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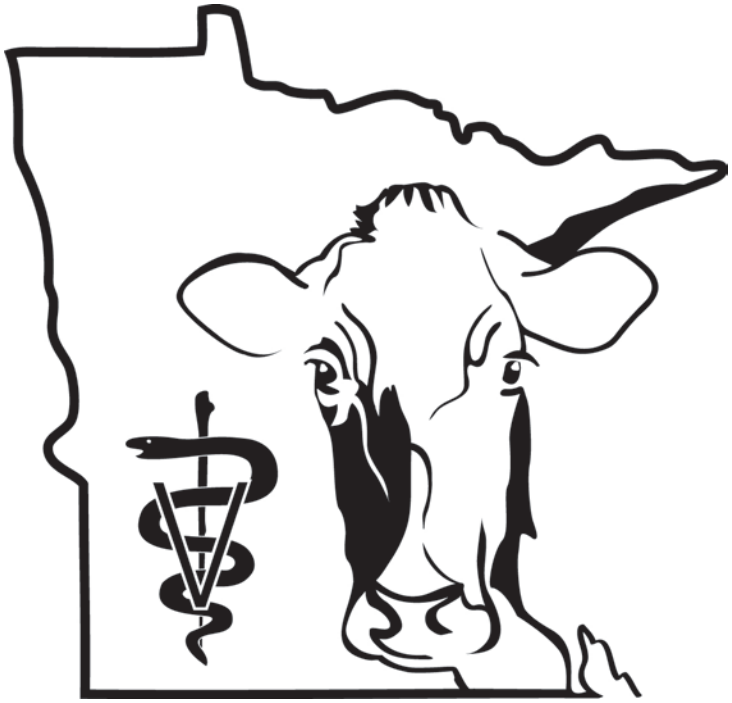

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Center for Excellence in XenoDiagnostics

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Multiple products are under investigation in clinical trials and research in xenotransplantation, which is the use of live animal cells, tissues, or organs as medical products in humans (Table 1). In addition the production of human proteins in the milk of transgenic goats and cows are in Phase I clinical trials and development stages of product production. Bovine-derived tissues and fluids constitute a significant raw material source for use in the manufacture of a variety of medical products. Medical products with bovine-sourced materials have been safely administered for a number of decades through a variety of routes. Some examples of biologic materials derived from bovine sources and for use in humans include bovine thyroxin, bovine lung lipids, bovine adrenal cells, and fetal bovine serum. The Biotechnology Industry Organization, in a White Paper response to the Draft Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation (August, 1996), concluded that certain organs and tissues can be obtained from healthy donor cattle in a safe, controlled, and documented fashion for use in specific xenogeneic therapeutic applications. Although this paper focuses on the safety issues in xenotransplantation the conclusions can be easily applied to the safety issues surrounding the use of transgenic animals for the production of human proteins. This report noted exceptions would include organ systems or epithelial tissue known to have significant microbial bioburden, such as the naso-pharynx and lower bowl (Harvey, J.,et al, 1997).

Table 1

Current Clinical Trials in Xenotransplantation		
Species	**Product**	**Disease**
Pig	Hepatocytes	Liver failure
	Whole liver	
	Transgenic liver	
Pig	Pancreatic Islets	Diabetes
Pig	Neuronal cells	Degenerative Neurologic Disease
Baboon	Bone marrow	HIV/AIDS
Cow	Adreanal cells	Refractory pain in terminal cancer

Current Clinical Trial Using Transgenic Animals		
Species	**Product**	**Disease**
Goat	Milk	Multiple indications

Risks Associated with Xenogeneic Bovine Cells, Tissues, and Transgenic Fluids

Developing a risk management program for known and putative infectious disease risks for bovine derived materials used in the production of medical products is one of the most important steps for the xenotransplantation and transgenic fields. The decision to utilize bovine-derived tissues in xenotransplantation therapy or bovine fluids (milk, urine) in transgenic derived products must be made by 1) thoroughly identifying the real and potential infectious disease risks associated with the donor source, 2) determining the consistency of the source material and , 3) determining the potential benefits of the product in relation to the infectious disease risks.

Zoonotic and Xenozoonotic Risks: Zoonotic risks are defined as diseases that are known to be transmitted from animal to man under natural conditions. They are caused by either bacterial, protozoan, rickettsial, or viral pathogens. Xenozoonotic risks are defined as diseases that may be transmitted from animal to man in a transplantation setting where there is a disruption of anatomical barriers. The known zoonoses associated with North American cattle are numerous (Radostits, 1994). Most of these diseases have been controlled through sound veterinary surveillance and good animal husbandry practices. Therefore, the risks of transmission of most of these diseases can be reduced to acceptable levels.

The risk associated with the presence in donor animals of certain retroviruses (including endogenous retroviruses, type D retroviruses, bovine syncytial virus, bovine immunodeficiency virus, and bovine leukemia virus) must be considered further. The list of retroviruses that may contaminate tissues, cellular products, or proteins intended for human use is extensive (Table 2). The three bovine retroviruses of major concern are the bovine leukemia virus (BLV), the bovine syncytial virus (BSV), and the bovine immunodeficiency virus (BIV). BLV much like its human and simian relatives HTLV-I and STLV-I infects and transforms its target cell and causes lymphoma in a small percentage of infected animals (Coffin, J.M., et al., 1997). Natural transmission occurs via the congenital route by the placenta or by infected milk and by horizontal transmission (Weiss, R., et al., 1984). The detection of BLV in *in vitro* studies in addition to infection and transformation of fetal lamb kidney cells includes PCR, IFA, and Mg^{++} dependent reverse transcriptase assays (Burny,A., et al., 1980 and personal communication, Dr.Gertrude Buehring, UC Berkeley). BIV infects large numbers of cattle but does not appear to always cause disease. BIV has been reported to be associated with persistent lymphocytosis, lymphadenopathy, and central nervous system lesions. (Coffin, J.M., 1997). The *in vitro* studies of BIV infection of fetal bovine lung cells demonstrates a similar growth kinetics and syncytial formation as HIV in human T-cell cultures (unpublished observations) and is easily detected by

the Mg^{++} dependent reverse transcriptase assay. In addition IFA and PCR assays are available for this potential xenozoonosis (personal communication, Dr. Susan Carpenter, Iowa State). Bovine syncytial virus (BSV), a foamy virus, is easily isolated from normal cattle and is detected by the Mn^{++} dependent reverse transcriptase assay in *in vitro* studies.

Table 2 **Family Retroviridae**

Type A	Type B	Type C	Type D	Type E	Type F
Intracisternal	*Oncovirinae*	*Oncovirinae*	*Oncovirinae*	*Lentivirinae*	*Spumavirinae*
	MuMTV (murine)	MuLV (murine)	SMRV (monkey)	HIV-1 (human)	Simian Foamy Viruses (monkey, human)
		PERV (murine)	MFRV (monkey)	HIV-2 (human)	BSV (bovine)
		BaEv (baboon)		SIV (monkey, human)	
		HTLV-I (human)		CAEV (caprine)	
		HTLV-II (human)		VISNA (ovine)	
		STLV (monkey)		BIV (bovine)	
		BLV (bovine)		EIAV (equine, human)	

Evaluation of relative risk: The ability of a virus to infect a human cell line, a primary human cell culture, a small animal model containing human cells (Scid-Hu mice), a primate model, and evidence for infection in humans would give a virus a risk score of five. A good example of a high risk virus with a score is rabies. Any virus with a score of four or five should be considered a potential xenozoonosis and should warrant close scrutiny. BLV has been reported to infect a human cell line and primates (Burny,A., et al., 1980) ; however, no risk evaluation has been reported for BIV or BSV. Since the infection of primary human cells by a virus is generally considered a good indicator of potential replication in primates and humans, the approach taken for risk assessment in this study was to attempt to infect primary human cells with BLV and BIV. This study would determine the risk associated with these two viruses in the xenotranplantation and transgenic settings. Positive controls for this study were $HIV\text{-}1_{SF2}$ and STLV-I for BIV and BLV respectively.

Risk Assessment Study

Question: Can BIV or BLV infect or transform human peripheral blood mononuclear cells enriched for monocytes/macrophages (PBMC)?

Method: Primary human PBMCs were stimulated with PHA for three days followed by supplemented media containing 10% IL-2. Additional PHA stimulated PBMCs were added to the culture at 13 days post inoculation and the cultures were maintained for 20 to 30 days. BIV was inoculated into the PHA stimulated PBMCs and BLV transformed fetal lamb kidney cells were gamma-irradiated with 10,000 rads and co-cultured with the PHA stimulated PBMCs. Positive controls were treated in a similar manner. Mock infected PBMCs served as negative controls. Cell transformation was quantitated by the trypan blue exclusion method and by measurement of cell aggregates. Cells and cell free supernatants were collected at 8, 13, 20, and 30 days post inoculation and stored at –80°C. Virus replication was detected using the Mg^{++} dependent reverse transcriptase assay for HIV and BIV. Briefly 10µL of samples were incubated with 50µL of buffered salt solution containing the template primer polyadenylic acid, oligo dT, Mg^{++}, NP-40 and [^{32}P]dTTP in 96 well plates. Plates were incubated at 37°C for 90± 10 minutes. 10µL from each well was doted onto Whatman DE81 filter paper. The filters were washed in 2XSSC, fixed in ethanol and dried. Incorporated radioactivity was quantitated using the Ambis Radioanalytical Imaging System (Potts, B.J., et al. 1990).

Results: HIV-1 infected and STLV-I infected and transformed the PBMCs. BLV infected and transformed the human PBMCs. BIV inoculated PBMCs had cytopathic effect; however, were RT negative suggesting that BIV did not replicate but there may have been another virus in the inoculum.

Summary: BLV has a significant risk of being a xenozoonosis and it is imperative that herds maintained for this purpose be free of the virus (Table 3). Although BIV did not appear to replicate in the PBMC culture additional studies should be done to determine the potential pathogenecity of this bovine virus for humans.

Table 3 Risk Assessment Study

TEST	BLV	BIV	Rabies
Growth in human Cell line	+	NT	+
Growth in primary Human cell culture	+	-	NT
Growth in small Animal model	NT	NT	+
Growth in Primates	+	NT	+
Growth in humans	NT	NT	+

NT not tested

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