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Serological survey of HIV and syphilis in pregnant women in Madagascar

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Abstract

OBJECTIVES Peripartal transmission of human immunodeficiency virus (HIV) and *Treponema pallidum*, the causative agent of syphilis, leads to severe consequences for newborns. Preventive measures require awareness of the maternal infection. Although HIV and syphilis testing in Madagascar could be theoretically carried out within the framework of the national pregnancy follow-up scheme, the required test kits are rarely available at peripheral health centres. In this study, we screened blood samples of pregnant Madagascan women for HIV and syphilis seroprevalence to estimate the demand for systemic screening in pregnancy.

METHODS Retrospective anonymous serological analysis for HIV and syphilis was performed in plasma samples from 1232 pregnant women that were taken between May and July 2010 in Ambositra, Ifanadiana, Manakara, Mananjary, Moramanga and Tsiroanomandidy (Madagascar) during pregnancy follow-up. Screening was based on *Treponema pallidum* haemagglutination tests for syphilis and rapid tests for HIV, with confirmation of positive screening results on line assays.

RESULTS Out of 1232 pregnant women, none were seropositive for HIV and 37 (3%) were seropositive for *Treponema pallidum*.

CONCLUSIONS Our findings are in line with previous studies that describe considerable syphilis prevalence in the rural Madagascan population. The results suggest a need for screening to prevent peripartal *Treponema pallidum* transmission, while HIV is still rare. If they are known, *Treponema pallidum* infections can be easily, safely and inexpensively treated even in pregnancy to reduce the risk of transmission.

keywords HIV, syphilis, pregnant, woman, Madagascar, screening

Introduction

Sexually transmitted diseases (STDs) including human immunodeficiency virus (HIV) infections and syphilis, which is caused by *Treponema pallidum*, are prevalent in Madagascar (Harms *et al.* 1994a,b; Behets *et al.* 1996). Neglect of the threat of STDs and of condom use facilitates STD acquisition in Madagascar (Andrianasolo *et al.* 2011), associated with underage sex (9–36%), sex with multiple partners (3–20%) and a low rate of laboratory testing (0–29%) among people at risk for acquiring HIV (WHO 2010: 303–357). Neglect of condom use is reported by 60–70% during casual sexual encounters (Khan *et al.* 2008), by 20–50% during professional sex (Xueref *et al.* 2003; WHO 2010: 341). Being married in association with occasional, non-professional sex work is a striking risk factor for STD acquisition, next to poor education, young age (Harijaona *et al.* 2009) and ambiguous discrimination between client and lover status of sexual partners (Stoebenau *et al.* 2009). Only 6% of university students in Antananarivo and 7% of men in rural Madagascan areas report consistent condom use during sexual intercourse (Leutscher *et al.* 2003; Rahamefy *et al.* 2008), while nearly half of the men questioned have sex with multiple partners (Leutscher *et al.* 2003).

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Consequently, STD infections were diagnosed in 37.5% of women and 26.8% of men in rural Madagascar in 2005. STD acquisition rates were highest in women aged 14–24 years (Leutscher *et al.* 2005), posing a high risk of vertical transmission.

Peripartal transmission of HIV or *Treponema pallidum* leads to severe consequences for newborns. Implementation of preventive and therapeutic procedures, including screening and specific therapy, can reduce the risk of transmission (Casalini *et al.* 2001). HIV and syphilis testing in Madagascar should be offered within the framework of the national pregnancy follow-up scheme as the assessment of HIV and syphilis in pregnancy is demanded by the Madagascar Action Plan (World Bank 2007) and recommended by the WHO (2002). However, the required test kits are rarely available at peripheral health centres (centres de santé de base), which accords with Madagascar being among the 10 most inequitable countries regarding access to maternal health interventions (Barros *et al.* 2012) and previously detailed inadequacies of the national health system (Harimanana *et al.* 2011). Low-threshold screening programmes are usually restricted to sentinel analyses on which available surveillance data are based (UNAIDS/WHO Epidemiological Fact Sheets on HIV and AIDS, 2008 Update).

The aim of the study was to identify serological parameters indicating the prevalence of HIV and syphilis infections in pregnant women at selected sites in Madagascar to estimate the demand for systematic HIV and syphilis screening in pregnancy.

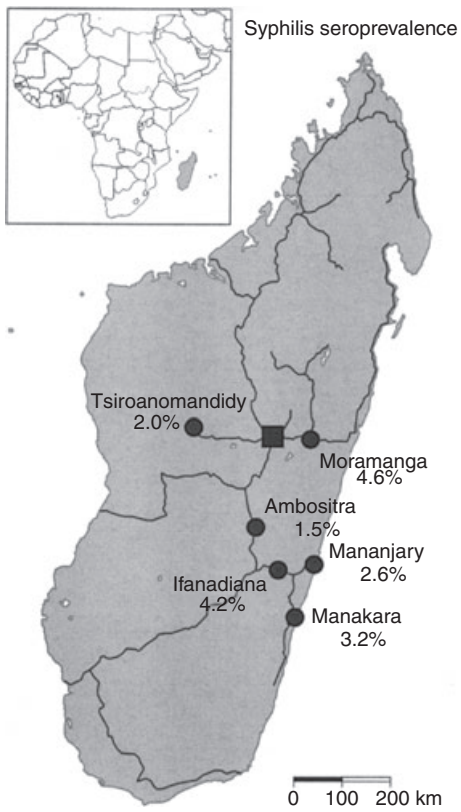

Figure 1 Local syphilis seroprevalences at the various study sites in Madagascar.

Materials and methods

Origin of the analysed samples

In the course of a study to identify malaria parasites in asymptomatic pregnant women, 1244 (EDTA) plasma samples were collected between May 2010 and July 2010 in six intermediated-sized locations with 18 000–36 000 inhabitants (see Figure 1 in the Results section): The two coastal cities of Mananjary (28 000 inhabitants) and Manakara (36 000 inhabitants), Ifanadiana (18 000 inhabitants), which lies on the ascending road from Mananjary and Manakara to the highlands, the two highland cities of Tsiroanomandidy (27 000 inhabitants) and Ambositra (30 000 inhabitants) and Moramanga (29 000 inhabitants), a highland city that lies on the road between the capital Antananarivo and Toamasina. The study sites were chosen to include sites at different elevations for malaria parasite assessment. Furthermore, a chikungunya/dengue seroscreening was initiated after a meeting at the WHO country office because of a previous chikungunya epidemic in the Manajary region (Schwarz *et al.* 2012).

The sample period at each site was 1 week. Blood was collected from all pregnant women presenting for routine pregnancy screenings to the local health centre who gave consent to their participation in the study. All participants signed an agreement to participate in the study. Ethical clearance for an anonymous retrospective screening for HIV and syphilis in the residual plasma samples as detailed above was obtained from the Ethical Committee of the University of Antananarivo, Madagascar.

Diagnostic workflow

Frozen (−20 °C) residual EDTA plasma samples of 1232 pregnant women presenting for routine pregnancy follow-up were screened for anti-HIV antibodies, HIV p24 antigen and anti-*Treponema pallidum* antibodies.

Determine™ HIV-1/2 Ag/Ab Combo rapid tests (Alere Ltd, Stockport, UK) with a sensitivity of 100% and a specificity of 99.49% for African samples (Alere Ltd) was

used for HIV screening. These fourth-generation tests, screening for both p24 antigen and antibodies against HIV, show better sensitivity for antibody responses than 'antibody-only'-based third-generation rapid tests in case of established HIV infections (Chetty *et al.* 2012). The sensitivity regarding early infections without antibody response is, however, poor with no more than 0–2% antigen detection (Chetty *et al.* 2012; Kilembe *et al.* 2012; Rosenberg *et al.* 2012). Positive screening results were confirmed using the line assay INNO-LIA™ HIV I/II Score (INNOGENETICS®, Ghent, Belgium) according to the diagnostic procedure of our institutes. If only antigen but no antibodies had been detected in the screening test, a subsequent PCR (Cobas® TaqScreen MPX Test; Roche, Basel, Switzerland) would have been added at a well-equipped German blood transfusion service.

To screen for syphilis, *Treponema pallidum* haemagglutination tests (TPHA) with a sensitivity of 98.7% and a specificity of 95.4% (Oxoid, Basingstoke, UK) were used. In an independent evaluation, the sensitivity was 92% for primary syphilis (35/38), 100% for secondary syphilis (10/10), early latent syphilis (28/28) and congenital syphilis (2/2), respectively, while specificity was 100% with no cross-reactions with samples from patients with leptospirosis (8), infectious mononucleosis (7), hepatitis (9), diabetes mellitus (11), rheumatoid arthritis (13), leprosy (11), tuberculosis (9), HIV/AIDS (12), systemic lupus erythematosus (4), rheumatic fever (3), as well as from elderly patients (9), pregnant women (29) and blood donors (164) (Rodriguez *et al.* 2002). Positive results were confirmed or rejected by line assays 'recom line' for IgG (Mikrogen GmbH, Neuried, Germany). An individual was counted as syphilis positive if the TPHA screening test was positive and the detected band pattern in the line assay provided at least an indeterminate result.

Epidemiological analysis

The age-dependent syphilis seroprevalence was compared for pregnant women in the age groups under 20 years of age, between 20 and 30 years of age and more than 30 years of age. Analysis was performed by chi-squared testing. Significance was accepted at $P < 0.05$. Risk factors for the acquisition of STDs had not been assessed.

Results

Study population

Among the 1244 included, sufficient plasma for HIV and syphilis tests was available for 1232 pregnant women. We investigated 190 samples from Mananjary, 251 from Manakara, 192 from Ifanadiana, 198 from Moramanga, 199 from Ambositra and 202 from Tsiroanomandidy. The right-skewed age distribution was similar in all study locations. Overall the median age was 25.3 years (range: 12.5–50.3).

Serological testing

HIV screening with Determine™ HIV-1/2 Ag/Ab Combo rapid tests led to two reactive results for antibodies but not for p24 antigen. Consecutive confirmation tests of these two samples with INNO-LIA™ HIV I/II Score line assays remained negative, so the diagnosis of HIV could not be confirmed in any sample (0% HIV prevalence).

Forty-five of 1232 samples tested positive in the TPHA screening. Confirmation tests with 'recom line' IgG-line assays confirmed this result for 37 samples (3% seroprevalence), while eight screening results could not be confirmed and were interpreted as 'false positive'. In detail, anti-*Treponema pallidum*-IgG was positive in 34 instances and indeterminate in further three instances.

Epidemiological analysis

Syphilis seroprevalences varied between 1.5% and 4.6% among the study locations. Seroprevalences were 2.6% (5 of 190) in Mananjary, 3.2% (8 of 251) in Manakara, 4.2% (8 of 192) in Ifanadiana, 4.6% (9 of 198) in Moramanga, 1.5% (3 of 199) in Ambositra and 2.0% (4 of 202) in Tsiroanomandidy (Figure 1). The age of the corresponding pregnant women was available for 1226 out of the 1236 analysed samples. Syphilis prevalences were by trend slightly higher (4.2%, $n = 15$) in 359 women over 30 than in 258 women under 20 years (2.3%, $n = 6$) ($P = 0.21$, chi-squared test) and 609 women between 20 and 30 years (2.6%, $n = 16$, $P = 0.19$, chi-squared test; Figure 2).

Discussion

The study demonstrated complete absence of HIV seroresponse and a moderate *Treponema pallidum* seroresponse of about 3% in more than 1200 Madagascan pregnant women presenting for routine pregnancy follow-up.

An anonymous study design was demanded for ethical considerations because STDs in general and HIV infections in particular are still associated with discrimination in Madagascar, even in medical settings (Andrianasolo *et al.* 2011). Specific risk factors for the acquisition of STDs could not be analysed. The residual samples were taken from a study without any focus on sexual behaviour, so no respective practices were recorded.

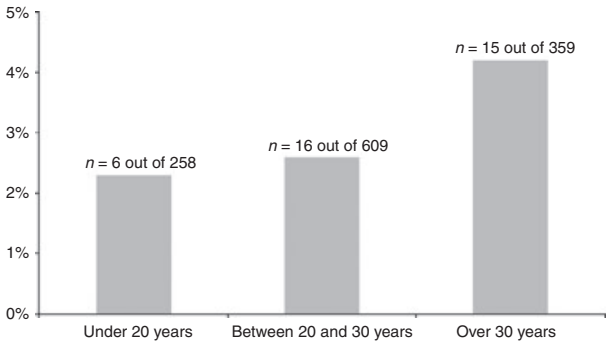

Figure 2 Age-dependent percentage of syphilis seroprevalence among pregnant Madagascan women. The differences were not significant.

Expectations of future analysis for STDs could not have attracted or deterred any of the pregnant women regarding participation in pregnancy follow-up, because neither the pregnant women nor the investigators knew about or anticipated these analyses at the time of presentation. A differential selection bias is thus unlikely. Moramanga and Ifanadiana, the places with the highest measured syphilis seroprevalence, are comparatively large cities on arterial roads from the capital to the coastline. Haulage traffic might pose a risk factor for STDs there.

The study was focused solely on prevalence data, so the analysis of syphilis activity markers such as IgM, TPHA quantification or lipoid antibody tests such as cardiolipin agglutination or Venereal Disease Research Laboratory test was deliberately avoided. The quantities of residual material would have been insufficient for more sophisticated serodiagnostic testing. In addition, serological evidence of active syphilis infections without the possibility to provide therapy because of the need for anonymization would have led to ethical conflicts.

Syphilis seroprevalence increased slightly with increasing age. Syphilis prevalence in each age group of our study population is still considerably lower than in Madagascan risk groups, as 18% *Treponema pallidum* seropositivity was detected among 316 registered female sex workers in Toliary in 1998 (Xueref *et al.* 2003). Four per cent of women from Antananarivo without history of sex work but with genital discharge syndrome were seropositive for syphilis in 2001. Young age, multiple sex partners and poor education were independent risk factors (Behets *et al.* 2001). Considering that our samples were taken from non-symptomatic pregnant women, a syphilis seroprevalence of 3% in this group suggests a further increase of syphilis infections in the last 10 years.

General HIV seroprevalence in Madagascar rose from 20/100 000 in 1989 to about 30/100 000 in 1992 to 70/100 000 in 1995 (Zeller *et al.* 1997; Ravaoarimalala *et al.* 1998). The 2010 UNAIDS report estimated that the total number of people living with HIV in Madagascar was 24 000 in 2009. With the Madagascan population of around 22 million this leads to a prevalence of 1.1 per 1000 (WHO 2010: 180). An HIV seropositivity of 0.9% was found in rural Madagascar in the 15–49 age group in 2005 (Leutscher *et al.* 2005). Our data suggest that HIV seroprevalence has at least not increased in non-risk groups since that time. The reasons for the differences in HIV spread on the two sides of the Mozambique channel remain unclear as specific risk factors are present in Madagascar (Leutscher *et al.* 2003; Khan *et al.* 2008; Rahamefy *et al.* 2008; WHO 2010: 303–357; Andrianasolo *et al.* 2011). Future epidemiological studies might focus on the differential distribution of high-risk sexual practices such anal intercourse or dry sex (i.e. sexual intercourse after deliberate drying of the female partner's vagina to increase friction-associated sensations associated with an increased risk of bleeding).

As HIV prevalence is still low in the assessed pregnant non-risk group, universally available routine screening for HIV during pregnancy follow-up is not a priority issue. In contrast, our data suggest that the establishment of an easily accessible syphilis routine screening for pregnant women in combination with a test-and-treat strategy should be considered for Madagascar regarding the relevant risk of peripartal syphilis transmission to foetuses and the comparatively easy and inexpensive treatment of active syphilis infections in pregnancy (Casalini *et al.* 2001).

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References

Andrianasolo RL, Rakotoarivelo RA, Randriarimanana D, Angijiro PG & Randria MJ (2011) Discrimination of HIV infected persons in medical settings in Madagascar. *Médecine et Maladies Infectieuses* **41**, 2–6.

Barros AJ, Ronsmans C, Axelson H *et al.* (2012) Equity in maternal, newborn, and child health interventions in Countdown to 2015: a retrospective review of survey data from 54 countries. *Lancet*, **379**, 1225–1233.

Behets F, Andriamahenina R, Andriamiadana J, May JF & Rasamindrakotroka A (1996) High syphilis and low but rising HIV seroprevalence rates in Madagascar. *Lancet* **11**, 401–402.

Behets F, Andriamiadana J, Rasamilalao D *et al.* (2001) Sexually transmitted infections and associated socio-demographic and behavioural factors in women seeking primary care suggest

Madagascar's vulnerability to rapid HIV spread. *Tropical Medicine & International Health* **6**, 202–211.

Casalini C, Signorini L, Beltrame A, Matteelli A & Carosi G (2001) Vertical transmission of human immunodeficiency virus (HIV) and other sexually transmitted infections (STI). *Minerva Ginecologica* **53**, 177–192.

Chetty V, Moodley D & Chuturgoon A (2012) Evaluation of a 4th generation rapid HIV test for earlier and reliable detection of HIV infection in pregnancy. *Journal of Clinical Virology* **54**, 180–184.

Harijaona V, Ramambason JD, Morisset R, Rasamindrakotroka A & Ravaoarinoro M (2009) Prevalence of and risk factors for sexually-transmitted infections in hidden female sex workers. *Médecine et Maladies Infectieuses* **39**, 909–913.

Harimanana A, Barennes H & Reinharz D (2011) Organizational analysis of maternal mortality reduction programs in Madagascar. *The International Journal of Health Planning and Management* **26**, e186–e196.

Harms G, Kirsch T, Rahelimiarana N *et al.* (1994a) HIV and syphilis in Madagascar. *AIDS* **8**, 279–280.

Harms G, Matull R, Randrianasolo D *et al.* (1994b) Pattern of sexually transmitted diseases in a Malagasy population. *Sexually Transmitted Diseases* **21**, 315–320.

Khan MR, Rasolofomanana JR, McClamroch KJ *et al.* (2008) High-risk sexual behavior at social venues in Madagascar. *Sexually Transmitted Diseases* **35**, 738–745.

Kilembe W, Keeling M, Karita E *et al.* (2012) Failure of a novel, rapid antigen and antibody combination test to detect antigen-positive HIV infection in African adults with early HIV infection. *PLoS One* **7**, e37154.

Leutscher PD, Behets F, Rousset D *et al.* (2003) Sexual behavior and sexually transmitted infections in men living in rural Madagascar: implications for HIV transmission. *Sexually Transmitted Diseases* **30**, 262–265.

Leutscher P, Jensen JS, Hoffmann S *et al.* (2005) Sexually transmitted infections in rural Madagascar at an early stage of the HIV epidemic: a 6-month community-based follow-up study. *Sexually Transmitted Diseases* **32**, 150–155.

Rahamefy OH, Rivard M, Ravaoarinoro M, Ranaivoharisoa L, Rasamindrakotroka AJ & Morisset R (2008) Sexual behaviour and condom use among university students in Madagascar. *SAHARA J: Journal of Social Aspects of HIV/AIDS Research Alliance/SAHARA, Human Sciences Research Council* **5**, 28–35.

Ravaoarimalala C, Andriamahenina R, Ravelojaona B *et al.* (1998) Aids in Madagascar. II. Intervention policy for maintaining low HIV infection prevalence. *Bulletin de la Société de Pathologie Exotique* **91**, 71–73.

Rodriguez I, Alvarez EL, Fernandez C & Miranda A (2002) Comparison of a recombinant-antigen enzyme immunoassay with *Treponema pallidum* hemagglutination test for serological confirmation of syphilis. *Memórias do Instituto Oswaldo Cruz* **97**, 347–349.

Rosenberg NE, Kamanga G & Phiri S (2012) Detection of acute HIV infection: a field evaluation of the determine® HIV-1/2 Ag/Ab combo test. *Journal of Infectious Diseases* **205**, 528–534.

Schwarz NG, Girmann M, Randriamampionona N, Bialonski A, Maus D, Krefis AC, Njarasoa C, Rajanalison JF, Ramandrisoa HD, Randriarison ML, May J, Schmidt-Chanasit J, Rakotozandrindrainy R (2012) Seroprevalence sampling of chikungunya, dengue and Rift Valley fever following a fever outbreak in Eastern Madagascar. *Emerging Infectious Diseases*, DOI: 10.3201/eid1811.111036.

Stoebenau K, Hindin MJ, Nathanson CA, Rakotoarison PG & Razafintsalama V (2009) "… But then he became my sipa": the implications of relationship fluidity for condom use among women sex workers in Antananarivo, Madagascar. *American Journal of Public Health* **99**, 811–819.

WHO (2002) Standards for Maternal and Neonatal Care Steering Committee. *Standards for Maternal and Neonatal Care 1.3. Prevention of mother-to-child transmission of syphilis. Integrated management of pregnancy and childbirth (IMPAC)*. WHO, Geneva. http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/prevention_mtct_syphilis.pdf.

WHO (2010) Joint United Nations Programme on HIV/AIDS (UNAIDS), 2010. Global Report: UNAIDS Report on the Global AIDS Epidemic 2010. WHO, Geneva. http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2010/20101123_globalreport_en.pdf.

World Bank (2007) Madagascar – Public expenditure review: implementation of the Madagascar Action Plan – analysis for results (Vol. 1 of 5): Executive summary (English). World Bank, Washington DC. http://documents.worldbank.org/curated/en/2007/06/8146598/madagascar-public-expenditure-review-implementation-madagascar-action-plan-analysis-results-vol-1-5-executive-summary.

Xueref S, Holianjavony J, Daniel R, Kerouedan D, Fabry J & Vanhems P (2003) The abscence of HIV seropositivity contrasts with a high prevalence of markers of sexually transmitted infections among registered female sex workers in Toliary, Madagascar. *Tropical Medicine & International Health* **8**, 60–66.

Zeller HG, Ramamonjisoa A, Boisier P *et al.* (1997) HIV infection in Madagascar in 1995. *AIDS*, **11**, 401–402.

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