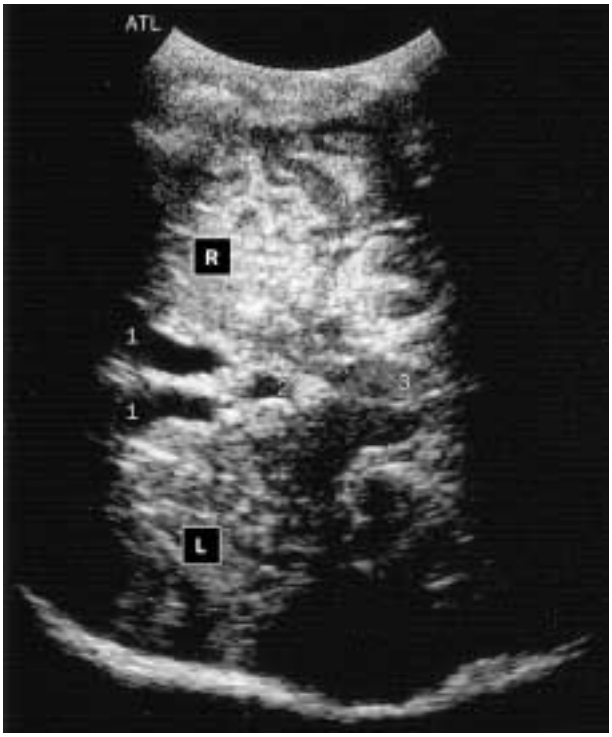


Figure 1: **PICHI transverse section of the brain with Levovist**
Brain structure is depicted both ipsilateral and contralateral to insonation through the temporal bone window (top of image). Ventricular horns (1), third ventricle (2), mesencephalon (3), and cortical gyri (4). Two 10 mm² regions of interest (R=right and L=left) are positioned symmetrically in the temporal lobes for analysis of harmonic microbubble enhancement.

Insonation was through the transtemporal bone window. After informed consent, a 12·5 mL bolus of Levovist, a galactose-based microbubble contrast agent (Schering AG, Berlin, Germany), was injected into the subcubital vein. Intermittent imaging was done, with one image acquired every two heart cycles. This technique amplifies contrast intensity,[2] the effect of which is attributed to decreased microbubble destruction by lower exposure time to ultrasound energy. Since amplification of contrast intensity with intermittent imaging is much greater at low flow velocities,[5] we expected that this would be particularly valuable for investigation of our patient (figure 1). The proportional harmonic microbubble enhancement was measured before and after bolus application in 10 mm² regions of interest positioned symmetrically in both temporal lobes with HDILab, a software quantification tool (ATL) that operates on unprocessed ultrasound signals.

Proportional enhancement curves for both temporal lobes showed a blunted response, and indicated poor tissue perfusion (figure 2). Arrival of microbubbles, as defined by an amplitude threshold of 10% of peak intensity in the respective

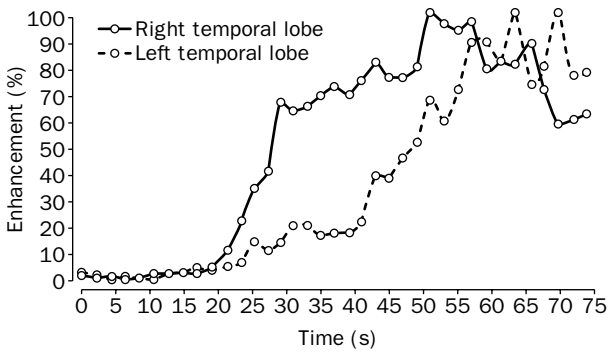


Figure 2: **Contrast enhancement of temporal lobes after bolus application of Levovist**

hemisphere is seen at 21 s in the right region of interest and at 25 s in the left region of interest after application of contrast agent. 65% total dB enhancement is attained within 8 s in the right lobe, whereas the left lobe shows corresponding enhancement 22 s later. Compared with the other techniques used for the assessment of reserve cerebrovascular capacity which show no great differences in perfusion between the right and left hemisphere, PICHI identified a delay of echocontrast enhancement in the left temporal lobe. These findings were confirmed by insonating the contralateral left temporal bone window 30 min later with the same procedure.

Our results with PCHI show good ultrasonographic visualisation of adult brain tissue. The depth penetration allowed simultaneous measurement of harmonic microbubble contrast enhancement in both temporal lobes, and thus provided a basis for qualitative comparison of perfusion characteristics with a single bolus injection of contrast agent. Although preliminary, the results provide evidence that PICHI may be sensitive in characterisation of brain perfusion. The subtle hemispheric differences in contrast enhancement in our patient may result from postoperative neovascularisation. Of particular interest is the question of whether PICHI can be used for quantitative measurement of perfusion—ie, to measure regional cerebral blood flow. PICHI also may provide an opportunity for bedside monitoring of patients with acute cerebral ischaemia, and open new avenues for the assessment of stroke therapy.

1 Chang PH, Shung KK, Levene HB. Quantitative measurements of second harmonic Doppler using ultrasound contrast agents. *Ultrasound Med Biol* 1996; **22:** 1205–14.
2 Firschke C, Linder JR, Wei K, Goodman NC, Skyba DM, Kaul S. Myocardial perfusion imaging in the setting of coronary artery stenosis and acute myocardial infarction using venous injection of a second-generation echocardiographic contrast agent. *Circulation* 1997; **96:** 959–67.
3 Krishnan S, O'Donnell M. Transit aperture processing for nonlinear contrast agent imaging. *Ultrason Imaging* 1996; **18:** 77–105.
4 Horowitz M, Yonas H, Albright AL. Evaluation of cerebral blood flow and hemodynamic reserve in symptomatic moyamoya disease using stable Xenon-CT blood flow. *Surg Neurol* 1995; **44:** 251–61.
5 Ohmori K, Cotter B, Kwan OL, Mizushige K, DeMaria AN. Relation of contrast echo intensity and flow velocity to the amplification of contrast opacification produced by intermittent ultrasound transmission. *Am Heart J* 1997; **134:** 1066–74.

Departments of Neurology (S Meairs MD, M Daffertshofer MD, C Eschenfelder MD, M Hennerici MD)**, and Radiology** (W Neff PhD)**, University of Heidelberg, Klinikum Mannhein, 68135 Mannheim, Germany**

Correspondence to: Dr Stephen Meairs
(e-mail: meairs@neuro.ma.uni-heifelberg.de)

Simian foamy virus infection among zoo keepers

Paul A Sandstrom, Kim Oanh Phan, William M Switzer, Terry Fredeking, Louisa Chapman, Walid Heneine, Thomas M Folks

We investigated 322 North American zoo workers in an anonymous serosurvey for antibodies to simian foamy viruses to establish the potential risk of zoonotic transmission by these retroviruses. 4 of 133 (3%) individuals who worked specifically with mammals including primates were seropositive, primarily with chimp-like viruses, indicating the importance of work practices to reduce exposure to these agents.

Foamy viruses represent a unique retrovirus genus endemic to several mammalian species, with infection rates as high as 70–90% for some species of captive non-human primates.[1] Despite a common evolution and long periods of

Work with	Total	Seropositive
Mammals including primates*	133	4 (3%)
Mammals other than primates	50	0
Other animals (eg, fish, reptiles, birds)	39	0
Other occupations (eg, secretaries, maintenance, clerks)	100	0

*Work with all mammals and/or primates.

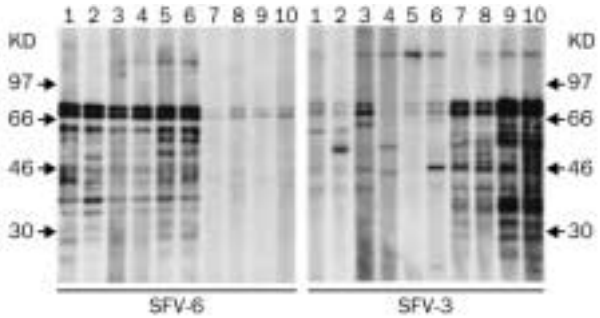
Simian-foamy virus zoonosis among zoo workers

cohabitation with old-world primates, foamy virus infection is not endemic in man. We have previously reported that people occupationally exposed to non-human primates in breeding colonies or through biomedical research activities show a substantial prevalence (4 of 235 [28%]) of zoonotic simian foamy virus (SFV) infection.[2]

Evidence of persistent SFV infection included seropositivity, proviral DNA detection by PCR and virus isolation. Sequence analysis indicated African green monkeys and baboons as the source of the human infections. Testing of archived serum samples indicates that these infections represent true persistent retroviral infections, traceable for over two decades in some infected individuals. All infected people reported some form of occupational injury involving either a non-human primate or its body fluids, although these injuries could not be aetiologically linked to the infection. Reports indicating that SFV is orally shed and transmitted among non-human primates suggest that zoonotic transmission to man by less intimate contact cannot be ruled out.[1] We raise the possibility that occupations requiring close contact with non-human primates may place individuals at risk of retroviral zoonosis. To identify if non-human primate exposure in a zoo setting places individuals at risk of simian retroviral zoonosis, and specifically SFV zoonosis, we did an anonymous survey of 322 zoo workers, to seek evidence of SFV infection.

Serum samples examined in this study had been previously collected by Antibody Systems Inc (Fort Worth, TX, USA), for research purposes from employees of several large North American zoos. Samples had a brief description of the occupation of the individuals. No unique identifiers were provided that could allow serology test results to be linked to an individual.

Preliminary serological screening of zoo worker serum samples was by western immunoblot assay with a combination of three antigenically distinct SFVs; SFV-6 (chimpanzee), SFV-3 (African green monkey), and SFV-2 (macaque). SFV antigens used in the serology assay were derived from whole-cell lysates of SFV-infected Cf2 T helper cell canine thymocytes, and individually prescreened by western immunoblot with serum samples from SFV-infected simians to ensure that nearly equivalent amounts of antigen were present



Western blot analysis of serum samples from zoo keepers
All four zoo keeper serum samples (figure; lanes 1–4) show much stronger reactivity to SFV-6, a pattern similar to that recorded with samples from SFV-infected chimpanzees (lanes 5 and 6), SFV-3 infected African green monkey serum samples (lanes 10), SFV-10 infected baboon samples (lanes 8), as well as serum samples from people infected with either SFV-3 or SFV-10 (lanes 7 and 9), react strongly with SFV-3, and only poorly with SFV-6.

in the final combined antigen preparation. This assay reliably detects SFV infection in a range of primate species including chimpanzees, African green monkeys, baboons, macaques, and people known to be infected with SFV of chimpanzee, African green monkey, and baboon origin. The format and experimental protocol for the western immunoblot assay used in this study has been described.[3] All control and test serum samples were analysed at a 1/100 dilution. Testing of the zoo-keeper samples for the presence of antibodies to SFV was carried out with the tester unaware of occupational data provided.

322 samples were analysed for evidence of antibodies to SFV. 78 were provided by individuals who described specifically working with primates, whereas 55 serum samples were from individuals whose occupations were likely to involve exposure to primates (ie, zoo veterinary services, work with all zoo mammals; table). Four serum samples had clear SFV-specific seroreactivity. All seroreactive individuals were contained within the group with occupations that had potential for exposure to zoo primates, resulting in a prevalence of 3% within this subgroup of zoo workers. No evidence of SFV infection was noted among zoo keepers working with animals other than primates, or among zoo workers not directly in contact with animals. Additional analysis of serum samples from the four seropositive zoo keepers against SFV-6 and SFV-3 antigen preparation separately revealed strong reactivity to SFV-6 in all four individuals, suggesting infection with chimpanzee-like variants (figure). SFV infection is not associated with detectable plasma viraemia, precluding the use of molecular approaches for phylogenetic determination of the zoonotic SFV.[4] The seroreactivity probably represents persistent SFV infections with the potential for secondary transmission among human beings and is of unclear clinical significance. The identification of this additional population with a substantial prevalence of SFV infection will be helpful in assessing the consequences of human SFV infection and quantification of the risks associated with primate exposure. There is no evidence indicating that SFV infections are pathogenic to non-human or human primates. The presence of SFV zoonosis among zoo workers underscores the importance of work practices that decrease exposure to these viruses, and suggests that assessment of protection practices among zoo keepers might be warranted.[5]

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1 Neumann-Haefelin D, Fleps U, Renne R, Schweizer M. Foamy viruses. *Intervirology* 1993; **35:** 196–207.
2 Heneine W, Switzer WM, Sandstrom P, et al. Identification of a human population infected with simian foamy viruses. *Nat Med* 1998; **4:** 403–07.
3 Matthews AL, Brown J, Switzer WM, Folks TM, Heneine W, Sandstrom PA. Development and validation of a western immunoblot assay for the detection of antibodies to porcine endogenous retrovirus. *Transplantation* 1999; **67:** 939–43.
4 Von Laer D, Neumann-Haefelin D, Heeney JL, Schweizer M. Lymphocytes are the major reservoir for foamy viruses in peripheral blood. *Virology* 1996; **221:** 240–44.
5 Lairmore MD, Kaplan JE, Daniel MD, et al. Guidelines for the prevention of simian immunodeficiency virus infection in laboratory workers and animal handlers. *J Med Primatol* 1989; **18:** 167–74.

HIV/Retrovirus Diseases Branch, Division of AIDS, Sexually Transmitted Disease, and Tuberculosis Laboratory Research, Mailstop G-19, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta GA, 30333, USA
(P A Sandstrom PhD, K O Phan BS, W M Switzer MPH, L Chapman MD, W Heneine PhD, T M Folks PhD) **and Antibody Systems Inc, Fort Worth TX, 76054, USA** (T Fredeking)

Correspondence to: Dr Thomas M Folks
(e-mail: tmfa@cdc.gov)