Journal of Medical Virology 53:13–18 (1997)

Transmission of Human and Feline Immunodeficiency Viruses Via Reused Suture Material

J.D. Druce,[1]* W.F. Robinson,[2] S.A. Locarnini,[1] M.T. Kyaw-Tanner,[2] S.F. Sommerlad,[3] and C.J. Birch[1]

[1]*Victorian Infectious Diseases Reference Laboratory, Fairfield, Victoria, Australia*
[2]*Department of Veterinary Pathology, University of Queensland, Brisbane, Queensland, Australia*
[3]*Department of Companion Animal Medicine and Surgery, University of Queensland, Brisbane, Queensland, Australia*

Several documented cases of human immunodeficiency virus (HIV) infection have involved unconventional or unknown modes of transmission of the virus. Some such cases have occurred within a surgical setting. We investigated the potential for transmission of HIV on suture material that had been reused following passage through an HIV-infected patient. Initial experiments were conducted in vitro using HIV. To provide stronger evidence that HIV could be transmitted via this route, further experiments were undertaken in vivo using a feline immunodeficiency virus (FIV)/cat model. Both methods indicated the possibility of transmission of virus if suture materials were reused. ***J. Med. Virol. 53:13–18, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: infection control; patient-to-patient transmission; surgical procedures

INTRODUCTION

Since the association of the human immunodeficiency virus (HIV) with acquired immunodeficiency syndrome (AIDS), the modes of transmission of the virus have been investigated widely. The two most common forms of transmission involve sexual or parenteral acquisition. The relative proportions of each vary greatly depending on geographical location. In general, sexual transmission is most common, although in Italy, Spain, and several cities (e.g., Edinburgh), intravenous drug use (IDU) is the predominant risk factor [Covell et al., 1993; Frischer et al., 1993; Rezza et al., 1993]. Needlestick injuries, transfusion of blood or blood products, and mother-to-infant or infant-to-mother transmission have also been reported [Peckham and Gibb, 1995; Gerberding and Henderson, 1992]. More recently, cases where the route of transmission is difficult to determine or where it has occurred in an unconventional way have been documented [Ciesielki et al., 1994; Chant et al., 1993]. These few cases, which have occurred in individuals attending dental or medical surgeries, are likely to have arisen from breaches of infection control guidelines, including the possible reuse of needles, syringes, or sutures [Penny, 1995; Collignon, 1995].

Needlestick injuries are a common cause of accidental exposure to HIV-infected blood. While the risk of HIV transmission is low (estimated at 0.3%), specific features of the injury, including the depth, volume, and titer of HIV in the blood, may alter this estimation [Gerberding and Henderson, 1992]. We reported previously the potential for HIV to be transmitted via incorrect handling of needles and syringes used to inject local anesthetics [Druce et al., 1995]. This led us to investigate a second potential route of transmission of HIV, namely the reuse of contaminated sutures. Commercially available suture equipment can be used to insert approximately 20 stitches. However, only a few stitches are normally required for minor surgery, leaving most of the suture thread unused. It seems possible that thread passed through the skin of an HIV-infected patient and then reused on a second uninfected patient may be able to transmit HIV. How often the practice of reuse of suture thread occurs is open to conjecture. In human medicine it is likely to be extremely rare, and is a practice that is forbidden under current (Australian) infection control guidelines. There are, however, anecdotal reports of its occurrence [Collignon, 1995].

To investigate the potential for transmission of HIV via suture material, both in vitro and in vivo experiments were used. Those carried out in vitro used a strain of HIV-1 deliberately brought into contact with the suture thread. Because of the limitations of such experiments in reproducing events similar to those

*Correspondence to: J.D. Druce, Victorian Infectious Diseases Reference Laboratory, Yarra Bend Road, Fairfield, Victoria 3078, Australia. E-mail: julian@hna.ffh.vic.gov.au

Accepted 18 March 1997

which might occur in a surgical setting, we also carried out transmission experiments involving feline immunodeficiency virus (FIV)-infected cats. FIV is a retrovirus classified within the lentivirinae subgroup of the family Retroviridae. Infection with the virus is associated with an AIDS-like syndrome in cats [Siebelink et al., 1990]. There are also similarities in the pathogenesis of HIV and FIV in their respective species [Siebelink et al., 1990; English et al., 1994]. These properties suggested that the FIV/cat model was an appropriate surrogate in which to investigate the transmission of HIV via suture material within a surgical setting.

MATERIALS AND METHODS

Viruses

The chronically infected T-lymphocyte line H9/HTLV-IIIB was used as the source of virus in all in vitro experiments involving cell-associated HIV. FIV isolate T91 (isolated from a domestic Australian cat) was used to infect cat 2 (see below) via subcutaneous administration [Greene et al., 1993].

Cells and Medium

For in vitro experiments, the MT-2 human T-lymphocyte line was used for reisolation of HIV from contaminated suture threads. These cells support the replication of HIV isolate HTLV-IIIB. RF-10, an RPMI-1640-based medium containing 10% fetal calf serum, was used for maintenance and passage of MT-2 and H9/HTLV-IIIB cells.

For experiments involving isolation of FIV from cats, interleukin 2 (IL-2) sensitive T-lymphoblastoid cells (L-31 cells, originally derived from an FIV-negative cat) were cultured in the presence of peripheral blood lymphocytes (PBLs) obtained from infected animals. RPMI-1640/IL-2 medium containing 5% fetal calf serum, 100 U/ml penicillin, 100 U/ml streptomycin, 5 μM 2-mercaptoethanol, 2 μg/ml polybrene, and 100 U/ml recombinant human IL-2 was used for maintenance of L-31 cells and PBLs derived from cats.

Detection of FIV-Specific Antibodies in Cats

The antibody status of each cat was determined using a whole blood agglutination test ("VetRed FIV," Agen Biomedical Ltd., Brisbane, Australia) used according to the manufacturer's protocol. This assay utilizes a synthetic peptide derived from the transmembrane protein (HIV gp41 equivalent) of FIV as the source of antigen.

Cats Used in Transmission Studies

Two FIV-infected cats were used as index cases for transmission studies involving the reuse of suture thread. At the time of surgery, one of these cats (cat 1) had been infected for at least 4 years. This animal was repeatedly FIV antibody-positive and FIV had been isolated from its PBLs on several occasions, but never from its plasma [Meers et al., 1992]. A second cat (cat 2) was infected with FIV isolate T91 by subcutaneous administration 28 days before the transmission experiments were undertaken.

In Vitro Transmission of HIV Via Suture Thread

H9/HTLV-IIIB cells were spiked into HIV antibody-negative unclotted human blood to a final concentration of 10^7 infected cells/ml. One centimeter lengths of silk and nylon suture thread (3/0 Ethicon and 3/0 Ethilon, Johnson & Johnson Medical Pty. Ltd., Sydney, Australia) were immersed in this blood for 30 seconds, then exposed to air for 20 minutes at room temperature before being immersed in either 2 ml of saline for 20 minutes at room temperature or in 70% ethanol for 20 minutes at room temperature. All sutures treated this way were then rinsed in phosphate-buffered saline (PBS) for 10 seconds. Each was then placed directly into separate wells of a tissue culture tray (24-well; Costar Corporation, Cambridge, MA) containing 10^5 MT-2 cells in 1 ml of RF-10 medium, and the cells were incubated at 37°C in 5% CO_2. The cells in all wells were observed for HIV-specific cytopathic effects (cpe) until day 10, at which time aliquots of supernate were assayed for p24 levels using a commercial assay (p24 EIA, Organon Teknika B.V., Boxtel, Holland).

In Vivo Transmission of FIV Via Suture Thread

To demonstrate in vivo transmission of FIV via suture thread, a submandibular lymph node was surgically removed from cat 1. The wound created was then sutured using 3/0 Ethicon thread which was then placed in a kidney dish containing 10 ml PBS for 20 minutes at room temperature. During this time, a full thickness skin incision was made in an FIV-negative cat (cat 3), and its wound was sutured using the same silk thread used to suture the wound in cat 1. A second infected cat (cat 2) underwent the same submandibular lymph node removal and suturing procedure as above. The suture thread was also immersed in PBS as above, then used to suture the wound created by a full thickness skin incision in a second FIV-negative animal (cat 4). The surgery carried out in both transmission studies (cat 1 to cat 3 and cat 2 to cat 4) was undertaken in separate operating theaters and involved the use of separate sets of surgical instruments, and total re-scrubbing and gowning of the surgeon.

Isolation of FIV From Cats

Blood samples from cats 3 and 4 were collected 2, 4, and 8 weeks after surgery and virus isolation attempted as previously described [Meers et al., 1992]. Briefly, the blood was centrifuged at 300*g* for 10 minutes, the plasma removed, and the cells at the interface were resuspended in Hank's balanced salt solution (HBSS). PBLs were obtained by centrifuging these cells through Ficoll Hypaque (Pharmacia Biotech, Uppsala, Sweden). For isolation attempts, 10^5 PBLs were mixed with 5×10^5 L-31 cells in RPMI-1640/IL-2 medium and incubated at 37°C in 5% CO_2. A total of 5×10^5 PBLs from the infected cats was also cultured alone in RPMI-1640/IL-2 medium. Medium changes

TABLE I. Infectivity of HIV on Suture Material Following Various Treatment Procedures

		Silk (day a)		Nylon (day a)	
Immersion into	Saline rinse	cpe[a]	p24[b]	cpe	p24
Not immersed	No	4+	1.71	4+	1.70
	Yes	4+	nt[c]	4+	nt
Saline	No	4+	nt	4+	nt
	Yes	4+	1.69	4+	1.71
70% ethanol	No	Neg	0.11	Neg	0.04
	Yes	Neg	nt	Neg	nt
Control cells		Neg	0.05	Neg	0.05

[a]Ratings for cpe were 1+, 2+, 3+, 4+ = 25%, 50%, 75%, 100% of cells infected, respectively. Neg = no evidence of HIV-specific cpe.
[b]p24 enzyme immunoassay cutoff = 0.06.
[c]nt = not tested.

were performed at days 7, 14, and 21, at which time the cells were harvested and analyzed by polymerase chain reaction (PCR) (see below).

To isolate FIV from plasma, 10 μl samples from each cat were added to 5×10^5 L-31 cells in 2 ml of growth medium and the cells incubated at 37°C in 5% CO_2. Medium changes were performed at 7, 14, and 21 days.

The suture thread remaining from each procedure was incubated in the presence of L-31 cells and FIV isolation attempted as described above. In each case, the cells were assayed for FIV proviral DNA by PCR as described below.

Detection of FIV Proviral DNA by PCR

Extraction and PCR amplification of FIV proviral DNA from cocultures and PBLs obtained directly from recipient cats at 2, 4, and 6 weeks after the transmission experiments were performed as previously described [Miller et al., 1988; Kyaw-Tanner et al., 1994]. Briefly, proviral DNA sequences were amplified using primers specific for the envelope region [Kyaw-Tanner et al., 1994]. Oligonucleotide primer pairs which encompassed the region between nucleotides 6299 and 6866 were used to amplify a fragment of 568 bases. A primer designated L6299 is located at positions 6299–6319 of env (gp120) and has the sequence 5′ AGGACCAGAAGAAGCTGAAGA 3′. Primer R6866 is located at positions 6866–6846 of env (gp120) and has the sequence 5′ TTCTGGTGCCCAACAATCCCA 3′. Purified double-stranded genomic DNA (1 μg) was added to 50 μl of a reaction mix containing a final concentration of 10 pmol of each primer and 200 μM of each dNTP in reaction buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin). The reaction mix was overlaid with paraffin oil and cycled on a thermoreactor (Corbett Research, Sydney, Australia). After denaturation of the mix at 94°C for 5 minutes, 2.5 units Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT) was added and the primers annealed at 60°C for 1 minute. Extension was carried out at 72°C for 2 minutes and denaturation at 94°C for 30 seconds. A final extension reaction at 72°C for 8 minutes was carried out at the end of cycle number 30.

PCR-amplified DNA samples were electrophoresed through 1.5% agarose (FMC BioProducts, Rockland, ME), transferred to hybond-N (Amersham, Buckinghamshire, UK) filters, and Ultraviolet (UV) cross-linked. The specificity of PCR-amplified FIV proviral DNA product was demonstrated by Southern blot analysis [Southern, 1975] using a digoxigenin (DIG) (Boehringer, Mannheim, Germany)-labeled probe. Following prehybridization for 2 hours in 5 × standard saline citrate (SSC), 1% DIG-blocking solution, 0.1% N-laurorylsarcosine, and 0.02% sodium dodecyl sulfate (SDS), the membranes were hybridized in the same buffer containing freshly denatured PCR-labeled probe at 68°C overnight. Filters were washed twice in 2 × SSC/0.1% SDS at room temperature, followed by high stringency washings in 0.1 × SSC-0.1% SDS at 68°C.

RESULTS

In Vitro Transmission on Suture Thread

HIV was isolated from contaminated silk and nylon suture threads exposed to HIV-infected lymphocytes in blood and then incubated in air for 20 minutes or soaked in saline (Table I). Observation by light microscopy of suture threads exposed to blood containing chronically infected cells revealed visible amounts of biological material, including cells, attached to the surface of the threads and penetrating the braided structure of the silk thread (Fig. 1). HIV could not be isolated from threads immersed in 70% ethanol for 20 minutes following exposure to these cells (Table I). HIV-specific cpe was confirmed by the presence of elevated p24 levels in culture supernates.

In Vivo Transmission to Cats

When the thread used to suture the FIV-positive cats was reused to suture the two uninfected cats, both recipient animals developed acute infection (Table II). At the time of surgery, donor cat 1 was FIV antibody-positive and positive for FIV in PBLs by PCR, but was negative for FIV in plasma by PCR. Donor cat 2 was FIV antibody-negative, but was positive for FIV in plasma and PBLs by PCR. While both recipient cats became infected, there was a difference in the time to development of markers of infection. One (cat 4) became positive for FIV markers in plasma and cells 2 weeks postsurgery. The second (cat 2) did not become unequivocally positive until 4–6 weeks postsurgery

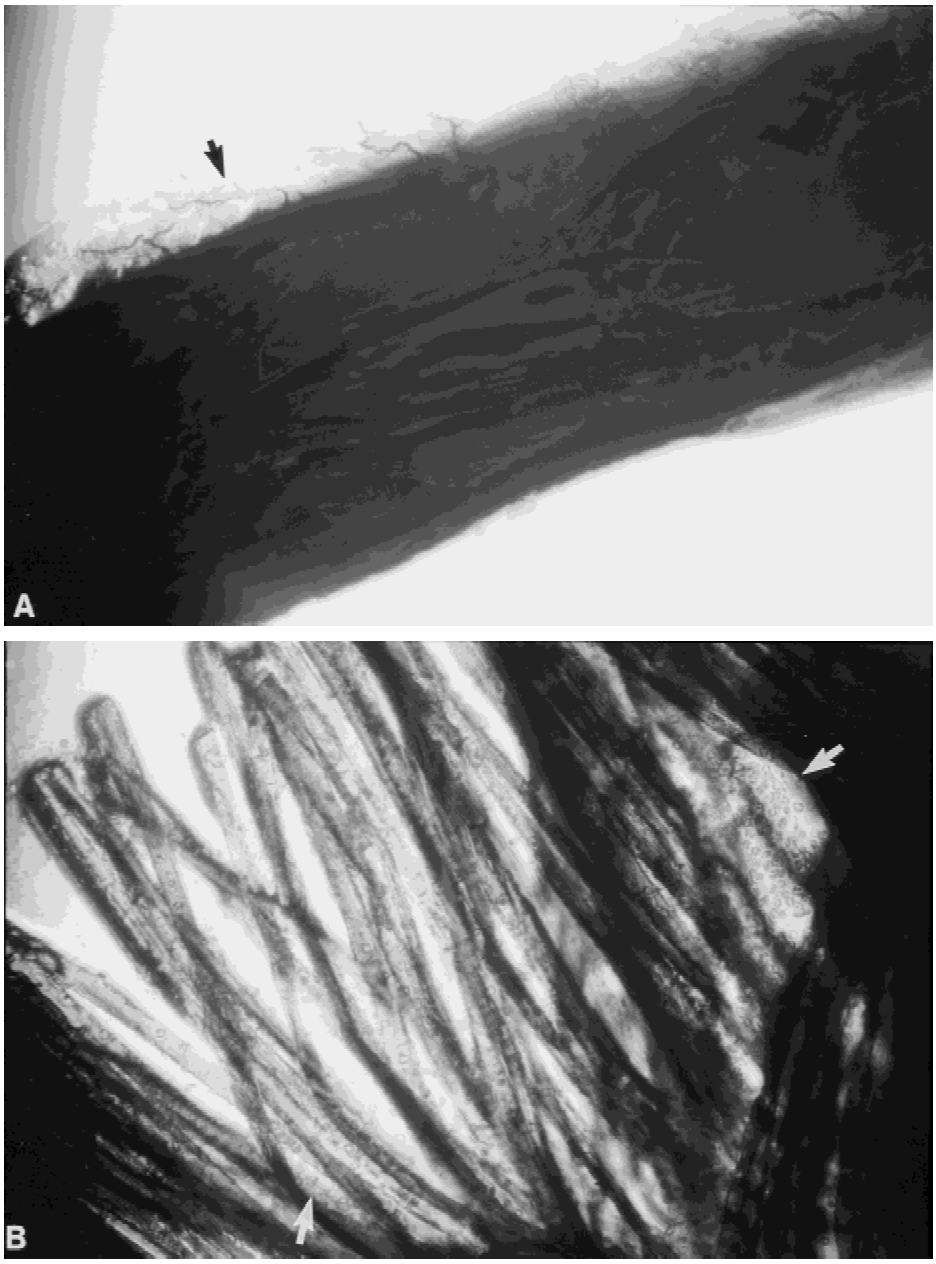

Fig. 1. **A:** Blood residue attached to braided silk suture thread following immersion into blood. **B:** Frayed silk suture following immersion into blood and a further 20 minute soak in saline. Trapped erythrocytes within the filament structure are indicated (arrows). ×400.

(Table II). Twelve weeks following the surgical procedures, no clinical signs associated with recent FIV infection were evident in the two recipient cats, and although FIV-positive by PCR, neither animal had developed antibodies to FIV by this time. FIV was isolated from the suture material used in the surgical operation from cat 2 to cat 4 following cocultivation with indicator cells but not from cat 1 to cat 3 (results not shown).

DISCUSSION

We established that transmission of FIV via surgical suture thread is possible. Because of the biochemical and biophysical similarities of HIV and FIV, it seems likely that HIV could also be transmitted by this route. The results also show that cell-associated HIV and FIV in blood retain their infectivity on suture material exposed to air or PBS for at least 20 minutes at room temperature. Exposure of the HIV-contaminated suture thread to 70% ethanol for 20 minutes resulted in complete inactivation of the virus.

The in vitro experiments were an attempt to mimic the events which could lead to transmission during a minor surgical procedure. However, they did not provide conclusive evidence that transmission might occur by this route. For example, the numbers of HIV-infected lymphocytes spiked into the blood were high,

TABLE II. Development of FIV Infection in Recipient Cats Following Suture With Surgical Silk Previously Used to Suture FIV-Infected Cats

	Week 0			Week 2			Week 4			Week 6			Week 8		
	PL[a]	CC[b]	PBL[c]	PL	CC	PBL	PL	CC	PBL	PL	CC	PBL	PL	CC	PBL
Cat 3 PCR[d]	−	−	−	−	−	−	−	−	+	+	+	+	ND	ND	+
Cat 4 PCR	−	−	−	+	+	+	ND	ND	+	ND	ND	ND	ND	ND	ND

[a]Result determined on incubation of plasma (PL) from recipient cat with L-31 cells.
[b]Result determined on coculture (CC) of PBLs from recipient cat with L-31 cells.
[c]Result determined on culture of PBLs from recipient cats in RPMI-IL-2.
[d]+ = FIV proviral DNA detected by PCR; − = no FIV proviral DNA detected by PCR; ND = not done.

and represented an in vivo situation in which all PBLs would be infected. Work by others has shown that only 1–10% of circulating lymphocytes are infected in HIV-positive individuals, even at times of maximum virus load [Pantaleo et al., 1991, 1993; Daar et al., 1991]. In addition, the in vitro method we used involved exposure of the suture material to infected blood, a situation less likely to occur in vivo because of the presence of components in local anesthetic which reduces localized bleeding. These in vitro experiments were also unable to mimic the situation where the amount of blood contaminating the suture thread is reduced as the thread is passed through the skin. Comparing the mechanics of contamination in vitro to those possibly occurring in surgical practice highlights the need for caution when extrapolating data from in vitro experiments.

To overcome the shortcomings of the in vitro model, the study was expanded to include the use of the FIV model. Cats at different stages of infection were used in these experiments, and although quantitation of virus load was not undertaken, our results suggest that transmission of FIV in the setting described is efficient whether virus is cell-associated or cell- and plasma-associated. That infectious virus was transmitted is evidenced by the fact that the indicator cells were positive for FIV after their cocultivation with plasma from the recipient cats.

In the surgical procedures used to study the potential for transmission in cats, we only investigated transmission via silk that had been soaked in PBS. Due to its braided multifilament composition, silk is more likely than nylon to retain infected cells or blood. This would allow for an increased likelihood of transmission if reused on subsequent patients. However, the in vitro experiments with HIV clearly show that HIV virions or HIV-infected cells remain associated with both nylon and silk thread for at least 20 minutes after immersion and after rinsing in saline. No attempt was made to quantitate the volume of blood transferred in the manner described, but it is likely to be small, and only small quantities of blood could be observed microscopically. After passage through skin this volume is likely to be even less.

We believe that reuse of suture thread is a very uncommon practice in human surgery, although there is anecdotal evidence that it does occur [Collignon, 1995]. The reuse of such thread is clearly a potential source of transmission of HIV, and is likely to be so for any bloodborne virus. The potential dangers of such a practice are obvious.

ACKNOWLEDGMENTS

The authors thank Dr. A. Vankammen for her assistance with cell culture.

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