

Copper content in *Areca catechu* (betel nut) products and oral submucous fibrosis

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Chewing areca nut, alone or as a component of betel quid, is widespread in the Orient and among some emigrant populations therefrom. Populations chewing areca nut have an increased incidence of oral cancer.¹ Areca nut is also causally linked to oral submucous fibrosis,² a potentially malignant condition of the mouth, pharynx, and oesophagus. Much effort has been expended in searching for the agents in areca nut which induce fibrosis. Recent evidence suggests upregulation of the copper-dependent extracellular enzyme lysyl oxidase by fibroblasts in oral submucous fibrosis is important, leading to excessive crosslinking and accumulation of collagen.³

We examined the dry weight of copper in eight areca nut products and two samples of raw areca nut, and the soluble copper in these products after extraction with water. A graphite-furnace atomic-absorption spectrophotometer with Zeeman background correction was used. Accuracy was confirmed by analysing digests of the National Bureau of Standards bovine liver (lot 1577a). Mean dry weight of copper was 302 (SD 92) nmol/g (range 205–535 nmol/g), much higher than reported in types of nut commonly consumed as snacks in Britain (range 22–173 nmol/g).⁴ 1.5–11.6% and 7.1–11.3% of copper was soluble when areca nut products were extracted *in vitro* in distilled water and *in vivo* in human saliva.

In a crossover trial, whole mouth saliva was collected from three adult Asian healthy volunteers (aged 25–49 years) who do not chew areca products. Each chewed 3 g of Pan Parag—a commercially available areca nut product, for 30 min. During chewing of the areca nut product, soluble concentrations of copper in saliva were significantly higher than stimulated salivary samples collected without areca-nut challenge ($p < 0.001$; χ^2 test). The concentration of copper in whole mouth saliva peaked after chewing for 10 min in two volunteers and at 20 min in the third, and dropped to control values 10 min after chewing stopped.

We suggest that substantial amounts of copper released from areca products induces lysyl oxidase activity upregulating collagen synthesis by fibroblasts, facilitating its cross linking and, thereby, inhibiting its degradation. To our knowledge, the concentration of copper in areca nut has not been recorded before. We are currently examining copper concentrations in tissue and body fluid samples from habitual chewers and in people with oral submucous fibrosis.

The daily intake of copper in American diets averages about 1.0 mg, about 60% of which is absorbed.⁵ Our data indicate that an adult Indian chewing areca daily will consume over 5 mg of copper per day, of which a substantial but unknown quantity will be absorbed, particularly in habitues known to swallow substantial amounts of quid juice. The role of copper from areca products in the pathogenesis of oral submucous fibrosis merits further investigation, particularly since it is thought to be involved in other fibrotic diseases such as scleroderma and liver fibrosis.

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Effect of low-molecular-weight heparin on serum potassium

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Heparin has been used in the prevention and treatment of thromboembolism ever since its discovery more than 50 years ago. Low-molecular-weight heparins have replaced unfractionated heparin for prophylaxis in patients undergoing surgery or who are immobile.¹ An effect of unfractionated heparin is hypoaldosteronism with hyperkalaemia caused by a direct inhibition of aldosterone biosynthesis and by inhibition of angiotensin-II with secondary hypoaldosteronism.^{2,3} High doses of low-molecular-weight heparins may also induce hypoaldosteronism and cause hyperkalaemia.^{4,5} It is not known, however, if low-dose low-molecular-weight heparin causes hyperkalaemia in patients at risk such as those with renal failure, diabetes mellitus, those treated with angiotensin-converting enzyme inhibitors (ACE inhibitors), or taking non-steroidal antirheumatic drugs.

We studied patients receiving nadroparin (Fraxiparin, Sanofi) 3075 U subcutaneously daily for prophylaxis. Serum potassium was measured by routine laboratory methods before nadroparin was started and after 4 days of treatment. Where possible, serum potassium was also measured 3 days after nadroparin was stopped. Patients with conditions or taking drugs that could affect serum potassium were excluded. There were five groups of patients (table). For statistical analysis a Kruskal-Wallis test for non-parametric data and an unpaired *t*-test were applied.

116 patients were enrolled; 35 patients were excluded (13 were taking diuretics, two β_2 -stimulants, one insulin, 12 were on special diets, one had diarrhoea, and six were on potassium supplements). The remaining 81 patients were

	n	K before (mmol/L)		K ⁺ after 4 days (mmol/L)		Increase (%)
		Mean (SD)	Range	Mean (SD)	Range	
Total	81	4.06 (0.38)	2.76–5.11	4.25 (0.40)*	3.12–5.70	5.1 (9.1)
No renal disease	46	4.01 (0.35)	3.20–4.88	4.17 (0.37)*	3.12–5.04	4.0 (7.8)
Renal failure	6	4.26 (0.57)	3.59–5.11	4.59 (0.62)	3.80–5.70	8.0 (9.1)
Diabetes mellitus	7	4.10 (0.25)	3.67–4.42	4.16 (0.27)	3.88–4.70	1.3 (7.4)
ACE-inhibitor treatment	10	4.18 (0.40)	3.21–4.79	4.42 (0.36)	3.97–5.17	6.6 (11.7)
NSAID treatment	12	3.98 (0.46)	2.76–4.57	4.29 (0.39)*	3.68–5.09	8.6 (12.0)

*p<0.05 compared with baseline value, Kruskal-Wallis test. NSAID=non-steroidal anti-inflammatory drugs.

Serum potassium before and during nadroparine

aged 62 (SD 19) years old. 4-day prophylaxis with nadroparin increased serum potassium by 5% from 4.06 (0.38) to 4.25 (0.40) mmol/L (p<0.001). The potassium increase was also significant in patients without renal diseases. The highest rise of serum potassium was observed (from 5.11 to 5.70 mmol/L) in an individual in the subgroup with renal failure (creatinine clearance 10–49 mL/min, serum creatinine 108–529 µmol/L). In diabetic patients (HbA1c 6.0–8.4%) potassium increase was marginal, which may be due to the fact that during the 4 days in hospital blood glucose fell under treatment from 7.0–15.5 mmol/L to 5.4–7.6 mmol/L, causing a shift of potassium into intracellular compartments. No correlation was found between age and potassium change. In 11 patients we had the opportunity to measure serum potassium again 3 days after nadroparin withdrawal and found a lower value than when they were on nadroparin in eight of them.

We conclude that even the lowest dose of nadroparin used for prophylaxis of thromboembolism increases serum potassium. Potassium should be monitored during the administration of low-molecular-weight heparin at least in patients with concentrations greater than 5 mmol/L.

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Enterotoxigenic *Escherichia coli* in infant feeding bottles

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Weaning foods are an important route for the transmission of enteropathogens. Practically all major bacterial enteropathogens have been isolated from these foods, particularly from milk. Diarrhoeagenic *Escherichia coli* is the predominant cause of diarrhoea in developing countries. The distinct categories of this group can be identified by DNA hybridisation probes and at least three of them by patterns of adherence to HeLa and HEp-2 cells cultivated in vitro. *E coli* strains showing aggregative adherence (EAggEC) have become increasingly recognised as agents of persistent diarrhoea, the most important diarrhoeal syndrome associated with malnutrition and mortality.¹ Our study reports the isolation of EAggEC from foods.

	Sample 1	Sample 2	Sample 3
<i>E coli</i> (bacteria/mL)	2400	1100	150 000
No isolates tested	3	18*	14
No EAggEC isolates (%)	3 (100)	1 (5.6)	1 (7.1)
AA adherence	Yes	Yes	Yes
EAggEC (bacteria/mL)	2400	62	10 650

*3 (16.7%) of these isolates reacted with ETEC LT-I probe.

EAggEC in lacteal contents of feeding bottles

100 samples of the lacteal contents of feeding bottles belonging to infants (median age 7 months) brought to the outpatient clinic of the Department of Pediatrics of the Universidade Federal de São Paulo were collected. The feeding bottles were prepared at home by mothers of low socio-economic class. The mothers were selected at random and were not aware of the collection of samples. The infants were taken for free clinical checkups and routine immunisations. The samples were collected before the infants were fed and analysed by *E coli* enumeration in conformity with standard methods.² Isolates were identified as *E coli* by biochemical tests and hybridised with isotopically (α -³²P[dATP]) labelled DNA probes associated with virulence factors of the distinct categories of diarrhoeagenic *E coli*. The following probes were used: *eaeA* (*E coli* attaching and effacing), EAF (enteropathogenic *E coli* adherence factor), *bfpA* (bundle-forming pilus), EAggEC, Inv (*E coli* invasiveness), LT-I (heat-labile enterotoxin type I), ST-Ih (heat-stable enterotoxin type I from *E coli* of human origin), ST-Ip (heat-stable enterotoxin type I from *E coli* of porcine origin), SLT-I (Shiga-like toxin I), and SLT-II (Shiga-like toxin II).³

26 of the 100 milk samples analysed were contaminated with *E coli*. From these samples, 146 isolates were recovered and submitted to colony hybridisation. None of them carried any of the gene sequences associated with enteroinvasive *E coli*, enterohaemorrhagic *E coli*, or enteropathogenic *E coli*. Three (11.5%) of the 26 contaminated samples carried *E coli* isolates which reacted with the EAggEC probe (table). These isolates (total of five) adhered to HeLa cells in the characteristic aggregative pattern (AA). In sample 2, enterotoxigenic *E coli* (ETEC LT-I) was also found.

Although the dose required for EAggEC to cause illness is still uncertain, two of the milk samples reached the bacterial concentration (10^3 – 10^4 per mL) required by *Vibrio cholerae* to induce illness in normal adult volunteers⁴ and showed the inocula (10^2 – 10^4 per mL) believed to be the usual infective ingestion in natural infection by contaminated food and water.⁵ Furthermore, proteinaceous foods as milk, acting as a natural buffer, may protect bacteria from gastric acid.⁴ Our study, reporting the isolation of EAggEC from foods, reconfirms that in developing countries weaning foods are usually contaminated with all major enteropathogens, thus accounting for a substantial proportion of diarrhoeal diseases and associated malnutrition among infants. In the light of these findings, within the strategies for diarrhoeal disease control, promotion of breast-feeding and improved weaning practices must be given high priority.

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Re-emergence of human monkeypox in Zaire in 1996

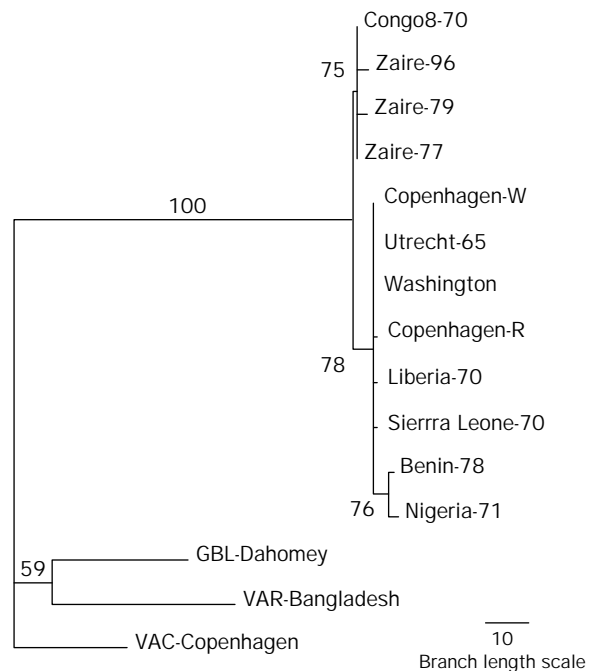
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Human monkeypox is a systemic exanthem, resembling smallpox, that occurs as a sporadic zoonosis in rural rainforest villages of western and central Africa. The disease is caused by an orthopoxvirus, which is transmitted to human beings by handling infected animals; serosurveys have implicated squirrels [*Funisciurus* and *Heliosciurus* spp] as the probable reservoir. Secondary human-to-human spread by aerosol or direct contact accounts for about 28% of cases; tertiary and quaternary chains of transmission are rare.¹

Between Feb 15, and Aug 31, 1996, 71 cases of human monkeypox, including six deaths, were reported in 13 villages in the Katako-Kombe health zone among a population of 15 698, in Sankuru subregion, Kasai Oriental, Zaire.² The outbreak had gone largely unrecognised until the end of July, when an abrupt increase in the number of reported cases led to a preliminary investigation, which found that 42 cases of human monkeypox, including three deaths, had occurred in a small village (population 346) where squirrels were often hunted by men and boys. Most cases were in people under 25 years of age. Among those examined during a preliminary investigation, none had a scar of smallpox vaccination. From February to July, one person in the village appeared to be the primary case-patient who may have been the source of a cascade of human-to-human transmission through eight members of his clan. During this time, monkeypox infections also occurred in other families living together and in a few clans in nearby villages, raising the possibility of other introductions of human monkeypox into the population.

Monkeypox was confirmed in 11 clinically suspect cases from crusted scabs, vesicular fluid, or serum collected from July to September, including three pairs of samples representing secondary contact cases in separate households. Virus-specific polymerase chain reaction (PCR) amplifications of genes for the monkeypox virus haemagglutinin (HA)³ and tumour necrosis factor receptor (TNFR; unpublished data) were positive for three of four available scab samples, and monkeypox virus was isolated in culture from two of the PCR-positive specimens. In addition, western blot assay showed orthopoxvirus genus-specific IgG in ten different patient sera, and an experimental enzyme-linked-indicator serum assay that used orthopoxvirus antigen peptides showed monkeypox-specific IgM in five of six sera tested.

The present cluster of cases constitutes a reemergence of human monkeypox on a scale greater in magnitude than the approximate 65 annual cases previously indicated for Kasai Oriental, Bandundu, and Equateur regions from 1981 to 1986, and it contains a more extensive occurrence of person-



Phylogenetic analysis of orthopoxvirus TNFR open reading sequences of the current Zairian isolate compared with cognate sequences of monkeypox virus strains from Zaire in previous years and selected non-Zairian monkeypox strains, variola (VAR), gerbilpox (GBL), and vaccinia (VAC) viruses

Nucleotide sequences of genome PCR-generated amplicons were determined using dye-terminated, primer-walking, fluorescence-based Sanger-type reactions⁴. Maximum parsimony analysis of aligned sequences provided bootstrap confidence intervals (values in bold) after 1000 heuristic search replicates weighted for a transition to transversion ratio of 2 (PAUP software version 3.1.1).

to-person transmission than previously recognised.¹ The extent of the outbreak in Katako-Kombe, which reported no cases during the previous surveillance, and the incidence of disease among household contacts, challenge previous modelling studies¹ that suggested prolonged episodes or sustained cascades of transmission of human monkeypox would be unlikely even after smallpox vaccination, which is protective, ceased. Alternately, the events may represent multiple introductions into the same population because of increasing encroachment of larger populations into the primary habitat of animals in this and other areas of Africa. Because sequence analyses have indicated that Zairian monkeypox strains have not diverged greatly from the first isolate from the area in 1970 (figure) and monkeypox and smallpox variola viruses are independently evolved species,⁵ notions of monkeypox virus mutating into variola virus are unfounded.

In light of the 1996 episode, an international team coordinated by WHO, Centers for Disease Control and Prevention, and the Zairian Ministry of Health began an investigation in February, 1997, to evaluate the outbreak and determine current risk factors for infection. More specific rapid diagnostic assays should enable more precise monitoring of fluctuations in the virus and epidemiological pattern of this zoonosis as changes occur in human demographics, sanitary practices, and reservoir animal distributions.

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Glutathione S-transferase theta 1 (*GSTT1*) gene defect in myelodysplasia and acute myeloid leukaemia

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Approximately 30% of cases of myelodysplastic syndrome (MDS) culminate in acute myeloid leukaemia (AML). Ten to 20% of cases of AML have a preceding history of myelodysplasia or evidence of tri-lineage dysplasia at the time of presentation, and this appears to be more frequent in older patients developing AML. Studies of lineage involvement have suggested that whereas AML in younger patients often involves only a single-cell lineage, in older patients a multipotential stem cell is usually involved.¹

Chen and colleagues² have reported that there is a high frequency of the *GSTT1* null genotype in US patients with MDS (46% compared with 16% in a control group). Glutathione S-transferases are involved in the metabolism and detoxification of a range of carcinogens and the above study suggests that MDS might be caused by failure to detoxify such carcinogens, a finding with important implications. We have sought to confirm these findings in UK patients with MDS and AML.

We studied the frequency of the *GSTT1* null genotype in a cohort of 100 young (<40 years) and 100 older (>70 years) patients with AML. These patients were entered into the MRC AML 10 and AML 11 trials and DNA was obtained from the MRC AML trials DNA bank. DNA from 57 patients with MDS and 100 haematologically normal controls (laboratory staff, general medical patients, or women attending the antenatal clinic) was obtained from University College London Hospitals DNA bank. The *GSTT1* null genotype was determined with a PCR-based technique producing a fragment of 480 bp.³ All cases in which no band was detected were repeated and the integrity of the DNA was shown by amplification of a 250 bp sequence from the β globin gene.

The results (table) indicate that the frequency of the *GSTT1* null genotype is similar in our control group to that previously for a UK population,⁴ and there is no difference in the frequency found in either the patients with MDS or AML. The reasons for the difference between the results of the US group and our study are not clear. It is possible that there are major differences in the pathogenesis of MDS in the two countries, although this would be surprising. However, a potential problem in surveys of the frequency of the *GSTT1* null genotype in a given disease is the fact that the frequency of this genotype varies markedly between racial groups, being particularly high in some Asian populations.⁵

Group	Null genotype
Controls	23/100 (23%)
AML: <40 years of age	22/100 (22%)
>70 years of age	21/100 (21%)
MDS	16/57 (28%)

AML=acute myeloid leukaemia, MDS=myelodysplastic syndrome.

Frequency of *GSTT1* genotype in AML, MDS, and controls

Studies purporting to show a difference in genotype frequency in a disease should show that the racial origins of patient and control groups are comparable.

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Glutathione S-transferase gene deletions in myelodysplasia

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Chen and colleagues reported on the frequency of null genotypes for the glutathione S-transferases *GSTM1* and *GSTT1* genes showing a significantly increased frequency of the null genotype for the *GSTT1* gene in patients with myelodysplastic syndrome (MDS).¹ These enzymes play a role in the metabolic pathway for carcinogens and it is important for Chen et al's findings to be confirmed. We determined the frequency of the null genotypes in a large group of controls (haematologically normal UK laboratory staff and UK general medical outpatients) and patients with primary MDS. We found no significant difference in frequency between controls and patients for either *GSTM1* (odds ratio 0.89 [95% CI 0.5–1.43]) or *GSTT1* (odds ratio 0.72 [95% CI 0.4–1.34]) null genotype frequency (table). There was no suggestion of any difference in frequencies within the different MDS subgroups. We also looked at eight cases of secondary MDS and found 4/8 to have the *GSTM1* null genotype. The reason for the discrepancy between our findings and those of Chen are not clear.² There is the possibility of racial heterogeneity in studies from the USA causing skewed results but other possibilities include, for example, different causes of MDS in different countries.

Group	<i>GSTM1</i> null genotype	<i>GSTT1</i> null genotype
Controls	54/112 (48%)	18/112 (16%)
RA/RAS	48/97 (49%)	17/97 (17%)
RAEB/RAEBt	22/37 (59%)	11/37 (29%)
CMML	21/32 (65%)	7/32 (22%)
Total MDS	91/166 (55%)	35/166 (21%)

Frequency of *GSTM1* and *GSTT1* genotypes in MDS patients and controls

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Apolipoprotein E genotype and cognition in the very old

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In addition to increasing the relative risk of developing Alzheimer's disease (AD),¹ the $\epsilon 4$ allele of *APOE* may adversely affect memory, learning ability, cognitive decline, and activities of daily living in non-demented elderly individuals.² We investigated the influence of *APOE* genotype on cognition in a prospective study of very old residents of a nursing home, the Jewish Home and Hospital for the Aged. We postulated that *APOE* genotype might have an effect on cognitive performance irrespective of diagnosis.

Patients were prospectively enrolled in the study, the sole entry criterion being consent for cognitive testing. There were no exclusion criteria. In most cases, extensive medical comorbidity precluded a definitive diagnosis of probable Alzheimer's disease.³ 419 patients had both a cognitive assessment with the mini-mental state examination (MMSE)⁴ and their *APOE* genotype was determined by laboratory personnel who were blinded to patient identity, available diagnoses, and cognitive testing results. DNA for *APOE* genotyping⁵ was derived from blood samples taken for clinical reasons. 79 patients were male (average age 83.72 years [SD 7.93]) and 340 were female (average age 87.12 years [SD 7.64]).

MMSE scores were significantly related to *APOE* genotype (MMSE $p < 0.001$ by ANOVA) and were not related to age differences among those with different *APOE* genotypes (age $p > 0.5$ [table]). These results show that in this prospectively ascertained population with substantial medical comorbidity, cognitive performance is adversely affected by *APOE* ϵ allele dosage. Independent of underlying diagnoses, we were able to detect a significant effect of *APOE* genotype on MMSE scores. In this population, age and *APOE* allele frequency are not related and therefore age bias resulting from grouping patients according to *APOE* genotype does not account for our finding.

The clinical outcome measures in this study were cognitive testing scores rather than specific medical diagnoses. The data suggest that *APOE* ϵ allele dosage may worsen cognitive dysfunction in general rather than being specific for AD alone. In addition, adverse effects of *APOE* ϵ allele dosage on neuronal function or repair could provoke early expression or exacerbation of cognitive symptoms associated with AD and would therefore result in both more frequent and earlier diagnosis of AD and a statistical association between AD and *APOE* ϵ gene dosage.

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<i>APOE</i> genotype	Mean MMSE	Mean age (yr)	n
$\epsilon 4/\epsilon 4$	5.54 (4.96)	87.08 (8.88)	13
$\epsilon 4/X$	12.86 (10.47)	86.51 (6.94)	100
X/X	16.50 (9.97)	84.45 (8.18)	306

$\epsilon 4/\epsilon 4$ =*APOE*- $\epsilon 4$ homozygotes; $\epsilon 4/X$ =*APOE*- $\epsilon 4$ heterozygotes; and X/X =no *APOE* $\epsilon 4$ allele.

Mean (SD) MMSE and age according to *APOE* genotype

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Spread of HIV-1 to children in Cambodia

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Little has been published about the HIV-1 epidemic in Cambodia.¹ We report, for the first time, HIV-1 infection in Cambodian children studied at the Kantha Bopha Children's Hospitals I and II, Phnom Penh.

From Nov 1, 1995, children younger than 5 years of age admitted to the Kantha Bopha Children's Hospitals and suspected of having tuberculosis were tested for antibodies to HIV-1 by ELISA (Genelavia or Genscreen, Sanofi Pasteur, Marnes-la-Coquette, France). Up to Jan 31, 1997, HIV-1 serology was done on 9026 children (83% of all admitted patients to hospital in this age group). 290 (3.2%) tested positive (table). In 205 of the 236 HIV-1-seropositive children younger than 18 months, serum was tested also for p24 antigen (Elavia Ag, Sanofi) and was positive in 51 (25%) (table). The mothers of 173 of the HIV-1-seropositive children were also tested for antibodies to HIV-1 and all but three were positive, suggesting that the main mode of HIV-1 acquisition in children is vertical. There was a history of previous transfusion in one of the three children with seronegative mothers.

Assuming that HIV-1 infection occurred only in the 54 seropositive children older than 18 months and in the 51 children younger than 18 months with detectable p24 antigen, the calculated prevalence of HIV-1 infection in children younger than 5 years of age and suspected of having tuberculosis when admitted to Kantha Bopha Children's Hospitals I and II would be 1.2%. If the 17% of children not included in the test were all to be HIV-1 negative, the prevalence would still be as high as 1%. These calculated prevalences may underestimate the true number of children with HIV-1 infection, because some of the children with indeterminate HIV-1-infection status and some of the children not tested have to be expected to be infected. This is substantiated when HIV-1 testing was extended to all children admitted to hospital: of 715 younger than 5 years of age, seroprevalence of those suspected (596) and of those not suspected (119) of having tuberculosis was 3.2% and 0.8%, respectively. Most remarkably, none of the 369 children older than 5 years of age was seropositive.

Because our investigation focused on children in hospital, it is not possible to generalise to all the country's children. WHO estimated in 1996 a seroprevalence of 1.97% in adults aged 15-49 years in Cambodia, the highest among Asian

Age (months)	HIV seropositive	p24 antigen positive (%) / total HIV seropositives tested
0-6	126	21 (17.5%) / 120
7-12	61	16 (33.3%) / 48
13-18	49	14 (37.8%) / 37
19-24	21	..
25-60	33	..

Ages of children younger than 5 testing positive for HIV-1

countries. At Kantha Bopha's blood bank we recorded in 1995 HIV-1 seropositivity in 211 (6.6%) of 3197 blood donors and in 1996 211 (7.5%) of 2834 blood donors. Similar prevalences of HIV-1 seropositivity for this target population have been noted at the National Transfusion Centre in Phnom Penh.² A sentinel surveillance programme in Cambodia in 1996 showed an HIV-1 seroprevalence of 40.9% in prostitutes and of 1.7% in pregnant women (data from the Health Ministry of Cambodia and WHO). This may indicate that vertical spread of HIV-1 to children will increase in the future. Because not all vertically infected children die within their first 5 years of life, lack of detection of HIV-1 infection in children older than 5 years of age suggests that infection has only recently been introduced in Cambodia and thus that HIV is spreading extremely rapidly.

Antiretroviral drugs are not yet available in Cambodia. Preventive measures are the only means to combat the HIV-1 epidemic. Our management of HIV-1-infected children is limited to testing and counselling of parents, switching to bottle feeding in breastfed infants, and providing children with cotrimoxazole for *Pneumocystis carinii* pneumonia prophylaxis.

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Pistachio-green stools and anaemia in infancy: early signs of cystic fibrosis?

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Stool characteristics may vary in healthy infants and are influenced by the type of feeding. Whereas almost all breastfed infants have yellow loose stools, formula-fed infants' stools may be green, brown, or yellow. In cystic fibrosis (CF), meconium ileus or other meconium abnormalities may be the earliest manifestations, while later in infancy the gross stool characteristics are generally described as frequent, voluminous, loose, greasy, and foul-smelling. I report two anaemic infants with striking pistachio-green stools who were later diagnosed with CF.

A 4-month-old breastfed girl was referred for anaemia and suspected occult intestinal bleeding. She was born after 40 weeks of gestation with no reported complications, birth weight 3400 g. Postnatally she had stayed in London with her English father's family where she had been investigated for anaemia and failure to gain weight. Haemoglobin was 7.1 g/dL, packed cell volume 22%, reticulocytes 4.2%, mean-cell volume and mean cell haemoglobin concentration at the lower end of normal, platelets $548 \times 10^9/L$, and serum iron, folate, and cobalamins were all normal. There were no signs of haemolysis. Several tests for faecal haemoglobin had been positive but she had not passed any visible blood. We recorded almost identical results 4 weeks later, and also that the serum

albumin was 24 g/L and the prothrombin complex 45%. Her body weight was then 5100 g. The stools were loose, smooth, and bright pistachio-green, without any remarkable odour or greasy appearance, and occasionally positive for haemoglobin. A rectoscopy, technetium scan, and bone marrow smear were all normal. No explanation for the anaemia was found and after a blood transfusion she maintained a normal haemoglobin and started to gain weight and length. The stools remained pistachio-green until about 8 months of age. At the age of 2 years and 9 months she was readmitted for pneumonia and a chest radiograph showed peribronchial opacities indicative of CF, which was confirmed by subsequent investigations including an abnormal sweat test.

An 8-week-old boy was born without complications after 37 gestational weeks with a birth weight of 2950 g. He was entirely breastfed and his mother reported that his stools invariably were green. He had had projectile vomits once or twice daily since 4 weeks of age and had therefore been sent for a barium meal (which was normal). Immediately after this he was seen by me in the casualty department and he then appeared anaemic and miserable but otherwise normal, except that his body weight was only 3710 g. His napkin (diaper) contained purée-like pistachio-green stools mixed with some of the contrast agent. No remarkable odour or greasy appearance was noticed. I admitted him for further investigations. Laboratory tests showed haemoglobin 7.5 g/dL, packed cell volume 26%, reticulocytes 9.8%, and serum albumin 14 g/L; all other blood tests were normal. One in eight stools were positive for haemoglobin. He had bacteriuria and was therefore put on co-trimoxazole and the vomiting improved. Since his haemoglobin dropped to 6.9 g/dL during the next few days he was transfused to 12.4 g/dL. Initial investigations revealed no bleeding source and a bone marrow aspirate was normal. He was readmitted 4 weeks later because his haemoglobin had dropped to 9.8 g/dL, packed cell volume to 32%, and reticulocytes to 2.8%. The serum albumin remained at 12 g/L and he had developed dorsal foot oedema. Liver enzymes were moderately raised. Investigations, including an abnormal sweat test, now confirmed the diagnosis of CF. The laboratory abnormalities improved and the oedema resolved soon after oral treatment with pancreatic enzymes had been started. His stools were more normal 1 month later.

Anaemia in infants with CF may be due to protein-energy malnutrition¹ or inadequate erythropoiesis,² or both. Protein-energy malnutrition may preferentially affect breastfed infants³ with CF and was present in both cases as indicated by hypoalbuminaemia and poor weight gain. The pistachio-green stools are harder to explain, but may be due to excretion of increased faecal bile acids following malabsorption⁴ of the relatively diluted and supersaturated bile (with cholesterol) that is produced in infancy. A defect in intestinal absorption of iron might contribute.⁵ If so, the association of anaemia, hypoproteinaemia, slow weight gain, with pistachio-green stools in infants with CF is plausible. Epidemiological studies are now needed.

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