some participants liked the notion of using representational difference analysis (RDA) or modified fluorescence *in situ* hybridization (FISH) methods to detect previously uncharacterized pig infectious agents in xenograft recipients, but others cautioned against the use of non-validated methods. Moreover, such methods are difficult to use, may be problematic, and are therefore unlikely to be suitable for routine monitoring. It may not be useful to be too specific on what tests should be employed. Some participants suggested working backwards, so that when an infectious agent is strongly suspected, then these “research methods” to identify and characterize the unknown entity can be used.

Xenozoonoses Outbreak Responses

Virtually all respondents indicated the need for SOPs at the national level for testing, sample collection, quarantine, and notification in conjunction with the federal, provincial and territorial health ministries, although the question came up as to who has

the authority to invoke such measures. As well, participants recognized the need for SOPs on how to manage archived samples. Alternatively, an archive steering committee could be consulted about the use of archived materials. SOPs should also provide information on how to deal with the animal sources, including recall, lookback, and traceback procedures in the event of an outbreak. This requirement is dealt with in the Proposed Canadian Standard for Xenotransplantation.

There is a need to develop SOPs at HC for clinical holds, moratoria, inspections, and international notification in the event of an outbreak; both sets of SOPs (national and local) should be in place before clinical trials are started in Canada.

Finally, most respondents agreed that both local and national laboratories should have the facilities and resources in preparation for zoonotic outbreaks, including the setting up of reference assays. However, this testing may be conducted through contracts with accredited laboratories. One person suggested that, in addition, the sponsor should have SOPs for responding to a potential zoonotic outbreak, assuming that some clinical trials may be multicentered. Generic emergency response plans could be tailored to incorporate particulars relating to xenotransplantation infectious disease risks, although until these agents are identified and further characterized it will be difficult to provide a xenotransplantation pathogen-specific emergency response plan.

The Proposed Canadian Standard does not deal with zoonoses outbreaks *per se*, but details in generic terms what to do if an adverse reaction/suspected infection occurs⁽⁸⁾. Again, it recommends the creation of SOPs in advance on issues such as quarantine, destruction, investigation, recall, and notification, but does not provide details on how and what to investigate.

III: CONCLUSIONS AND SUMMARY

An enhanced xenotransplantation surveillance system is one of many precautionary measures to reduce infectious disease risks to recipients, their offspring and third parties. A critical review of the literature along with an analysis of the survey results and discussion suggests that the current regulatory framework in Canada may benefit from the development of an interim policy on xenotransplantation clinical trials to deal with enhanced surveillance issues. Additionally, international harmonization on the surveillance of xenotransplantation clinical trials to reduce the infectious disease risks to recipients and third parties also seems warranted.

Although an appraisal of the literature on PERVs seems to indicate that there is at least a theoretical risk of PERV transmission to humans through xenotransplantation, recent evidence suggests this risk might be minimized in the near future. For example, there is preliminary evidence from Dr. Clive Patience, a principal scientist at BioTransplant Inc., that pig herds could be bred that do not produce infective, replication-competent PERVs⁽³³⁾. However, whether special breeding or cloning of animals may, in fact, eliminate the production of replication-competent endogenous or endemic viruses remains to be seen.

There remain several important issues about pre-clinical safety assessment, including the requirement for studies in NHPs (efficacy and safety) versus infectious disease risks (transmissibility of pig infectious agents to primates) and minimal follow-up times in animals, before clinical trials are considered in Canada.

In summary, national and international cooperation is needed to effectively devise and adopt enhanced surveillance measures for xenotransplantation that will identify, contain, and minimize infectious disease risks to recipients, their offspring and third parties worldwide. A number of problems and points to consider have been outlined in this report for future national and international discussion.

IV: REFERENCES

1. *National Forum on Xenotransplantation: clinical, ethical and regulatory issues, November 1997.* URL: <http://www.hc-sc.gc.ca/hpb-dgps/therapeuti/zfiles/english/btox/reports/frmrpt_e.pdf>.
2. *Notice to interested parties: intent to develop a regulatory framework for xenografts.* URL: <http://www.hc-sc.gc.ca/hpb-dgps/th...es/english/btox/notices/noi_e.html>. Date of access: February 6, 1999.
3. Notice to hospitals from the Therapeutic Products Programme. March 29, 1999. *The clinical use of viable animal cells, tissues, or organs to treat patients.* URL: <http://www.hc-sc.gc.ca/hpb-dgps/th...x/notices/noticetohospitals_e.html>.
4. *Xenotransplantation (Factsheet).* Revised July 1999. URL: <http://www.hc-sc.gc.ca/hpb-dgps/th.../english/fact-sht/fact_xeno_e.html>.
5. *Adverse Drug Reaction (ADR) Expedited Reporting Summary Form (to be used for ADRs occurring during clinical trials).* URL: <http://www.hc-sc.gc.ca/hpb-dgps/th...zfiles/english/forms/ct_adr_e.html>.
6. *Preparation of an application for investigational testing (Devices).* URL: <http://www.hc-sc.gc.ca/hpb-dgps/therapeutic/zfiles/english/guides/meddev/test_md3_e.pdf>. Date of access: February 22, 1999.
7. *Mandatory and voluntary problem reporting for medical devices.* URL: <http://www.hc-sc.gc.ca/hpb-dgps/therapeutic/zfiles/english/guides/meddev/manrpt2_e.pdf>. Date of access: September 14, 1998.
8. *Proposed Canadian Standard for Xenotransplantation.* URL: <http://www.hc-sc.gc.ca/hpb-dgps/therapeut/zfiles/english/btox/Standard/xeno_std_e.html>.
9. *PHS Guideline on Infectious Disease Issues in Xenotransplantation.* (61 Federal Register 49919). URL: <<http://www.fda.gov/cber/gdlns/xeno.txt>>. Date of access: September 23, 1996.
10. *PHS Guideline on Infectious Disease Issues in Xenotransplantation..* URL: <<http://www.fda.gov/cber/gdlns/xeno0500.pdf>>. Date of access: May 26, 2000.
11. *Agency information collection activities; proposed collection; comment request, Food and Drug Administration, HHS, Docket No. 96M-0311 and see Federal Register website,* URL: <http://frwebgate.access.gpo.gov/cgi-bin/...bname=2000_register&docid=00-13340-filed>.
12. *Guidelines for reporting adverse events associated with vaccine products.* CCDR 2000;26(S1). See Website: <http://www.hc-sc.gc.ca/hpb/lcdc/publicat/ccdr/00vol26/26s1/index.html>.
13. *Council of Europe, Working Party on Xenotransplantation (CDBI/CDSP-XENO). Report of the state of the art of xenotransplantation.* URL: <<http://www.coe.fr/dase/en/qoflife/publi/artreport/tableart.htm>>.
14. *United Kingdom Xenotransplantation Interim Regulatory Authority: Draft report of the Infection Surveillance Steering Group of the UKXIRA.* URL: <<http://www.doh.gov.uk/surveil.htm>> last updated August 4, 1999; for other related documents, URL: <<http://www.doh.gov.uk/ukxira.htm>>.

15. WHO. *Xenotransplantation: guidance on infectious disease prevention and management WHO/EMC, (1998)*. URL: <<http://www.who.int/emc-documents/zoonoses/whoemczoo981c.html>> and for listing see index at URL: <<http://www.who.int/emc-documents/index.html>>.
16. Yoo D, Giulivi A. *Xenotransplantation and the potential risk of porcine viruses for xenogeneic transmission*. Can J Veterinary Res 2000;64:193-203.
17. Labrique AB, Thomas DL, Stoszek SK et al. *An emerging infectious disease*. Epidemiol Rev 1999;21:162-79.
18. Weiss RA, Griffiths D, Takeuchi Y et al. *Retroviruses: ancient and modern*. Arch Virol Suppl 1999;15:171-77.
19. Patience C, Takeuchi Y, Weiss RA. *Infection of human cells by an endogenous retrovirus of pigs*. Nature Medicine 1997;3:282-86.
20. Takeuchi Y, Patience C, Magre S et al. *Host range and interference studies of three classes of pig endogenous retrovirus*. J Virol 1998;72:9986-91.
21. Denner J. *Immunosuppression by retroviruses: implications for xenotransplantation*. Ann NY Acad Sci 1998;862:75-86.
22. Martin U, Kiessig V, Blusch JH et al. *Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells*. Lancet 1998;352:692-94.
23. Van Der Laan LJW, Lockey C, Griffeth BC et al. *Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice*. Nature 2000;407:501-4.
24. Tacke SJ, Kurth R, Denner J. *Porcine endogenous retroviruses inhibit human immune cell function: risk for xenotransplantation?* Virology 2000;268:87-93.
25. Clemenceau B, Lalain S, Martignat L et al. *Porcine endogenous retroviral mRNAs in pancreas and a panel of tissues from specific pathogen-free pigs*. Diabetes Metab 1999;25:518-25.
26. Paradis K, Langford G, Long Z et al. *Search for cross-species transmission of porcine endogenous retrovirus in patients treated with living pig tissue*. Science 1999;285:1236-41.
27. Pitken Z, Mullon C. *Evidence of absence of porcine endogenous retrovirus (PERV) infection in patients treated with a bioartificial liver support system*. Artif Organs 1999;23:829-33.
28. Switzer WM, Shanmugam V, Chapman et al. *Polymerase chain reaction assays for the diagnosis of infection with the porcine endogenous retrovirus and the detection of pig cells in human and nonhuman recipients of pig xenografts*. Transplantation 1999;68:183-88.
29. Martin U, Steinhoff G. *Porcine endogenous retroviruses (PERV): in vitro artifact or a big problem for xenotransplantation?* DTW-Dtsch-Tierarztl-Wochenschr 1999;106:146-49.
30. Patience C, Patton GS, Takeuchi Y et al. *No evidence of pig DNA or retroviral infection in patients with short-term extracorporeal connection to pig kidneys*. Lancet 1998;352:699-701.
31. Heneine W, Tibell A, Switzer WM et al. *No evidence of infection with porcine endogenous retrovirus in recipients of porcine islet-cell xenografts*. Lancet 1998;352:695-98.
32. Deacon T, Schumacher J, Dinsmore J et al. *Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease*. Nature Medicine 1997;3:350-53.
33. Patience C. *Sessional report on xenotransplantation: potential for infection*. The XVIII Meeting of the International Congress of the Transplantation Society, Rome, Italy, August 28, 2000.

APPENDIX A: List of Participants

Chair:

Dr. Marian Laderoute

Research Manager, Blood Zoonotics Unit
Bloodborne Pathogens Division
Bureau of Infectious Diseases
Laboratory Centre for Disease Control
Health Canada

Tunney's Pasture, LCDC Bldg. AL 060 1E2
Ottawa, Ontario, Canada K1A 0L2

tel: (613) 941-6087 **fax:** (613) 952-6668 **email:** marian_laderoute@hc-sc.gc.ca

Dr. Francisca Agbanyo

Senior Biologist/Evaluator
Blood and Tissues Division
Bureau of Biologics and Radiopharmaceuticals
Therapeutic Products Programme
Health Canada

Tunney's Pasture; Room 3356, AL: 0603C3
Ottawa, Ontario K1A 0L2

tel: (613) 954-5705 **fax:** (613) 941-5841 **email:** francisca_agbanyo@hc-sc.gc.ca

Dr. Upton Allen

Associate Professor
Division of Infectious Diseases
Department of Paediatrics
University of Toronto
The Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8

tel: (416) 813-8129 **fax:** (416) 813-5032 **email:** upton.allen@sickkids.on.ca

Dr. Karl-Friedrich Bopp

Council of Europe
Health Division
67075 Strasbourg Cedex
FRANCE

tel: + 33 388 41 2214 **fax:** + 33 388 41 2726 **email:** karl-friedrich.bopp@coe.int

Dr. Daniel Butler

Ontario Veterinary College
Department of Clinical Studies
University of Guelph
Guelph, Ontario N1G 2W1
tel: (519) 824-4120 ext. 4012 **fax:** (519) 767-0311 **email:** dbutler@uoguelph.ca

Dr. Louisa Chapman

Medical Epidemiologist
National Center for Infectious Diseases
Centers for Disease Control & Prevention
Mailstop G-19, 1600, Clifton Rd., N.E.
Atlanta, GA 30333 USA
tel: (404) 639-1028 **fax:** (404) 639-0868 **email:** lec3@cdc.gov

Dr. Michael Coulthart

Chief, Host Genetics and Prion Diseases
Bureau of Microbiology
Canadian Science Centre for Human & Animal Health
Laboratory Centre for Disease Control
Health Canada
1015 Arlington St., Winnipeg, Manitoba R3E 3R2
tel: (204) 789-6026 **fax:** (204) 789-5021 **email:** mike_coulthart@hc-sc.gc.ca

Dr. Francisco Diaz-Mitoma

Chief, Regional Virology Laboratory
Children's Hospital of Eastern Ontario
401 Smythe Road
Ottawa, Ontario K1H 8L1
tel: (613) 737-2736 **fax:** (613) 738-4825 **email:** diaz@cheo.on.ca

Dr. Claude Dubuc

Senior Biologist
Vaccines Division
Bureau of Biologics and Radiopharmaceuticals
Therapeutic Products Programme
Health Canada
Tunney's Pasture; Room 3340, AL: 0603C3
Ottawa, Ontario K1A 0L2
tel: (613) 954-1402 **fax:** (613) 941-5841 **email:** claud_dubuc@hc-sc.gc.ca

Ms. Susie ElSaadany

Statistician

Bloodborne Pathogens Division

Bureau of Infectious Diseases

Laboratory Centre for Disease Control

Health Canada

Tunney's Pasture, LCDC Bldg. AL 060 1E2

Ottawa, Ontario, Canada K1A 0L2

tel: (613) 952-6925 **fax:** (613) 952-6668 **email:** susie_elsaadany@hc-sc.gc.ca

Dr. Ezzat Farzad

Medical Specialist

Bloodborne Pathogens Division

Bureau of Infectious Disease

Laboratory Centre for Disease Control

Health Canada

Tunney's Pasture, LCDC Bldg. AL 060 1E2

Ottawa, Ontario, Canada K1A 0L2

tel: (613) 952-8228 **fax:** (613) 952-6668 **email:** ezzat_farzad@hc-sc.gc.ca

Dr. Claude Gardell

A/Manager

Vaccines Division

Bureau of Biologics and Radiopharmaceuticals

Therapeutic Products Programme

Health Canada

Tunney's Pasture; Room 3340, AL: 0603C3

Ottawa, Ontario K1A 0L2

tel: (613) 957-4133 **fax:** (613) 941-5841 **email:** claud_gardell@hc-sc.gc.ca

Dr. David Grant

Surgical Director

Multi-Organ Transplant Program

Toronto General Hospital

621 University Ave., NU-10-114

Toronto, Ontario M5G 2C4

tel: (416) 340-5230 **fax:** (416) 595-1905 **email:** david@grant-web.com

Mr. Martin Houghton

UKXIRA Secretariat

Department of Health

UK Xenotransplantation Interim Regulatory Authority

Room 311, Wellington House

133-155 Waterloo Rd.

London, UK SE1 8UG

tel: 0171 972 4822 **fax:** 0171 972 4852 **email:** martin.houghton@doh.gsi.gov.uk

Dr. Atul Humar

Assistant Professor, Department of Medicine
University of Toronto
Infectious Diseases and Multi-Organ Transplantation
Toronto General Hospital - University Health Network
200 Elizabeth Street; NUW 9-132
Toronto, Ontario M5G 2C4
tel: (416) 340-4800 ext. 6752 **fax:** (416) 340-5047 **e-mail:** atul.humar@uhn.on.ca

Dr. Rima Khabbaz

Deputy Director
Division of Viral and Rickettsial Diseases (MS A-30)
National Center for Infectious Diseases
Centers for Disease Control and Prevention
1600 Clifton Rd., N.E., Room 117-B, Building 3
Atlanta, GA USA 30333
tel: (404) 639-2775 **fax:** (404) 639-3163 **email:** rfk1@cdc.gov

Mr. Andre La Prairie

Manager, Blood, Tissue, Organ & Xenograft Project
Bureau of Policy & Co-ordination
Therapeutic Products Programme
Health Canada
2nd Floor, Holland Cross, Tower B
1600 Scott Street
Ottawa, Ontario K1A 1B6
tel: (613) 941-5513 **fax:** (613) 941-6458 **email:** andre_la_prairie@hc-sc.gc.ca

Dr. Khazal Paradis

Imutran Ltd (Novartis Pharma)
P.O. Box 399
Cambridge, CB2 2YP
United Kingdom
tel: 44 788 793- 2108 **fax:** 44 1223 847 467 **email:** khazal.paradis@pharma.novartis.com

Dr. Charlotte Rigby

Laboratories Directorate
Canadian Food Inspection Agency
59 Camelot Drive
Nepean, Ontario L1A 0Y9
tel: (613) 225-2342, ext. 4002 **fax:** (613) 228-6656 **email:** crigby@em.agr.ca

Dr. Elettra Ronchi

Chief Co-ordinator of Biotechnology & Health Activities

OECD - DSTI - BU

2 rue André Pascal

Paris, 75016 FRANCE

tel: 33-1-4524-1828 **fax:** 33-1-4524-1825 **email:** elettra.ronchi@oecd.org

Dr. Erling Rud

Chief

National Laboratory for HIV Pathogenesis

Bureau of HIV/AIDS, STD and TB

Laboratory Centre for Disease Control

Health Canada

AL 0603B, Ottawa, Ontario K1A 0L2

tel: (613) 957-8049 **fax:** (613) 780-8987 **email:** erling_rud@hc-sc.gc.ca

Dr. Eilleen Tackaberry

Research Services Division

Bureau of Biologics & Radiopharmaceuticals

Banting Research Centre Room 205

Therapeutic Product Programme

Health Canada

Tunney's Pasture; AL: 2201C

Ottawa, Ontario K1A 0L2

tel: (613) 952-6820 **fax:** (613) 941-8933 **email:** eilleen_tackaberry@hc-sc.gc.ca

Dr. Jean Tchervenkov

Royal Victoria Hospital

General Surgery

687 Pine Avenue West, Suite S10.30

Montreal, Quebec H3A 1A1

tel: (514) 842-1231 Ext. 4042 **fax:** (514) 843-1434 **email:** tchervenkov@hotmail.com

Dr. Dan Tucker

Veterinary Services

Imutran Ltd (A Novartis Pharma AG Company)

PO Box 399

Cambridge UK CB2 2YP

tel: (011) 44 1223 840834 **fax:** (011) 44 1223 840444

email: dan.tucker@pharma.novartis.com

Dr. Dongwan Yoo

Assistant Professor of Virology

Department of Pathobiology

Ontario Veterinary College

University of Guelph

Guelph, Ontario N1G 2W1

tel: (519) 824-4120 **fax:** (519) 767-0809 **email:** dyoo@ovc.uoguelph.ca

Dr. Shimian Zou

A/Chief CABBI

Bloodborne Pathogens Division

Bureau of Infectious Diseases

Laboratory Centre for Disease Control

Health Canada

Tunney's Pasture, LCDC Bldg. AL 060 1E2

Ottawa, Ontario, Canada K1A 0L2

tel: (613) 946-8819 **fax:** (613) 952-6668 **email:** shimian_zou@hc-sc.gc.ca

Health Canada Observers:

Mr. Adam Probert and Dr. Farid Hindieh

Appendix B: Discussion and Survey

What data elements are critical to xenotransplantation surveillance?

- demographics of donor (s)
- demographics of recipient
- immunosuppressive regime
- transfusion/transplantation history
- previous or current infectious diseases and cancer
- serious adverse events
- other adverse events
- links to archived samples
- links to infectious disease testing & results
- links to animal sources
- exposure frequency & duration
- type of graft
- tolerance induction protocol (if applicable)
- others

How to define “adverse events”.

- draft Proposed Canadian Standards for Xenotransplantation definition: an undesirable outcome directly or indirectly related to the xenograft (e.g. infection, disease transmission, graft failure)
- any notable change in the recipient’s health or well-being, reversible or not, requiring medical attention or intervention, inclusive of death, disability, disease transmission, infection, or hospitalization whether directly or indirectly related to the xenograft protocol

What are “serious” adverse events”?

- death
- cancer
- disabilities including chronic fatigue
- any event requiring hospitalization
- infection

- graft rejection
- others

What adverse events might be linked to infection?

- death
- cancer/autoimmunity
- disabilities, including chronic fatigue, coma, paralysis, mental confusion, behavioural changes
- graft rejection or failure of graft to thrive
- rash, fever, GI changes, hepatitis, pneumonia, weight changes, etc.,
- others

By what criteria should an adverse event warrant an investigation for potential zoonosis?

- death
- cancer/autoimmunity
- disabilities, including chronic fatigue, coma, paralysis, mental confusion, behavioural changes
- graft rejection or failure of graft to thrive
- rash, fever, GI changes, hepatitis, pneumonia, weight changes, etc.,
- others

What should be the timelines for reporting data to registry and for adverse events?

- the Standards indicate 72 hours for data collection
- recipient adverse events should be reported immediately (or without delay), but which is taken to mean no later than 72 hours
- animal sources adverse events should also be reported immediately, but no later than 72 hours
- for infection/potential zoonosis within 24 hours
- other considerations

What recipient samples are needed for monitoring?

- baseline, routine monitoring and that for investigative purposes for local and national archives
- plasma (at least 5 aliquots of 0.5 mL times 2)
- serum (at least 5 aliquots of 0.5 mL times 2)
- PBMC (at least 3 aliquots of 1×10^6 times 2)
- urine (early morning specimen 3 mL)
- other considerations

What donor animal samples are needed for monitoring by National Archives?

- plasma (at least 5 aliquots of 0.5 mL)
- serum (at least 5 aliquots of 0.5 mL)
- PBMC (at least 5 aliquots of 1×10^7)
- xenograft sample/biopsy
- other considerations

What samples are needed for monitoring of close contacts by National Archives?

- plasma (at least 5 aliquots of 0.5 mL)
- serum (at least 5 aliquots of 0.5 mL)
- PBMC (at least 5 aliquots of 1×10^6)
- baseline, routine and for investigative purposes
- other considerations

What samples are needed for monitoring of health care workers by National Archives?

- plasma (at least 5 aliquots of 0.5 mL)
- serum (at least 5 aliquots of 0.5 mL)
- PBMC (at least 5 aliquots of 1×10^6)
- baseline, routine and for investigative purposes
- other considerations

What schedule for recipient sampling would be best? (choose ONE of the following)

- baseline, weekly for first 3 months, then monthly for next 9 months, then every 6 months for 2 years, then yearly (and for any serious adverse event)
- BASELINE, every two weeks for first 3 months, then monthly for next 3 months, then annually (and for any serious adverse event)
- other:

What schedule for close contact sampling would be best? (choose ONE of the following)

- baseline, monthly for first 3 months, then annually (and for any serious adverse event investigation)
- BASELINE, every 3 months, then annually (and for any serious adverse event investigation)
- other:

What pre-clinical workup on the donor herd is needed?

- screening for various pig pathogens, and sentinel animal herd program reveals no pathogens
- as above but some sentinel animals are immunosuppressed for 2 months, with no pathogens identified
- NHP are inoculated with animal donor blood and cells/tissues, and health is monitored for at least 2 years
- immunosuppressed NHP are inoculated with animal donor blood and cells/tissues, and health is monitored for at least 2 years
- coculture of donor samples with various relevant human indicator cell types (includes PBMC and activated PBMC) with screening for retroviruses, herpesviruses, and looking for viruses with EM, cytopathic effects, and other non-specific means for unknown pathogens
- other
- screen herds for evidence of viremia for endogenous retroviruses (PERVs, reverse transcriptase activity), for endemic herpesviruses (specifically pCMV & circoviruses as well as DNA polymerase for herpes viruses), hepatitis E, and others such as:
- employ EM or other visual techniques for virion production

What surrogate markers for adverse effects potentially related to zoonosis might be useful?

- RT-PCR for alpha-fetoprotein (AFP) on PBMC for early detection of cancer, immunosuppression (related to cancer or infection)
- RNase L activity: activated protein secreted in urine
- ribosomal RNA for bacteria
- other surrogate markers of viral infection such as:

What methods might be used to detect unknown zoonotic agents?

- representational difference assay, used to pick up HHV-8 in Kaposi's sarcoma (PCR based driver to amplify new sequences not found in pre-sample)
- modified FISH using recipient lymphocyte chromosome preparations pre and post xenografting, label pre and post cDNA (cut with certain restriction enzymes) from PBMC with different fluorochromes
- other

What preparation is needed to deal with a xenozoonotic outbreak?

- develop SOPs at National Registry/Archives for testing, sample collection, quarantine and notification in conjunction with F/P/T health ministries
- develop SOPs at Health Canada (TPP) for clinical holds/moratorium/inspections & international notification

- have laboratory facilities & resources (budget, staff, contracts, etc.) ready for testing/sequencing at local and national levels
- other