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**Early mixed feeding and breastfeeding beyond six months increase the risk of postnatal HIV transmission: ANRS 1201/1202 Ditrane Plus, Abidjan, Côte d'Ivoire**

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*Abbreviations*

ANRS, Agence Nationale de Recherches sur le Sida; CI, Confidence Interval; HIV, Human Immunodeficiency Virus; scZDV, short-course Zidovudine; sdNVP, Nevirapine single dose; sc3TC, short-course Lamivudine; SD, Standard Deviation; WHO, World Health Organization.

*Running title*

Pattern and duration of breastfeeding and postnatal HIV transmission

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## Abstract

**Objective.** To evaluate the risk of postnatal HIV transmission among women in Abidjan, Côte d'Ivoire offered alternatives to prolonged breastfeeding, and to assess the impact of the breastfeeding pattern and duration on this risk.

**Methods.** In 2001-2003, HIV-infected pregnant women received peri-partum antiretroviral prophylaxis and were counselled antenatally regarding infant feeding options: formula feeding, or exclusive breastfeeding with early cessation from 4 months of age. The primary outcome was HIV postnatal transmission by 18 months of age, defined by a positive HIV test after a negative test  $\geq 30$  days. The effect of the pattern (mixed feeding, defined as breastmilk plus food-based fluid, solid food or non-human milk) and duration (less vs. more than 6 months) of breastfeeding on postnatal transmission was assessed.

**Results.** Of 622 live-born infants who were HIV-uninfected at or after 30 days, 15 were infected postnatally, 13/324 among breastfed, and 2/298 among formula-fed infants. The 18-month probability of remaining free from HIV-infection was 0.95 [95%CI, 0.92-0.97] and 0.99 [95%CI, 0.97-1.00] in the breastfeeding and formula-feeding groups respectively ( $p < 0.001$ ). In adjusted analysis, breastfeeding for more than 6 months and mixed feeding during the first month of life were independently associated with a 7.5 (AOR 95%CI, 2.0-28.2,  $p = 0.003$ ) and a 6.3 (95%CI, 1.1-36.4,  $p = 0.04$ )-fold increase of postnatal transmission among breastfed children.

**Conclusions.** Mixed feeding during the first month of life and breastfeeding beyond six months are strong determinants of HIV transmission and should be avoided when replacement feeding after breastfeeding cessation can be safely and sustainably provided.

**Key words.** HIV; mother-to-child transmission; breastfeeding; Africa

## **Introduction**

The efficacy of peri-partum antiretroviral regimens in preventing mother-to-child transmission of HIV around delivery has been demonstrated in Africa (Leroy et al., 2005). However, postnatal HIV transmission remains responsible for at least 40% of paediatric HIV infections in settings where prolonged breastfeeding is widely practised (Breastfeeding and HIV International Transmission Study Group (BHITS), 2004). Modifications of breastfeeding practices in terms of duration (complete avoidance of breastfeeding or early weaning) and pattern (promotion of exclusive breastfeeding) aim to reduce this risk (Becquet and Newell, 2007, Rollins et al., 2004). Although exclusive breastfeeding has been reported to carry a lower risk of postnatal HIV transmission than breastfeeding with introduction of other fluids, feeds or milk (Coovadia et al., 2007, Iliff et al., 2005), the effect of both breastfeeding pattern and duration on this risk has not yet been quantified.

The aim of this study was to evaluate the risk of postnatal HIV transmission among HIV-infected West-African women counselled on alternatives to prolonged breastfeeding, and to assess the effect of the breastfeeding pattern and duration on this risk.

## **Population and methods**

### ***Study design and follow-up procedures***

The inclusion procedures and research design of the ANRS 1201/1202 Ditrane Plus study have been described in detail (Becquet et al., 2005b, Leroy et al., 2007). Briefly, this study was an open-labelled cohort, based on women attending any one of six community-run health facilities in Abidjan, Côte d'Ivoire. Between March 2001 and July 2003, pregnant women aged 18 years and over, diagnosed as HIV-infected within one of these selected facilities, were eligible for enrolment into the study. All women included were offered peri-partum antiretroviral and post-partum nutritional interventions to prevent mother-to-child transmission of HIV. First, they received peri-partum antiretroviral zidovudine with or without lamivudine and single dose nevirapine (Dabis et al., 2005). Second, they were counselled antenatally on infant feeding options: complete avoidance of breastfeeding or exclusive breastfeeding with early cessation from the fourth month. Replacement feeding from birth or from breastfeeding cessation until 9 months of age, as well as the utensils needed, were provided free of charge. In all cases, the staff supported women's choice and counselled them accordingly. Vitamin A supplementation was provided to all children according to WHO recommendations.

Antiretroviral therapy was not available in Côte d'Ivoire before the end of 2004. Maternal antiretroviral therapy was therefore not available during the course of the study. However, eligible women included in the Ditrane Plus were referred to the MTCT-Plus initiative in Abidjan to receive antiretroviral treatment for their own health.

Between birth and the second birthday, 19 clinic visits were scheduled for clinical, nutritional, psychosocial, and biological follow-up of both mothers and infants. At each visit, infant feeding practices were recorded using structured questionnaires with both 24 hours and seven days recall histories (Gaillard et al., 2001). Infant feeding practices were recorded by trained field workers who were not involved in nutritional counselling (Becquet et al., 2005b, Leroy et al., 2007).

### ***Diagnosis of HIV infection***

Blood samples were collected from children at day 2, weeks 4, 6, and 12, and then every 3 months until 2 months after complete cessation of breast-feeding (Alioum et al., 2001, Alioum et al., 2003). Blood samples were stored and processed in Abidjan, at the CeDRoS reference laboratory. An HIV-antibody test was performed at age 18 months in all children. Paediatric HIV infection was defined by a positive plasma HIV-1 RNA PCR at any age, or positive HIV serology if aged 18 months or more.

There is a 'window period' during which it is not possible to detect HIV infection using available HIV testing technology (Fowler and Newell, 2002). It is therefore difficult to determine whether paediatric HIV infection has occurred before or during delivery, or through early breastfeeding during the first few weeks of life (Fowler and Newell, 2002, Leroy et al., 1998). We used the following definitions to determine the timing of HIV transmission. Peri-partum transmission of HIV was diagnosed at 4 weeks of age, and could have occurred in late pregnancy, intra-partum, or early postnatally during the first weeks of life. Late postnatal HIV transmission (beyond 4 weeks of age) was assessed in children with a negative RNA PCR from a sample obtained at age  $\geq 30$  days. (Alioum et al., 2001). Three children had a first positive test around 2 months of age with no previous negative test or with a last negative test at 2 days of age, and were considered as HIV-infected with unknown timing of infection (although it is more likely their infection occurred during the peri-partum period).

### ***Statistical analysis***

The primary outcome was the cumulative risk of late postnatal HIV transmission at 18 months of age. The time of acquisition of infection was estimated to be mid-way between the date of the last negative and first positive test (Alioum et al., 2003). The Turnbull's extension of the Kaplan-Meier procedure to interval-censored data is recommended when the length of intervals between HIV tests is long, but a standard Kaplan-Meier approach is satisfactory if most intervals are short (less than 3 months) (Alioum et al., 2003), which was the case in our study (data not shown). We therefore used the Kaplan-Meier method to assess the risk of postnatal transmission among live-born infants who were HIV-uninfected in the peri-partum period and with known mode of feeding; the association with maternal and infant variables was quantified in univariate and multivariate stepwise Cox regression analyses. Univariate analysis included maternal baseline socio-demographic, biologic, and clinical characteristics; and multivariate analysis included all variables with  $p < 0.25$  in the univariate analysis.

Some women did not express their choice regarding the infant feeding practice of their forthcoming infant antenally; and some children were not breastfed in the first 24 hours of life. As a result, the allocation to the breastfeeding or formula-feeding group was based on the feeding mode 2 days after delivery and reported by the mother during the interview.

The effects of the pattern of breastfeeding during the first month or the first 2 or 3 months of life ('exclusive breastfeeding vs. not', and 'mixed feeding vs. not') and breastfeeding duration (less vs. more than 6 months) on postnatal transmission were assessed in the breastfeeding group. We used WHO infant feeding definitions to allow a better comparability of results between studies (Table 1). A child who was breastfed at a given age while having never received any other food or drink was considered as exclusively breastfed (WHO, 2004). A child who was breastfed at a given age having ever received food-based fluid, solid food or non-human milk was considered as mixed fed (WHO, 2004). Breastfed children having been given water or water-based drinks (tea, fruit juice) only were predominantly breastfed, and considered as non-exclusively breastfed and non-mixed fed. The effect of the introduction of any fluids or solids in addition to breastmilk on postnatal transmission was assessed with the variable 'exclusive breastfeeding vs. not', whereas the effect of the introduction of food-based fluid, solid food or non-human milk was assessed with the variable 'mixed feeding vs. not' (see the footnote below Table 2). All statistical analyses were carried out using SAS software (version 9.1; SAS Institute, <http://www.sas.com>).

The Ditrane Plus study was granted ethics permission in Côte d'Ivoire from the ethics committee of the National AIDS Control Programme, and in France from the institutional review board of the French ANRS.

## Results

Of the 808 HIV-infected pregnant women enrolled in the Ditrane Plus study, 34 with a non-confirmed HIV-1 status, or infected with HIV-2 only, were excluded, 44 were lost to follow-up before delivery, and 730 gave birth to 763 infants. Of these, 33 second- and third-born babies of multiple births and 19 stillbirths were excluded, as were 21 neonates not tested for HIV infection, 3 with unknown timing of infection, 42 infected in the peri-partum period, 2 with unknown mode of feeding and 21 with no infant feeding recall histories in the first month of life.

Among the 622 infants exposed to the risk of acquiring HIV postnatally and included in the present analysis, 298 constituted the formula-feeding, and 324 the breastfeeding group. Baseline socio-demographic, clinical, and biological characteristics differences between these two groups are presented in Table 2. Compared to breastfeeding mothers, formula-feeding mothers had a significantly higher level of education, were less likely to live in crowded accommodation (typical shared housing), and more likely to have access to tap water at home rather than outside home. The other characteristics were comparable between the two groups.

In the breastfeeding group, the median duration of breastfeeding was 124 days (inter-quartile range, 97-199), and 28% of children were still being breastfed beyond 6 months of age. The probability of being exclusively breastfed from birth (95% CI) was 0.23 (0.19-0.28), 0.15 (0.11-0.19), and 0.01 (0.001-0.02) at 1, 3 and 6 months of age, respectively. By 3 months of age, most infants had been predominantly breastfed, and 22% had ever been exposed to any type of mixed feeding: 19% had received breastmilk and non-human milk as part of their weaning process, while the remaining 3% had been fed with breastmilk plus solids and were not in the process of being weaned.

At 18 months of age, there were no significant differences in morbidity (diarrhoea, respiratory infection, malnutrition), hospitalization and mortality between the breastfed and formula-fed children (Becquet et al., 2007).

Follow-up was stopped before 18 months of age for 43 (14%) children in the formula-feeding group and 65 (20%) children in the breastfeeding group ( $p=0.06$ ). HIV testing was performed at least once postnatally for all 43 lost-to-follow-up children in the formula-feeding group,



and the median age at their last available blood sample for HIV testing was 365 days (interquartile range, 183-542). Among the 65 children in the breastfeeding group, the last available blood sample for HIV testing was collected at least 2 months after breastfeeding cessation for 38 (69%), which allowed the assessment of their definitive negative HIV status since they were no longer at risk of acquiring HIV infection postnatally.

At age 18 months, 15 children had acquired HIV postnatally, 13 in the breastfeeding group, and 2 in the formula-feeding group. The overall estimated risk of late postnatal transmission was 8.7 /100 child-years of breast-feeding [95% CI, 4.4-13.0]. The cumulative estimate of the probability of remaining free from HIV-infection at 18 months of age was 0.95 [95% CI, 0.92-0.97] and 0.99 [95% CI, 0.97-1.00] in the breastfeeding and formula-feeding groups respectively (log-rank test,  $p<0.001$ ). As shown in Figure 1, the rate of postnatal transmission was similar for children who were formula-fed or breastfed for less than 6 months (log-rank test,  $p=0.22$ ), whereas significantly higher rates were observed among children breastfed for longer than 6 months (log-rank test comparing to formula-fed and breastfed for less than 6 months children pooled together,  $p<0.001$ ).

The determinants of postnatal HIV transmission were assessed among breastfed children, and are presented in Table 3. In multivariate analysis, maternal employment ( $p=0.05$ ) and short course zidovudine and lamivudine boosted with single dose nevirapine ( $p=0.05$ ) were associated with a decreased risk of postnatal HIV transmission; breastfeeding beyond six months and mixed feeding during the first month of life were associated with a 7.5 ( $p=0.003$ ) and a 6.3 ( $p=0.04$ )-fold increase of postnatal transmission risk respectively. Exposure to mixed feeding in month two or three of life was not significantly associated with postnatal transmission (data not shown). Similarly, exclusive breastfeeding as opposed to breastfeeding plus any liquids or feeds in month one, two or three of life was not significantly associated with a reduced risk of postnatal acquisition of HIV.

## **Discussion**

We have quantified the impact of modifying breastfeeding pattern and duration on the risk of postnatal HIV transmission. In our study, mixed feeding during the first month of life and breastfeeding beyond six months of age were strong independent risk factors for acquisition of HIV postnatally. These results are consistent with previously published studies from other African settings reporting the association between mixed feeding (Coovadia et al., 2007,

Coutsoudis et al., 1999, Iliff et al., 2005) or prolonged breastfeeding (Taha et al., 2007) with increased HIV transmission risk.

Women included in the Ditrane Plus cohort were representative of the general population of pregnant women in Abidjan because they had been recruited antenatally among all attendees of community-run health facilities located in poor areas, with no other selection criteria than being HIV infected and at least 18 years old, and accepting the study protocol (Becquet et al., 2005b, UNICEF, 2000). Emphasis was placed on the use of standardised questionnaires for the collection of infant feeding data, frequent visits scheduled over the follow-up period, and interviews conducted by trained field workers other than those who counselled the women on infant feeding practices. This strategy contributed to minimising maternal recall bias that could have impaired the estimation of breastfeeding pattern and duration (Bland et al., 2003). However, the following limitations need to be addressed. First, there were relatively few postnatal transmission cases and it is therefore possible that associations did not reach statistical significance through lack of statistical power. Second, follow-up was stopped before 18 months of age for 17% of the cohort, these lost-to-follow-up children having participated in the study for a median of one year. However, a definitive postnatal HIV status was ascertained for all lost-to-follow-up children in the formula-feeding group and more than two thirds of the lost-to-follow-up children in the breastfeeding group had ceased breastfeeding when they left the study. A definitive HIV status was therefore also ascertained for these children as they were no longer at risk of acquiring HIV infection postnatally. As reported from another study (Nduati et al., 2000), follow-up tended to be better among non-breastfeeding mothers; possibly explained by the mother's socio-demographic characteristics or the health care workers' attitude toward this latter group (Becquet et al., 2005a), and could be a possible source of bias.

Women recruited in the Ditrane Plus study were counselled antenatally on alternatives to prolonged breastfeeding. On the one hand, women choosing not to breastfeed were encouraged to practise exclusive formula-feeding from birth, which was effectively the case for 85% of them: at 12 months, only 15% of the formula-fed children had been exposed to any breastmilk (Leroy et al., 2007). Only two cases of postnatal HIV transmission occurred in this group, both explained by mixed feeding practises. On the other hand, emphasis was placed on safer breastfeeding practices among women choosing to breastfeed, with the promotion of both exclusive breastfeeding and early weaning. The breastfeeding duration was particularly short in this context (median of 4 months) (Becquet et al., 2005b). Exclusive breastfeeding was practised by only a few women in the first 3 months, which did not imply a

high mixed-feeding rate however, since most of the complementary feeds introduced by that age were water-based fluids (hence most these infants were predominantly breastfed). As a consequence, only 8% of breastfed children had been exposed to mixed feeding in the first month of life; the breastfeeding pattern carrying the highest risk of HIV transmission was therefore avoided for most of the children.

In our study, the risk of postnatal transmission of HIV at 18 months was 5%, which is much lower than the rates previously reported in populations practising prolonged breastfeeding: 12.1% in Zimbabwe and 9.3% in a meta-analysis of 9 African studies (Breastfeeding and HIV International Transmission Study Group (BHITS), 2004, Iliff et al., 2005), and 9.8% at 24 months of age in an earlier study in Côte d'Ivoire (Leroy et al., 2003). Our intervention consisting in promoting the use of formula feeding from birth or the practice of a short duration of breastfeeding is likely to have led to the low postnatal transmission rates; the shorter the breastfeeding duration, the lower the risk of postnatal HIV transmission.

Exposure to mixed feeding (i.e. breastmilk plus food-based fluid, solid food or non-human milk) during the first month of life was associated with a significant 6-fold increase in the risk of postnatal transmission. However, exclusive breastfeeding in the first month of life was not significantly associated with a lower risk when compared with the predominant and mixed feeding taken together. The introduction of food-based fluid, solid food or non-human milk during the first month of life seemed therefore to have a stronger impact on the risk of postnatal acquisition of HIV than the introduction of water based fluids. This could be due to the fact that contaminants or bacteria contained in early introduced complementary feeds may damage the infant's immature gut, impair mucosal integrity, and thus facilitate postnatal transmission of HIV (Kourtis et al., 2003, Willumsen et al., 1997). It is therefore crucial to promote and support optimal feeding practices among HIV-infected women (Coovadia and Bland, 2007). There is increasing evidence that high rates of exclusive breastfeeding can be achieved in programmes concentrating on hospital policies and practices such as the Baby Friendly Hospital Initiative (Coutinho et al., 2005, Kramer et al., 2001), and/or on support in the community (Coovadia et al., 2007, Coutinho et al., 2005, Morrow et al., 1999) so that mothers can receive continuing help.

## **Conclusions**

We have previously reported that, in this urban African setting, and with appropriate nutritional counselling and care, alternatives to prolonged breastfeeding were safe (Becquet et al., 2007), socially acceptable and feasible (Becquet et al., 2005b, Leroy et al., 2007),

allowing optimal long-term effectiveness of peri-partum antiretroviral prophylaxis. We here show that this strategy also leads to low HIV postnatal transmission rates. Ideally, emphasis should now be placed on the provision of quality infant feeding counselling, commencing antenatally, and adequate replacement feeding after breastfeeding cessation in African communities, so that early mixed feeding and breastfeeding beyond six months of age can be avoided among HIV-infected women, and the risk of acquisition of HIV through breastmilk significantly reduced (WHO, 2007).

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## CONFLICT OF INTEREST AND ROLE OF THE FUNDING SOURCE

None of the authors had any conflict of interest to declare. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## AUTHORS CONTRIBUTIONS

Study concept and design: VL, FD, MTK. Obtained funding: FD, VL. Field work and data collection: RB, DKE, HM, CAB, LB, IV. Statistical analysis: RB. Interpretation of results: RB, VL. First drafting of the manuscript: RB. Critical revision of the manuscript for important intellectual content: RB, VL, DKE, LB, FD.

## References

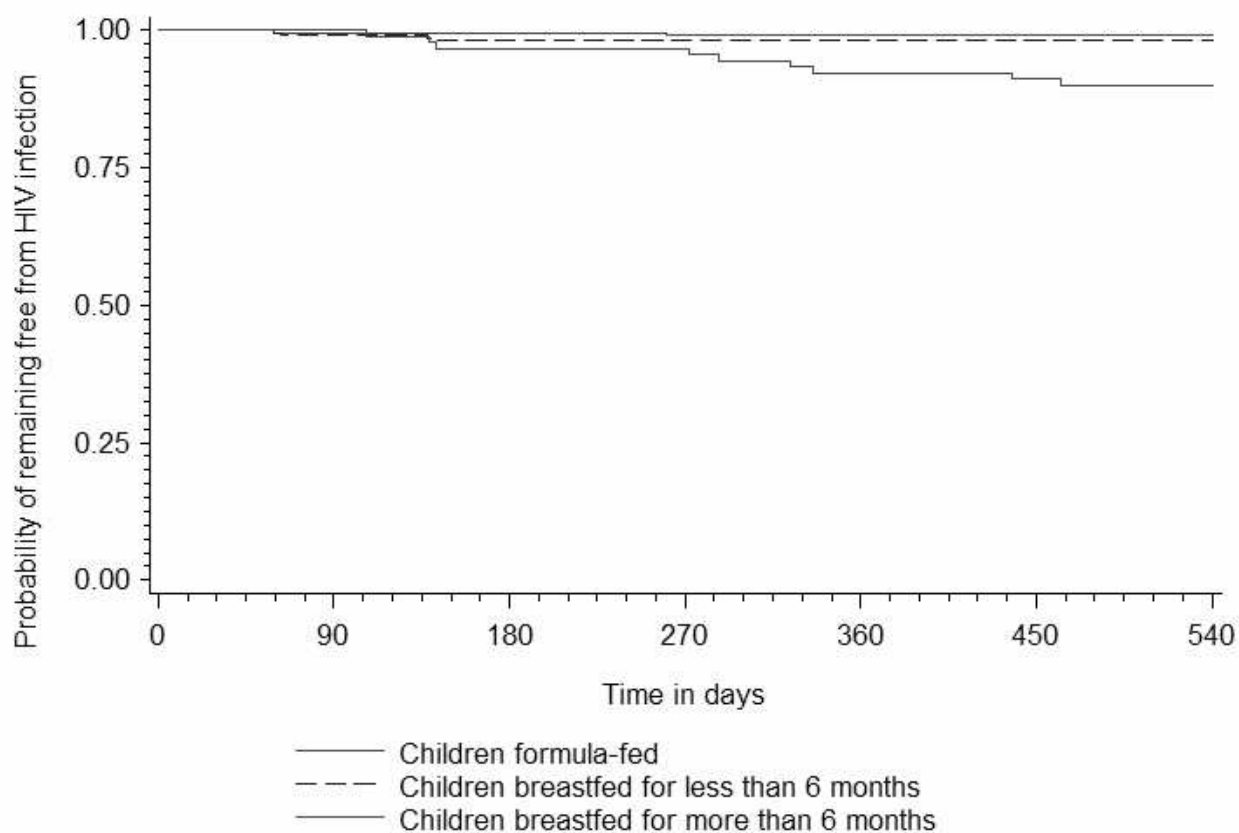
- Alioum, A., Dabis, F., Dequae-Merchadou, L., Haverkamp, G., Hudgens, M., Hughes, J., Karon, J., Leroy, V., Newell, M. L., Richardson, B. & Weverling, G. J. (2001) Estimating the efficacy of interventions to prevent mother-to-child transmission of HIV in breast-feeding populations: development of a consensus methodology. *Stat Med*, 20, 3539-56.
- Alioum, A., Cortina-Borja, M., Dabis, F., Dequae-Merchadou, L., Haverkamp, G., Hughes, J., Karon, J., Leroy, V., Newell, M. L., Richardson, B. A., van Weert, L. & Weverling, G. J. (2003) Estimating the efficacy of interventions to prevent mother-to-child transmission of human immunodeficiency virus in breastfeeding populations: comparing statistical methods. *Am J Epidemiol*, 158, 596-605.
- Becquet, R., Ekouevi, D. K., Sakarovitch, C., Bequet, L., Viho, I., Tonwe-Gold, B., Dabis, F. & Leroy, V. (2005a) Knowledge, attitudes, and beliefs of health care workers regarding alternatives to prolonged breast-feeding (ANRS 1201/1202, Ditrane Plus, Abidjan, Cote d'Ivoire). *J Acquir Immune Defic Syndr*, 40, 102-105.
- Becquet, R., Ekouevi, D. K., Viho, I., Sakarovitch, C., Toure, H., Castetbon, K., Coulibaly, N., Timite-Konan, M., Bequet, L., Dabis, F. & Leroy, V. (2005b) Acceptability of exclusive breastfeeding with early cessation to prevent HIV transmission through breastmilk, ANRS 1201/1202 Ditrane Plus, Abidjan, Côte d'Ivoire. *J Acquir Immune Defic Syndr*, 40, 600-8.
- Becquet, R., Bequet, L., Ekouevi, D. K., Viho, I., Sakarovitch, C., Fassinou, P., Bedikou, G., Timite-Konan, M., Dabis, F. & Leroy, V. (2007) Two-year morbidity–mortality and alternatives to prolonged breast-feeding among children born to HIV-infected mothers in Côte d'Ivoire. *PLoS Medicine*, 4, e17.
- Becquet, R. & Newell, M. L. (2007) Prevention of postnatal HIV infection: infant feeding and antiretroviral interventions. *Current Opinion in HIV and AIDS*, 2, 361-366.
- Bland, R. M., Rollins, N. C., Solarsh, G., Van den Broeck, J. & Coovadia, H. M. (2003) Maternal recall of exclusive breast feeding duration. *Arch Dis Child*, 88, 778-83.
- Breastfeeding and HIV International Transmission Study Group (BHITS) (2004) Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. *J Infect Dis*, 189, 2154-2166.
- Coovadia, H. M. & Bland, R. M. (2007) Preserving breastfeeding practice through the HIV pandemic. *Trop Med Int Health*, 12, 1-18.
- Coovadia, H. M., Rollins, N. C., Bland, R. M., Little, K., Coutsooudis, A., Bennish, M. L. & Newell, M. L. (2007) Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding: the first six months of life. *Lancet*, 369, 1107-16.
- Coutinho, S. B., de Lira, P. I., de Carvalho Lima, M. & Ashworth, A. (2005) Comparison of the effect of two systems for the promotion of exclusive breastfeeding. *Lancet*, 366, 1094-100.
- Coutsooudis, A., Pillay, K., Spooner, E., Kuhn, L. & Coovadia, H. M. (1999) Influence of infant-feeding patterns on early mother-to-child transmission of HIV-1 in Durban, South Africa: a prospective cohort study. South African Vitamin A Study Group. *Lancet*, 354, 471-6.
- Dabis, F., Bequet, L., Ekouevi, D. K., Viho, I., Rouet, F., Horo, A., Sakarovitch, C., Becquet, R., Fassinou, P., Dequae, L., Welffens-Ekra, C., Rouzioux, C. & Leroy, V. (2005) Field efficacy of Zidovudine, Lamivudine and single-dose Nevirapine to prevent peripartum transmission of HIV. The ANRS 1201/1202 Ditrane Plus study, Abidjan, Cote d'Ivoire. *AIDS*, 19, 309-18.

- Fowler, M. G. & Newell, M. L. (2002) Breast-feeding and HIV-1 transmission in resource-limited settings. *J Acquir Immune Defic Syndr*, 30, 230-9.
- Gaillard, P., Piwoz, E. & Farley, T. M. (2001) Collection of standardized information on infant feeding in the context of mother-to-child transmission of HIV. *Stat Med*, 20, 3525-37.
- Iloff, P., Piwoz, E., Tavengwa, N., Zunguza, C., Marinda, E., Nathoo, K., Moulton, L., Ward, B. & Humphrey, J. (2005) Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. *AIDS*, 19, 699-708.
- Kourtis, A. P., Butera, S., Ibegbu, C., Beled, L., Duerr, A., Wu, Z., Viisainen, K., Wang, Y. & Hemminki, E. (2003) Breast milk and HIV-1: vector of transmission or vehicle of protection? *Lancet Infect Dis*, 3, 786-93.
- Kramer, M. S., Chalmers, B., Hodnett, E. D., Sevkovskaya, Z., Dzikovich, I., Shapiro, S., Collet, J.-P., Vanilovich, I., Mezen, I., Ducruet, T., Shishko, G., Zubovich, V., Mknuk, D., Gluchanina, E., Dombrovskiy, V., Ustinovitch, A., Kot, T., Bogdanovich, N., Ovchinkova, L., Helsing, E. & for the PROBIT Study Group (2001) Promotion of Breastfeeding Intervention Trial (PROBIT): A Randomized Trial in the Republic of Belarus. *JAMA*, 285, 413-420.
- Leroy, V., Newell, M. L., Dabis, F., Peckham, C., Van de Perre, P., Bulterys, M., Kind, C., Simonds, R. J., Wiktor, S. & Msellati, P. (1998) International multicentre pooled analysis of late postnatal mother-to-child transmission of HIV-1 infection. Ghent International Working Group on Mother-to-Child Transmission of HIV. *Lancet*, 352, 597-600.
- Leroy, V., Karon, J. M., Alioum, A., Ekpini, E. R., Van De Perre, P., Greenberg, A. E., Msellati, P., Hudgens, M., Dabis, F. & Wiktor, S. Z. (2003) Postnatal transmission of HIV-1 after a maternal short-course zidovudine peripartum regimen in West Africa. *AIDS*, 17, 1493-1501.
- Leroy, V., Sakarovitch, C., Cortina-Borja, M., McIntyre, J., Coovadia, H., Dabis, F. & Newell, M. L. (2005) Is there a difference in the efficacy of peripartum antiretroviral regimens in reducing mother-to-child transmission of HIV in Africa? *AIDS*, 19, 1865-75.
- Leroy, V., Sakarovitch, C., Viho, I., Becquet, R., Ekouevi, D. K., Bequet, L., Rouet, F., Dabis, F. & Timité-Konan, M. (2007) Acceptability of formula-feeding to prevent postnatal transmission of HIV-1 infection in Abidjan, Côte d'Ivoire, Ditrane Plus ANRS 1201/1202. *J Acquir Immune Defic Syndr*, 44, 77-86.
- Morrow, A. L., Guerrero, M. L., Shults, J., Calva, J. J., Lutter, C., Bravo, J., Ruiz-Palacios, G., Morrow, R. C. & Butterfoss, F. D. (1999) Efficacy of home-based peer counselling to promote exclusive breastfeeding: a randomised controlled trial. *Lancet*, 353, 1226-31.
- Nduati, R., John, G., Mbori-Ngacha, D., Richardson, B., Overbaugh, J., Mwatha, A., Ndinya-Achola, J., Bwayo, J., Onyango, F. E., Hughes, J. & Kreiss, J. (2000) Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *Jama*, 283, 1167-74.
- Rollins, N., Meda, N., Becquet, R., Coutoudis, A., Humphrey, J., Jeffrey, B., Kanshana, S., Kuhn, L., Leroy, V., Mbori-Ngacha, D., McIntyre, J., Newell, M. L. & Ghent IAS Working Group on HIV in Women and Children (2004) Preventing postnatal transmission of HIV-1 through breast-feeding: modifying infant feeding practices. *J Acquir Immune Defic Syndr*, 35, 188-195.
- Taha, T. E., Hoover, D. R., Kumwenda, N. I., Fiscus, S. A., Kafulafula, G. & Nkhoma, C. (2007) Late postnatal transmission of HIV-1 and associated factors. *J Infect Dis*, 196, 10-14.

- UNICEF (2000) Multiple Indicator Cluster Survey (MICS-2000), Côte d'Ivoire.
- WHO (2004) HIV transmission through breastfeeding: a review of available evidence. Geneva, Switzerland, World Health Organisation.
- WHO (2007) Consensus statement of the WHO HIV and infant feeding technical consultation held on behalf of the Inter-agency Task Team (IATT) on prevention of HIV infections in pregnant women, mothers and their Infants, Geneva, October 25-27, 2006.
- Willumsen, J., Darling, J. C. & Kitundu, J. A. (1997) Dietary management of acute diarrhoea in children: effect of fermented and amylase-digested weaning foods on intestinal permeability. *J Pediatr Gastroenterol Nutr*, 24, 235-41.



**Figure 1.** Probability of remaining free from HIV infection by infant feeding practices. ANRS 1201/1202 Ditrane Plus, Abidjan, Côte d'Ivoire, 2001-2005 (N=622).



Probability of remaining free from postnatal HIV infection (95%CI)	Age 6 Months	Age 12 Months	Age 18 Months
Formula-feeding (n=298)	0.99 (0.98-1.00)	0.99 (0.97-1.00)	0.99 (0.97-1.00)
Breastfeeding $\leq$ 6 months (n=232)	0.98 (0.95-0.99)	0.98 (0.95-0.99)	0.98 (0.95-0.99)
Breastfeeding > 6 months (n=92)	0.97 (0.90-0.99)	0.92 (0.84-0.96)	0.90 (0.81-0.95)

**Table 1.** World Health Organization infant feeding definitions.

<b>Infant feeding practice</b>	<b>Definition</b>
Exclusive breastfeeding	Giving a child no other food or drink, including no water, in addition to breastfeeding with the exception of medicines, vitamin drops or syrups, and mineral supplements
Predominant breastfeeding	Breastfeeding a child but also giving small amounts of water or water-based drinks. Neither food-based fluid nor solid food nor non-human milk are allowed under this definition
Mixed feeding	Breastfeeding a child while giving non-human milk or food-based fluid or solid food
Artificial feeding	Feeding a child on artificial feeds (including non-human milk such as infant formula and powdered animal milk), and not breastfeeding at all

Source: Indicators for assessing breast-feeding practices (WHO, 1991); Breastfeeding counselling, a training course (WHO, 1993); HIV transmission through breastfeeding: a review of available evidence (WHO, 2004).

**Table 2.** Baseline characteristics according to infant feeding practices. ANRS 1201/1202 Ditrane Plus, Abidjan, Côte d'Ivoire, 2001-2005 (N=622).

	Breastfeeding group (n=324) N (%)	Formula-feeding group (n=298) N (%)	p-value
Median maternal age (IQR)	26.7 (5.4)	27.2 (5.1)	0.22
Maternal education			
none	141 (43.5)	73 (24.5)	<0.001
primary school	112 (34.6)	126 (42.3)	
secondary school or higher	71 (21.9)	99 (33.2)	
Mother employed in formal economic sector	172 (53.1)	150 (50.3)	0.49
Water access			
tap inside home	84 (25.9)	124 (41.6)	<0.001
tap outside home	240 (74.1)	174 (58.4)	
Type of housing *			
individual	96 (29.6)	128 (43.0)	<0.001
shared	228 (78.4)	170 (57.0)	
Peri-partum antiretroviral regimen *			
scAZT+sdNVP	150 (46.3)	179 (60.1)	<0.001
scAZT+sc3TC+sdNVPsd	174 (53.7)	119 (39.9)	
Median maternal CD4 count (IQR)	400 (270-535)	405 (249-600)	0.21
CD4 count < 200 cells/mm <sup>3</sup>	45 (14.0)	47 (15.8)	0.53
Female child	159 (49.1)	145 (48.7)	0.92
Multiple pregnancy (twins or triplets)	12 (3.7)	19 (6.4)	0.13
Low birth weight (< 2.5kg)	87 (26.9)	69 (23.1)	0.29

\* Shared housing: typical housing with several houses organized around a yard where inhabitants live in crowded accommodation and share kitchen and restroom ; scZDV: short-course Zidovudine ; sdNVP: Nevirapine single dose ; sc3TC: short-course Lamivudine.

**Table 3.** Determinants of postnatal transmission of HIV among breastfed children (N=324).

Variables	N	18-month cumulative risk of postnatal transmission	95% CI	Univariate analysis			Multivariate analysis <sup>a</sup>		
				Hazard ratio	95% CI	p	Adjusted hazard ratio	95% CI	p
Maternal age	324	-	-	0.97	0.9-1.1	0.55			
Maternal education									
none or primary school	253	4.95	2.8-8.8	1	-	0.56			
secondary school	71	3.11	0.8-11.9	0.64	0.2-2.9				
Mother employed in the formal economic sector									
yes	172	2.68	1.0-7.0	0.40	0.1-1.3	0.12	0.31	0.1-1.0	0.05
no	152	6.57	3.5-12.3	1	-		1	1	
Water access									
tap inside home	84	1.41	0.2-9.6	1	-	0.23			
tap outside home	240	5.63	3.2-9.7	3.57	0.4-27.9				
Type of housing *									
individual	96	2.32	0.6-9.0	1	-	0.25			
shared	228	5.50	3.1-9.7	2.42	0.5-10.9				
Peri-partum antiretroviral regimen *									
scAZT+NVPsd	150	8.08	4.6-14.1	1	-	0.02	1	-	0.05
scAZT+sc3TC+NVPsd	174	1.27	0.3-5.0	0.16	0.04-0.7		0.22	0.05-1.0	
Maternal CD4 count (10 <sup>6</sup> cells/ml)									
< 200 cells/mm <sup>3</sup>	87	2.78	0.4-18.1	2.16	0.1-34.5	0.24	4.29	0.2-75.6	0.15
[200-500] cells/mm <sup>3</sup>	182	6.61	3.7-11.6	5.58	0.7-43.2		8.22	0.9-73.6	
≥ 500 cells/mm <sup>3</sup>	95	1.16	0.2-8.1	1	-		1	-	
Female child									
yes	159	3.52	1.5-8.3	0.65	0.2-2.0	0.45			
no	165	5.52	2.8-10.8	-	-				
Multiple pregnancy									
yes	12	no case	-	-	-	-			
no	312	4.72	2.8-8.0						
Low birth weight (< 2.5kg)									
yes	87	5.13	1.9-13.1	1	-	0.68			
no	237	4.30	2.2-8.1	1.28	0.4-4.1				
Breastfeeding duration									

< 6 months	232	1.89	0.7-5.0	1	-	0.01	1	-	0.003
≥ 6 months	92	10.08	5.4-18.5	5.01	1.5-16.3		7.46	2.0-28.2	
Exclusive breastfeeding during the first month of life <sup>b</sup>									
yes	75	4.35	1.4-12.9	0.93	0.2-3.4	0.92			
no	249	4.61	2.5-8.4	1	-				
Exposure to mixed feeding during the first month of life <sup>c</sup>									
yes	27	7.85	2.0-27.9	2.03	0.5-9.2		6.26	1.1-36.4	
no	297	4.25	2.4-7.6	1	-	0.27	1	-	0.04

\* Shared housing: typical housing with several houses organized around a yard where inhabitants live in crowded accommodation and share kitchen and restroom ; scZDV: short-course Zidovudine ; sdNVP: Nevirapine single dose ; sc3TC: short-course Lamivudine.

<sup>a</sup> Variables included in multivariable modelling were maternal employment, peri-partum antiretroviral regimen, maternal CD4 count, breastfeeding duration and exposure to mixed feeding during the first month.

<sup>b</sup> Exclusive breastfeeding

- yes: breastmilk only
- no: breastmilk plus any liquids or feeds

<sup>c</sup> Mixed feeding

- yes: breastmilk plus food-based fluid, solid food or non-human milk
- no: breastmilk only or breastmilk plus water or water-based drinks

## **Précis**

Among the 622 mother-infant pairs included in the study in Côte d'Ivoire, the very early practice of mixed feeding and breastfeeding beyond six months of age were strong risk factors for acquisition of HIV postnatally.