SEROEPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM*, HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN T-CELL LEUKEMIA VIRUS INFECTIONS IN MOTHERS AND THEIR INFANTS IN ZIMBABWE

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ABSTRACT

Matched blood samples from parturients and their neonates were screened for malaria parasites and analyzed for *P. falciparum* immunoglobulin G (IgG), IgM, IgE, human immunodeficiency virus-1 (HIV-1) and human T-cell leukemia viruses-I/II (HTLV-I/II) by enzyme-linked immunosorbent assay (ELISA) and Western immunoblot. Three paired sera were positive for *P. falciparum* parasites, and the ELISA positivity rates for *P. falciparum* IgG, IgM, and IgE antibodies for maternal sera were 50.2%, 91.2% and 22.7%, respectively whereas those for cord sera were 24.9%, 14.9% and 2.7%, respectively. Overall cord antibody positivity rates were independent of maternal antibodies. *P. falciparum* IgG antibody rates by ELISA were not influenced by parity for parities 1 to 6 or greater but were related to parity when parity was grouped into parity 1 and 2 or greater. IgG antibody production was not significantly related to maternal age.

P. falciparum IgG, IgM and IgE antibody positivity rates by immunoblot were 78.1%, 39.6% and 1.33% for maternal sera and 72.5%, 14.3% and 1.33% for cord sera, respectively. Matched maternal and cord sera with very strong to strong IgG immunoblot reactivities showed near homology for each

pair. The most prevalent and strong antigen complexes in the positive IgG immunoblots were 195- to 170-, 91- to 75- and 48-kDa. There was no homology for each pair in the immunoblots of *P. falciparum* IgM antibody positive sera. The antigen complexes that were most frequent and strongly recognized by IgM immunoblot positive sera were 91- to 75- and 21-kDa. *P. falciparum* IgM and IgG antibodies were co-positive in 33% of the maternal sera tested by immunoblot.

The HIV-1 seropositivity rates for maternal sera were 48.7% and 34.9% by ELISA and Western blot respectively. Women aged 31 to 35 years had the highest rates of HIV-1 infection. The positivity rates for HTLV-I/II in the parturients were 87% by ELISA and either negative (48.4%) or indeterminate (56.2%) by Western immunoblot for HTLV-I/II antigen, suggesting possible early infection with the viruses.

P. falciparum IgG antibody reactivity with parasite antigen was observed in 49.1% and 91.7% of matched pairs that were HIV-1 IgG positive and HIV-1 IgG negative respectively in immunoblot assays. Less bands of lower intensity were formed on immunoblots of the former than the latter. These differences in reactivities were highly significant ($z=3.97$, $p<0.00$) and suggestive of *P. falciparum* IgG antibody immunosuppression by HIV-1 infection.

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CHAPTER 1

INTRODUCTION

MALARIA

Malaria is the most common infectious disease in sub-Saharan Africa with about 90 million clinical cases (1, 2) and 1-2 million deaths reported annually especially in young children (3). The degree of malaria endemicity varies between countries and different localities within a country. Children less than 5 years of age are the most affected in regions of very high endemicity; in areas of low endemicity all age groups are affected (4).

Several studies in areas endemic for malaria have demonstrated that the pathology caused by the parasite is almost absent in adults who show very low levels of parasitemia. Acquired immunity in these adults which is incomplete, non-sterilizing and acquired by repeatedly exposure to infective mosquito bites has been termed "premunition" (5). This kind of protection is dependent mainly on IgG antibodies as demonstrated by the in vivo effect in humans of passively transferred African adult IgG (6-9).

Cerebral malaria is a common complication of *Plasmodium falciparum (P. falciparum)* infection in areas of low

transmission in Africa and has a peak prevalence in children of 3-4 years old (4). Severe anemia is the most frequent complication in highly endemic areas. Its onset in African children is in infancy and progresses until the age of three years (10, 11). It is one of the major causes of death in many parts of Africa especially in areas with high transmission rates (12).

MALARIA AND PREGNANCY

P. falciparum malaria infection is more prevalent (4-12 times) in pregnant women than in those who are not pregnant (13, 14). Pregnancy reduces immunity to malaria resulting in increased susceptibility to severe clinical illness (13, 15, 16). The mortality rate due to cerebral malaria in pregnancy is about 40% (17). The major maternal complications include severe hypoglycemia, anemia, and acute pulmonary edema (18, 19). Chronic malaria causes severe anemia in pregnancy often complicated by folate deficiency. Pregnant women with little or without prior exposure are particularly prone to severe falciparum malaria infection. Infection in the second or third trimester gives rise to high maternal mortality, increased rates of miscarriage, preterm delivery, intrauterine death and delivery of low birthweight babies

(19-21). Malaria anemia may affect birthweight by impairing oxygen transport to the fetus (22).

The placental intervillous spaces provide favorable conditions for the growth of *P. falciparum* (23-26). Sequestration of mature stages of the parasite within the placenta seems to be a common occurrence and is thought to interfere with materno-fetal transplacental exchange (27). It is also associated with the infiltration of macrophages, damage to the syncytiotrophoblast, pigmentation and thickening of the basal lamina of the trophoblast (28-30). More severe pathological alterations have been reported in primiparous women and mothers with fewer pregnancies than in multiparous woman with many pregnancies (21, 29, 31). Malaria infected placentas are lighter in weight than normal ones and their function is most likely impaired (29). In pregnancy the presence of parasites in the placenta and cord blood could result in low birthweight by retarding growth in utero or by inducing preterm labor (15, 21, 23, 24, 28, 32-35). Low birthweight correlates with increased neonatal mortality and morbidity (13, 14, 36-38). On rare occasions, especially in non-immune pregnant women, malaria parasites can cross the placental barrier and enter the fetal circulation giving rise to congenital malaria (39). Under normal circumstances the fetus is usually well protected

from malaria infection by maternal antibodies and in addition fetal haemoglobin is by and large resistant to the malaria parasite (40). Congenital malaria may account for the high incidence of abortion, miscarriage, stillbirth, death among neonates and reduction in birthweight (41).

Fever, a common recurrent malaria clinical feature, could increase catabolism, limit calorific intake and lead to decreased availability of energy to the foetus with consequent impairment of fetal growth (22). These complications seem unaffected by maternal parity in non-immune women but in semi-immune pregnant women living in endemic areas parity seems to influence susceptibility. Primiparous women are at a greater risk of complications than multiparous women (42, 43).

The increased risk to severe malaria in pregnancy is believed to be due to immune depression related either to high levels of cortisol and/or decreased cellular immunity particularly in the third trimester (44-46). Immunity to malaria in pregnancy increases with parity and in areas of high transmission the number of parasitized erythrocytes in the placenta is greater than in the peripheral blood (13). Placental infection is most frequent and heaviest in primigravidae and it is in this group that malaria reduces birthweight the most (21). Before pregnancy, women residing in endemic areas acquire a certain level of immunity to

malaria. It has also been thecrized that when primiparous women become pregnant, new uteroplacental vasculature is formed. The uteroplacental tissues have no prior exposure to malaria and are therefore immunologically naive and permit colonization by the parasite (13, 21, 47). Immunity to malaria develops within the uterus during the course of the infection. After delivery immunologic memory of the infection is retained within this organ and helps to protect future placentas. Repeated exposure to malaria in subsequent pregnancies increases this uteroplacental immunity which protects the woman from getting severe infections (46). In Zimbabwe a high proportion of women infected with malaria in the first and second trimesters of pregnancy aborted whilst 50% of those studied in the third trimester went into premature labor (48).

Several isotypes of specific antibodies are produced during *P. falciparum* infection and protection seems to be primarily associated with IgG, however it is also associated with IgM and may be most effective when both isotypes are present (49). IgM antibodies have been demonstrated in cord sera an indication that fetuses and neonates can mount humoral immune responses against malaria parasites and hence providing evidence for congenital malaria (50-53).

Neonates residing in malaria endemic areas are known to be resistant to *P. falciparum* malaria because of many factors. Some of these factors are low para-aminobenzoic acid level in maternal milk which is the main source of the infant's diet, the persistence of fetal hemoglobin in red blood cells and the presence of transferred maternal antimalarial antibodies (54-56).

The transfer of immunoglobulins (Ig) across the placenta is both active and highly selective, and the relative proportions of Ig isotype varies in cord and maternal serum (53, 57, 58). *P. falciparum*-specific IgG1 to IgG4 isotypes, IgM and IgE have been demonstrated in sera from malaria patients and people living in malarious endemic regions (59-61). IgG is the only isotype that crosses the placenta (50, 51, 58) to give a titer in cord serum that is almost the same as that in maternal serum. A study investigating the isotype distribution of protected and unprotected subjects (62) showed that isotypes IgG1 and IgG3 were found to predominate in protected subjects. However, in non-protected subjects such as in adults and children having their first episode of malaria, IgG2 and IgM predominated respectively. IgG from protected subjects was shown to cooperate efficiently with blood monocytes, whereas IgG from non-protected groups did not. The authors hypothesized that

non-protected subjects had antibodies to the epitopes critical for protection, but that these antibodies were non functional. In a study by Chizzolini and others (53), the isotypes IgG1 and IgG3 predominated in matched maternal and cord sera. IgG4 gave the lowest titer levels and in another study (60) this subclass was reported missing in some immune adults.

P. falciparum-specific antibodies such as IgG, IgM and IgE were associated with malaria-related histologic placental changes (53). Previous immunohistologic studies demonstrated the deposition of IgG and complement ($C1_q$, C3, C4 and C9) in trophoblasts of falciparum malaria-infected placentas (28, 30). In other studies, the deposition of IgE complexes in the placental fetal blood vessels was inversely related to the degree of parasitemia in the placenta (63). *P. falciparum* IgE antibodies were also found in Papua New Guinea populations exposed to malaria (61) as well as in mothers and their neonates (58). The authors suggested that the presence of this class of antibody in cord blood could have been a response to immune stimulation-allergic sensitization. However, the human fetus is capable of producing IgE by the 10th to the 11th week after conception (64). In another study by Perlmann and others (65), higher levels of IgE anti-malarial antibodies were demonstrated in

cases of cerebral than uncomplicated malaria. What causes the elevation of IgE in malaria and the importance of IgE in terms of protection and/or pathogenesis in *P. falciparum* malaria is unknown.

HUMAN IMMUNODEFICIENCY VIRUS

The World Health Organization (WHO) estimates that about 70% of the world's 4.5 million acquired immunodeficiency syndrome (AIDS) cases in adults as well as children are from Africa (66). Nine million adults in sub-Saharan Africa were believed to have been infected with HIV by the end of 1993 (67) and as of mid 1995, the estimate was about 18.5 million adults and more than 1.5 children worldwide (66). In the adult population, infection has gradually increased since 1984, reaching 20-30% in the worst affected urban centers (68). The rate of spread of infection in the urban population varies between regions, being rapid in Rwanda, Uganda, Malawi, Tanzania and Zambia but slow in Kenya, Mali and Zaire (68). This difference in the rates of infections is dependent on various factors such as time since the introduction of infection in a specified area, rates of sexual partner changes, patterns of sexual contact between age groups and between individuals in urban and rural populations as well as the frequency with which males

have sexual contact with female prostitutes (68). Most studies conducted in Africa have shown transmission probabilities per partnership of 20% from male to female and 11% from female to male (68). AIDS is the leading cause of adult mortality in many African countries such as the Ivory Coast, Zaire and Uganda and one of the main determinants of infant mortality, and this situation is likely to develop in many African countries as the HIV/AIDS epidemic unfolds (69-72).

Many studies have demonstrated peak-age specific prevalence and incidence in women aged 20-29 years and men aged 30-39 years (73). This difference in peak-age prevalence/incidence is thought to be due to sexual contact between younger women and older men. Youth in various African countries are becoming sexually active at a younger age than before, especially high school students who frequently practise commercial sex (74). The mean age at first sexual intercourse in the Ivory Coast is now 15 years. In Zimbabwe, almost 16% of male high school students reported having had sex with prostitutes (74).

Numerous serological surveys conducted in Africa between 1985 and 1990 have established that the main mode of spread of HIV on that continent was heterosexual and hence infection rates are high in persons with, or at risk for, other sexually transmitted diseases. Some of the risk

factors responsible for heterosexual transmission in Africa include increased number of sexual partners (75-78), both ulcerative and nonulcerative sexually transmitted diseases (73, 76, 79-82), lack of circumcision in men (81, 83) and a history of prostitution or contact with a prostitute (76, 77, 79, 81, 84).

Infection rates in women commercial sex workers are estimated at 90% in large African cities such as Nairobi (76) and Abijan (70). Most commercial sex workers have no other form of employment, have very little education and are motivated by the desire for improvement in their economic status (85). Many monogamous women (married or not married) in Africa are unable to reduce their risk of contracting HIV infection because they feel that they do not have a say in condom use or other preventive measures during sexual intercourse. Many of them are thus at risk of HIV infection through their partners who, even in marriage, continue to have extra marital sex (86). In Kigali, Rwanda, 25% of women with one life sex partner were infected with HIV (87).

Sexually transmitted diseases enhance transmission and infection with HIV and on the other hand the manifestations of some sexually transmitted diseases are influenced by HIV infection. Studies in Kinshasa showed that the incidence of genital ulcer infection, including that due to chancroid,

was higher in HIV positive than in HIV negative prostitutes (82). Other behavioral factors that have been identified as risk factors for HIV infection in women are receptive anal intercourse (88) and oral contraceptives (89-93). It was hypothesized that oral contraceptives may induce cervical ectopy thus rendering women more susceptible to HIV infection through the cervical columnar epithelium (90).

Tuberculosis remains the most common cause of preventable adult death in developing countries. It is a common manifestation of AIDS in Africa (94-97) and this is due to the widespread prevalence of *Mycobacterium tuberculosis* infection before the HIV epidemic. Ten percent of the admissions at Mulago hospital in Kampala, Uganda in 1984 were tuberculosis diagnosed cases (98). In Abijan, Ivory Coast, tuberculosis was the cause of death in 32% of 247 HIV positive persons (99) and in 50% of those patients with "slim disease" (the HIV wasting syndrome), the etiology was that of tuberculosis.

In adults, infection with *Mycobacterium tuberculosis* usually precedes HIV infection and in the HIV-infected adult, tuberculosis usually results from the reactivation of latent tuberculosis infection (100). HIV infection in children is ordinarily acquired perinatally (101) and consequently precedes tuberculosis infection. In HIV-

infected children tuberculosis is mainly primary rather than reactivation of disease. In the Ivory Coast and Zambia (102), the prevalence of HIV infection in children diagnosed with tuberculosis was 11.8% and 37% respectively. The highest rates of infection were found in children 1-4 years of age, the group in which clinically diagnosed tuberculosis was most common (103).

AIDS predisposes to several parasitic diseases such as *Pneumocystis carinii* pneumonia and *Toxoplasma* encephalitis. The intracellular coccidia, *Cryptosporidium parvum*, and *Isospora belli* have been found as opportunists (104-107) in many African AIDS patients suffering from chronic diarrhoea. These coccidia are believed to be involved in the pathogenesis of wasting ("slim") disease in AIDS patients (107). Infections with other common gastroenteritis organisms such as *Salmonella*, *Giardia* and amoeba are also common in AIDS patients in Uganda (108).

HUMAN IMMUNODEFICIENCY VIRUS AND PREGNANCY

Perinatal transmission is the major route of acquiring HIV infection in children. WHO estimated that at the end of 1992, about 4 million women of child-bearing age in Africa had been infected with HIV (41). Rates of perinatal transmission of 25-48% have been reported from African

countries (69, 109-111). In the worst afflicted parts of Africa, especially the urban areas HIV infection rates in pregnant women 15-40 years of age are as high as 20%-30% or more (112). In Abijan 11% of women attending maternal and child health care centers were HIV positive (113). The rate of vertical transmission from mother to the newborn is about 30% (114) and the life expectancy of an HIV infected infant is 1-2 years (114) whereas the average duration from infection to death in adults is about 5-8 years for Africa. An examination of 173 children in Kampala showed that about 25% of both healthy children and children with acute diarrhea had *Cryptosporidia* in their stools (115) as a result of environmental exposure to this parasite. Infections with other gastrointestinal organisms such as *Salmonella, Shigella, Giardia* and amoeba are also common in Ugandan patients with AIDS (108).

AIDS is the leading cause of death in young children in Africa (41) as well as other parts of the world (116) and about 80-95% of these AIDS cases are the consequence of HIV transmission from mother to child (69, 117). The three routes involved in the transmission of HIV from mother to child are intrauterine (118, 119), intrapartum (120, 121) and postpartum through breastfeeding (122, 123).

The mechanisms of intrauterine transmission are poorly understood and it is not clear why only a certain proportion of fetuses is infected (119, 124-126). There is in vivo (127) and in vitro (128, 129) evidence that HIV-1 can cross the syncytiotrophoblast and infect the cytotrophoblast and placental macrophages. HIV-1 infected placental macrophages may protect the fetus, or the other hand, they may act as a reservoir for the virus and transmit it to the fetal circulation (129, 130). HIV antigens have been found in amniotic fluid, and in fetal and placental tissue as early as the first trimester of pregnancy suggesting that there is a possibility of the fetus being exposed to HIV infection during early pregnancy (128, 131).

Maternal characteristics associated with an increased risk for HIV transmission to the fetus include late stage of the disease during pregnancy (132), low $CD4^+$ T-cell count (121), severe chorioamnitis (69), low anti-gp120 antibodies in the mother's serum (133), absence of antibodies against specific domains of gp120 (134) and gp41 (135). Other risk factors that have also been implicated in HIV-1 vertical transmission are maternal response to HIV, antigenemia, recent infection, high levels of circulating HIV, and maternal disease progression (69, 133, 134, 136-140). Vertical transmission of HIV is also associated with a

history of sexually transmitted diseases (141) such as syphilis, gonorrhea, chancroid, chlamydia, herpes and genital warts.

The antibody response in humans infected with HIV-1 has been extensively studied in an effort to elucidate the role of the humoral immunity in HIV-1 infection and in disease progression. The methods that have been and are mostly used are those that detect HIV IgG antibody which is the predominant isotype in serum. Many studies have reported the distribution of IgG subclasses of antibodies to the various HIV-1 proteins and peptides in relationship to different stages of disease (139, 142-145). Using a flow cytometry-based assay for the detection of antibodies in human serum or plasma, it was found that IgG1 antibodies were the most frequently reactive isotype to each of the HIV-1 virus proteins (146). Intermediate frequencies of reactivities were observed with IgA1, IgG2, IgG3, and IgM classes and subclasses. IgA2, IgE and IgG4 antibodies were the least frequently detected. About 50% of the sera tested had IgD antibody reactivity with HIV-1 proteins. The most frequent antibody-antigen complex found in the transmitting mothers was IgG2-gp160. The complexes IgG1-IgG2, IgD-IgG2 antibodies to gp160 and IgA1-IgE antibodies to p66 were observed more frequently in transmitters in comparison to non-transmitters. The authors of this study suggested that the

increased activity of class and subclass antibodies in transmitting mothers was possibly due to antibody-mediated enhancement which may occur during HIV-1 transmission from mother to child.

Passive in utero transfer of maternal anti-HIV antibody to the fetus prevents the accurate serological diagnosis of HIV-1 in neonates and infants below the age of 15 months. A study on the ontogeny of anti-HIV antibody production in HIV--1 infected infants (147) showed that 60% of these infants produced small amounts of HIV-1-specific IgG. By 6 months of life more than 85% of the infected children produced antibody amounts that were almost equal to those of adults. During the first 2 months of life acquisition of HIV-1 antigen-specific IgG synthesis appeared to be sequential as well as selective, with antibody production to only one or two HIV-1-specific structural antigens. In general, antibodies to envelope proteins, in particular to gp160 which was produced very early and increased steadily during infancy, predominated at all age groups. On the other hand, antibodies to gp120 and gp41 appeared later but increased more rapidly. The investigators theorized that this ability to produce HIV-1-specific IgG at birth confirms that fetal B cells which are usually "naive" are capable of responding to certain antigens or antigen like moieties.

Children suffering from malaria anemia and women with obstetric complications in Africa receive blood transfusions more than in the industrialized countries (148). The causes of anemia are multifactorial and include *P. falciparum* infection, iron and folate deficiency, hookworm, and hemoglobinopathies (149-151). A study conducted in Kinshasa, (152) showed that 28% of all units of blood transfused in one month in 1990 had not been screened for HIV. Amongst all institutions transfusing blood, 42% were incapable of screening and even if blood were screened, laboratory errors as well as blood donors in the "window period" may have contributed to a high risk of infection in transfusion recipients.

HUMAN T-CELL LYMPHOTROPIC VIRUSES

Human T-cell lymphotropic virus-I (HTLV-I) has been shown to be the causative agent of adult T-cell leukemia (ATL), a mature T cell malignancy (153) and a chronic myelopathy known both as tropical spastic paraparesis (TSP) and HTLV-I-associated myelopathy (HAM) (154, 155). HTLV-II, in contrast, is rare and is associated with atypical T-cell variants of hairy cell leukemia (HCL) (156).

High HTLV seropositivity rates have been reported from many parts of the sub-Saharan Africa (157-159). HTLV-I

seroprevalence rates of 9.1% were reported from rural Gabon (160) and 14% from Equateur region, Zaire (159, 161). Cases of TSP/HAM were reported in an area of high HTLV-I seroprevalence in northern Zaire (162). In other regions of sub-Saharan Africa, only sporadic cases of TSP/HAM (155, 163, 164) and of HTLV-I associated ATL (165-167) have been reported. In the southern African region, various HTLV-I seroprevalences have been reported, with 0% and 5.2% in the north and east of the Republic of South Africa respectively (168) and 9% in Kavango territory in Namibia (169). Studies conducted in Pretoria, in the Republic of South Africa have shown prevalence rates of 0.2% in pregnant women (170).

MALARIA AND HUMAN IMMUNODEFICIENCY VIRUS

HIV infection is prevalent in many areas of sub-Saharan Africa that are also endemic for malaria, in particular *P falciparum* infection (68), and both are responsible for significant morbidity and mortality in many countries. Both malaria and HIV infections cause functional abnormalities of cell mediated immunity with a lowering of the CD4/CD8 lymphocyte ratio (171, 172). There has been some concern that this impaired immunity could increase the risk or severity of infection with *P. falciparum* in HIV-infected individuals with depleted CD4 lymphocytes with a consequent

decreased response to standard anti-malaria treatment (173). On the other hand repeated malaria episodes could enhance progression of HIV-related illness (172). Cross-sectional studies in children (174, 175), adults (176-179) and mother and child (180) in Africa have demonstrated that there is no association between HIV and malaria. Malaria may cause severe anemia requiring transfusion and this may result in transmission of HIV infection if blood units are not screened for HIV antibodies (175).

MALARIA, HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN T-CELL LYMPHOTROPIC VIRUS IN ZIMBABWE

Zimbabwe is situated in south central Africa, just above the Tropic of Capricorn with the 20° latitude south almost dividing the country into two halves (APPENDIX A). The population of the country in 1992 was estimated at 10.4 million people, 5.3 million females and 5.1 million males (181). The distribution of the population by province is shown in APPENDIX B (181). The national average population growth rate between 1982 and 1992 was 3.13%.

The district of Chiredzi (APPENDIX C) (181), the largest in Masvingo province, is located in the southeast of Zimbabwe and shares borders with Mozambique in the southeast and the Republic of South Africa in the south. Its

population in 1992 was 183,228 comprising 95,422 females and 87,806 males (181). The district is further divided into 33 wards that are listed in APPENDIX D (181). Some demographic indicators, based on the 1992 census, for the district of Chiredzi compared with those of the Province of Masvingo are shown in APPENDICES E and F (181).

MALARIA

Malaria is a notifiable disease and it is a serious public health problem in Zimbabwe with high morbidity and mortality rates. It is estimated that 5-6 million people in Zimbabwe are at risk of contracting malaria each malaria season (182). Malaria is amongst the top ten causes of admission in many health care centers in Zimbabwe and it accounts for up to 10% of the total hospital bed occupancy. In 1988 and 1989 it was the main cause of over 50% of parasitic infections and infectious diseases requiring hospital admission (APPENDIX G) (182). Malaria contributes to 1-4.5% of all registered deaths in Zimbabwe (APPENDIX H) (182).

Malaria endemicity in Zimbabwe varies from meso- to hyperendemic in the northern and southern lowveld to hypoendemic in the central high altitude region. For the purposes of malaria epidemiology, Zimbabwe may be divided

into seven well defined zones as shown in APPENDIX I (183). The climate may be divided into three main seasons, the cool dry season, (April to August), hot Dry season (August to October) and hot wet season (November to March). Zimbabwe is on the southern boundary of the distribution of malaria in Africa and in common with Swaziland (184) and South Africa (185) in the south (APPENDIX A). The major factors influencing malaria epidemiology are altitude and the related temperature changes. The prevalence of malaria is directly related to the changes in altitude, with the highest prevalence of 30.5% in the Zambezi Valley (<600 meters) in the north decreasing to 1.2% on the central plateau and rising to 7.8% in the southeastern lowveld which encompasses Chiredzi district (APPENDICES I and J) (183). Malaria is markedly seasonal at all altitude levels, most transmission taking place between the months of February and May annually. Little transmission occurs during the winter months between July to August and the hot dry season August to October.

The intensity of malaria transmission is also influenced by season, temperature and altitude (186, 187) which all affect the survival and migration of *Anopheles gambiae* (s.l.). *An. gambiae* population peaks at the end of the rainy season in March (186, 187) with a consequent peak

in malaria occurring about a month later at the end of March/beginning of April (APPENDIX K) (183). APPENDIX L shows malaria blood slides submitted and examined in Chiredzi district by month in 1993 (188). The peak of slide submission and malaria positivity rate (26.2%) occurred in March, at the same time as in the rest of the country.

HUMAN IMMUNODEFICIENCY VIRUS

The epidemic of heterosexually transmitted human immunodeficiency virus type-1 (HIV-1) is currently a major health problem in Zimbabwe. At the end of March 1991, the official count for full blown acquired immunodeficiency syndrome (AIDS) cases in the country was 7,718 with an estimate of at least 500,000 people infected with HIV (189). The cumulative AIDS cases by province for Zimbabwe for March 1987 to March 1993 are shown in APPENDIX M (190). Harare province, the most densely populated, reported more cases of AIDS (4,791) than the other provinces. Analysis by age group and sex of cumulative AIDS cases (APPENDIX N) (190) reported during that same period showed that the age groups 20-29 years and 30-39 years were the most affected with more females in the former group and more males in the latter group. The 0-4 year age group had the third highest AIDS positivity rates and infection in this group was acquired by

peri- and postnatal transmission. Overall, more males between the age of 30-60+ years had AIDS compared with females in the same age group whereas more young women in the age group 15-29 were infected in comparison to males of the same age. The reason for this difference in positivity rates is that younger women have sex with older men who can provide them with money, clothes and food.

The pattern of AIDS related cases (ARC) by age group and sex for March 1991 to March 1993 (APPENDIX O) (190) follows the same pattern as that of cumulative AIDS cases by age group and sex. The number of sexually transmitted diseases (STD) reported quarterly for 1992 decreased throughout the country (APPENDIX P) (190). Factors contributing to this decrease are unknown. The number of tuberculosis cases for the same year by quarter also increased on average (APPENDIX Q) (190).

In conclusion the number of HIV and AIDS cases continue to rise in Zimbabwe. More ARC and TB infections are being reported in most provinces of Zimbabwe as the HIV epidemic evolves.

In a prospective study carried out in Bulawayo, Zimbabwe, of children less than 13 years of age, who died either at home or on their way to the hospital, it was found that 57(86%) of the 66 children who were dead on arrival and 94(77%) of the 122 children who died after admission were 2

years old or less (191). The main cause of death in these two groups was AIDS. Pneumonia associated with HIV was the second main cause of death in both groups. The HIV positivity rate of the children who were dead on arrival was 31(47%) and those who died after admission was 50(41%). Nine children who were brought to hospital and with previously undiagnosed AIDS were infected with either TB or other pathogenic bacteria.

A seroprevalence study of HIV infection amongst antenatal women in greater Harare in Zimbabwe conducted in 1990 (192) showed that 18% of 1008 blood samples tested for HIV antibodies were positive. The rate of infection in women below 20 years old and those between 20-29 years was higher than those between 30 years and above (19% and 20.4% in comparison to 8.8%). HIV infection was high amongst mothers who were not registered for antenatal health care, women who were either single, divorced or cohabiting, and those with a shorter duration of marriage. Women with more than three children had a lower infection rate of 7.5% compared to 17% for parity 0, and 20.5% for parity 1 to 3. Education, income and type of occupation also influenced their HIV status with those earning above 600 Zimbabwe dollars per month and more than 11 years of education comprising the lower risk group. Those who were classified as unskilled workers had the highest rates of infection (28.6%).

Another study carried out in Mutoko among pregnant women and patients with STDs showed that 25% of the former group were HIV positive (193). The highest positivity rates were found in women between 25-30 years. Those who were pregnant for the second or third time had positivity rates of more than 35%. Fifty percent of those with STDs were HIV positive with men having higher rates than women. HIV positivity rates were also higher in single patients than in married patients. HIV positivity rates for those with genital ulcers was 67%.

Part of a survey of the causes of perinatal mortality at Mpilo maternity hospital tested for the presence of HIV-1 antibodies in 220 neonatal deaths and mothers of 221 stillbirths (194). The HIV positivity rates in neonatal deaths and stillbirths were 23.6% and 15.4%. respectively. Perinatal deaths arising from congenital malformations, birth asphyxia, pregnancy induced hypertension, placental abruption and other non infectious causes had positive rates averaging 8.1%. Deaths from septicaemia had a higher rate of 39.3% and the highest rate of 72.2% was found in deaths from congenital infection other than syphilis, an indication that maternal HIV infection contributes to perinatal mortality by predisposing to neonatal septicemia caused by Gram positive cocci (staphylococci and streptococci) and Gram negative enterobacteria and congenital infection with rubella,

cytomegalovirus and toxoplasmosis (194). Maternal HIV infection was estimated to increase the stillbirth rate by 1.6 times and neonatal mortality rate by 2.7 times.

HUMAN T-CELL LYMPHOTROPIC VIRUSES-I and II

The prevalence of HTLV-I in Zimbabwe among blood donors is 0.3% (195). This study is the only one that has been conducted on the prevalence of HTLV in Zimbabwe and the investigators did not test for HTLV-II.

RATIONALE AND OBJECTIVES OF THE CURRENT STUDY

Malaria coupled with HIV-1 infections are currently a major public health concern to health authorities at all levels in Zimbabwe. Malaria causes high rates of mortality and morbidity, especially in pregnant women and children High rates of HIV-1 infection in pregnant women (25%) and children have also been reported from Zimbabwe. In the low lying parts of Zimbabwe such as Chiredzi district, malaria transmission occurs seasonally and may be co-endemic with the retroviral infections HIV-1, HIV-2, HTLV-I and HTLV-II. Studies conducted to date in Zimbabwe have not addressed the seroepidemiology of malaria in any population group; neither have they determined the co-endemicity of these five diseases.

i. MAIN OBJECTIVE

The main objective of this study was to determine the antibody prevalence rates of *P. falciparum*, HIV-1 and HTLV-I/II in mothers and their newborn babies in the district of Chiredzi Zimbabwe.

ii. SPECIFIC OBJECTIVES

The specific objectives were:-

a. To determine the prevalence of malaria in mothers and their newborn babies in Zimbabwe by blood smear microscopy.

b. To characterize, by enzyme immunosorbent assay (ELISA) and immunoblot, the *Plasmodium falciparum* IgG, IgM, and IgE antibodies in matched maternal and cord sera from Zimbabwe.

c. To determine the seroprevalence of the retroviruses HIV-1, HTLV-I and HTLV-II in maternal and cord sera from Zimbabwe using ELISA and immunoblot techniques.

d. To determine if malaria and HIV-1 co-exist in mothers and their neonates and if so the type of relationship between these two infections.

CHAPTER 2

MATERIALS AND METHODS

STUDY AREA

The study proposal was approved by the Zimbabwe Ministry of Health and Child Welfare and by the Medical Research Council of Zimbabwe. The study was conducted from April 1993 to March 1994 in Chiredzi district (one of the eight districts in Masvingo Province) which is located in the southeast of Zimbabwe at an elevation of 600 meters above sea level (APPENDICES C and I) (181, 183). Some of the main demographic indicators for Chiredzi district compared with those of Masvingo province are shown in APPENDIX E (181). These demographic indicators are subject to changes from year to year depending on the amount of rain that fall in Chiredzi district. The major economic factor which determines population densities in Chiredzi district is sugar cane farming which in turn is dependent on irrigation and hence good rains.

The employees of the major sugar cane estates in the district, Hippo Valley, Triangle and Mkwasine (APPENDIX D) are mainly transmigrant workers drawn from the adjacent districts (APPENDIX C). Another factor which had an influence on the population statistics of Chiredzi district

was the civil unrest in Mozambique, which resulted in the establishment of Chambuta refugee camp with a population of 19005 (APPENDIX D), within the district, thus temporarily inflating the population of the district. The refugees were repatriated as soon as civil order was restored in Mozambique.

Malaria transmission in this district is meso- to hyperendemic, markedly seasonal, a pattern which is seen throughout Zimbabwe. The peak of slide submission and incidence rate (26.2%) for 1993 occurred in March (APPENDIX L) (188). Over 90% of all the malaria cases presenting at the health centers in the district are caused by *P. falciparum* (183). Chiredzi district was chosen as a study area because of its high malaria prevalence rates in all age groups (for example mean prevalence rate of 7.5% for the 1993 malaria season) in comparison to adjacent districts such as Bikita, Zaka and Mwenezi which had mean prevalence rates of 1.1%, 0.2% and 0.7% respectively (188). Chiredzi district also has high infection rates of AIDS related illnesses such as sexually transmitted diseases, pneumonia, diarrhea and tuberculosis (personal communication with medical personnel at various health care centers in Chiredzi district).

Four health care centers, Chiredzi district hospital, Chilonga clinic, Chipiwa clinic and Rupangwana clinic were selected for the study because of their accessibility either by road or telephone/radio from De Beers Research laboratory where serum and placental tissues were processed. Chiredzi district hospital, located in Chiredzi town, is a referral hospital that has 220 beds with an average admission of about 600-650 patients per month. It has the capability to perform most uncomplicated surgical procedures. The other three health care centers are small with a bed capacity of at least six beds and they refer all complicated cases to Chiredzi district hospital.

De Beers Research Laboratory (part of Blair Research Institute) where the samples were processed is part of the Ministry for Health and Child Welfare, Zimbabwe, and is about two kilometers from Chiredzi district hospital.

SAMPLE COLLECTION

Samples were collected between April 1993 to March 1994. At the beginning of the study, the Chiredzi district medical officer, medical officers and health care personnel in charge of maternity wards at the four selected health care centers were informed in great detail about all aspects of the study which was anonymous to the parturient women.

Placentas with the cord still attached and clamped tightly at the end proximal to the baby were collected from 277 parturient women within 6 hours of delivery. Each placenta and attached cord were placed in a coded plastic bag and stored in a cooler box until they were collected for processing at De Beers Research Laboratory. The same code that was on each plastic bag was also written on the parturient's case notes. The information in the case notes was used to complete a questionnaire on the parturients and their neonates.

i. PARASITOLOGY

Matched placental crush, thick and thin placental and cord blood smears were made and air dried. The thin blood smears were fixed with methanol before staining. All the smears were stained with 3% buffered Giemsa stain and examined under the light microscope for malaria parasites. A smear was considered negative when examination of 200 high power (1000x) magnification fields failed to show parasites.

ii. SERUM

Maternal blood from the placenta and placental tissues were collected prior to cord blood collection. The cord was then carefully severed proximal to the placenta, washed with

copious amounts of tap water to remove maternal blood on the outside and dabbed dry with a paper towel. The cord blood was then expressed into a tube. Matched maternal and cord blood samples that were collected were centrifuged at 15 minutes at 2500 rpm. The serum from each sample was aliquoted into 0.2 ml aliquots and stored at -30°C until shipped to Honolulu, Hawaii for serologic analysis. The number of paired sera tested for each immunoglobulin varied and was dependent on the quantity of sera available when each assay was done.

MALARIA SEROLOGY

i. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

a. Coating Microtiter Plate Wells With Antigen (196) - the *P. falciparum* antigen used in this assay was kindly provided by the Parasitology Laboratory (PL) in the Department of Tropical Medicine and Medical Microbiology, John A Burns School of Medicine, University of Hawaii at Manoa. The antigen was prepared from Uganda Palo Alto strain (FUP) (197) that has been maintained in continuous culture in PL since 1977. The antigen was diluted 1:500 in pH 9.6 coating buffer (1.59 g Sodium carbonate, 2.93 Sodium bicarbonate, and 0.2 Sodium azide in distilled water at pH 7.2). Using a repeating pipette, 100 µl of the diluted

antigen (1:500) were placed into each well on the microtiter plates (Falcon, Becton, Dickinson and Co.), the plates were covered with a lid, incubated at 37°C for one hour and then overnight at 4°C to allow antigen to adsorb onto the plates. The plates were washed three times with phosphate buffered saline with Tween (PBS-T).

b. Blocking Microtiter Plate Wells - 100 µl of bovine serum albumin diluted 1:100 in PBS-T were placed in each well and the plate incubated at 37°C for one hour. After the incubation period, the plate was washed three times with PBS-T.

c. Adding Serum - matched maternal and cord sera collected at the selected health care centers in Chiredzi were thawed and diluted 1:100 in PBS-T for IgG/M antibody assays. The paired sera were tested undiluted for IgE antibody assay. The number of matched maternal and cord sera tested for IgG, IgM and IgE were 233, 228 and 75 respectively. One hundred microliters of each serum sample diluted appropriately were pipetted in duplicate into the antigen coated wells. The controls used were Papua New Guinea *P. falciparum* IgG/IgM positive maternal and cord sera, Papua New Guinea paired sera that were positive for *P. falciparum* IgE and normal Hawaiian sera that were negative for malaria (taken from subjects that had never been exposed

to malaria). The plate was then incubated at 37°C for one hour and washed three times with PBS-T after incubation.

d. Adding Conjugate - 100 µl anti-human immunoglobulin conjugated to alkaline phosphatase (1:1000 dilution) was pipetted into each well and the plate incubated at 37°C for one hour then washed three times with PBS-T. The following anti-human immunoglobulin conjugates were used to test for malaria antibodies in the matched sera:

-Mouse monoclonal anti-human IgG, Fc (gamma heavy chain specific) alkaline phosphatase (AP)-labeled (clone JDC-2, Southern Biotechnology Associates, Inc.).

-Mouse monoclonal anti-human IgM (µ heavy chain specific) alkaline phosphatase (AP)-labeled (clone SA-DA4, Southern Biotechnology Associates, Inc.).

-Monoclonal anti-human IgE alkaline phosphatase (AP) conjugate developed in mouse (mouse IgG2b isotype clone GE-1, Sigma Immuno Chemicals).

e. Adding Substrate - the substrate, *p*-nitrophenyl phosphate (pNPP) was diluted 1:1000 in coating buffer pH 9.6 and 50 µl added to each well. Color development in the wells was stopped with 3M sodium hydroxide after 30 minutes and the absorbance of the wells at 405 nm determined immediately in a Dynatech MR650 ELISA reader. The dilutions used in this assay were those giving optimum sensitivity

from checkerboard titrations of known normal control sera as well as positive Papua New Guinea sera. The cut off point for positivity in these assays was an optical density reading greater than 2 standard deviations above the mean of the negative controls.

ii. IMMUNOBLOT ANALYSES

The method of Desowitz *et al.* (58) using whole parasite (FUP strain) saponin-extracted antigen in Laemmli's reduced buffer was followed for immunoblot analyses.

a. Immunoblot Strip Preparation - *P. falciparum* antigen was heated to 90°C for 90 seconds and sonicated. It was then mixed with an equal volume of Laemmli's reduced sample buffer and electrophoresed in 10% polyacrylamide gels without sodium dodecyl sulfate at 150 volts for one hour using the mini-Protean II (BioRad) electrophoretic apparatus. Rainbow colored protein molecular weight markers (Amersham LIFE SCIENCE) of weight range 14.3-220 kDa were used as standards and these contained myosin (220 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.3 kDa).

The polyacrylamide gel was equilibrated in transfer buffer (25 mM Tris, 192 mM Glycine, 20% Methanol) of pH 8.3

for 10 minutes and nitrocellulose pressed onto the gel using a BioRad Mini Transfer apparatus. Transfer of proteins from the polyacrylamide gel to nitrocellulose was carried out at a constant voltage of 100 volts for one and half hours with a BioRad 250/2.5 power supply. After transfer the nitrocellulose was washed with PBS-T and blocked with blotto (5% powdered milk in PBS-T) overnight. The nitrocellulose was washed and cut into 3mm wide strips which were stored frozen.

b. Sera Immunoblot - 91 randomly picked matched maternal and cord sera that included 37 pairs that were IgG ELISA negative were further tested for *P. falciparum* IgG and IgM antibody by immunoblot. Seventy five randomly picked pairs that included the only IgE positive pair were also tested for *P. falciparum* IgE antibody. The nitrocellulose strips were each put in a well on the blotting tray and blotto added to each well before adding 1 ml of matched maternal and cord sera (each diluted 1:100 for Immunoglobulins G/M and 1:5 for immunoglobulin E) to the wells. The contents were incubated at room temperature for one hour on a rotator platform. Each strip was washed three times with 1 ml PBS-T for five minutes at each wash. Anti-human immunoglobulin conjugated to alkaline phosphatase was diluted 1:1000, 1 ml added to each well and incubated as

before for one hour. The conjugates used in these analyses were the same as those used for ELISA. After washing in the same buffer as before, the strips were developed with the enzyme substrate nitroblue tetrazolium (NBT) in aqueous dimethylformamide (DMF)/5-bromo-4-chloro-3-indolyl phosphate in DMF (BCIP) (BioRad).

HUMAN IMMUNODEFICIENCY VIRUS-1 SEROLOGY

Two hundred and seventy seven paired maternal and cord sera were tested for HIV-1 IgG antibody using a commercial ELISA kit (Organon Teknika, Durham, North Carolina). Sixty three pairs which included repeatedly positive, non-reactive, positive borderline and negative borderline sera by ELISA were confirmed by Western blot (Organon Teknika, Durham, North Carolina). The ELISA and immunoblot tests were interpreted in accordance with the manufacturer's recommendations sent with the kits.

HUMAN T-CELL LEUKEMIA VIRUS SEROLOGY

Commercial ELISA (rp21e enhanced) (Cambridge Biotech Corporation, Worcester, Massachusetts) kits were used to test for IgG antibodies to HTLV-I in 169 matched maternal and cord sera. HTLV-I/II Western blot kits (Cambridge Biotech Corporation, Worcester, Massachusetts) were used to

test further a subsample of 48 pairs which included repeatedly reactive, negative and indeterminate sera by ELISA with absorbance readings of 0 to greater than 1. The Western blot kit includes only one negative control for both HTLV-I and II. The interpretation of the ELISA and immunoblot results were as recommended by the kit manufacturer.

DATA ANALYSES

PARADOX 4 data management system (Sybex Inc., Alameida, CA) was used to store the data obtained in this study. EPI INFO 6, Version 6 (CDC, Atlanta, GA and WHO, Geneva Switzerland) was used to analyze the data and also to generate TABLES 1 to 14 and HARVARD GRAPHICS, Version 3 (Software Publishing Corporation and Bitstream Inc.) created FIGURES 1, 2 and 4. The statistic tests used were the Kruskal-Wallis (equivalent to Chi square) to compare differences in mean values for two groups, Kruskal-Wallis One Way of Analysis of Variance to compare mean values for many groups, the z-test for population proportions and the Pearson's correlation test to assess the relationship between P. falciparum IgG antibody and HIV-1 infection. These statistical tests are shown at the end of each table where applicable.

CHAPTER 3

RESULTS

SELECTED STUDY POPULATION STATISTICS

The age range of the 277 parturients studied was 15 to 45 years, with a mean of 25.2 years and a mode of 20 years (TABLE 1). Their parity varied from 1 to 10, parity 1 (34.6%) being the most frequent (TABLE 2). The age distribution of the women who delivered during the study period is shown in FIGURE 1. There were more parturients (30%) in the 15 to 20 year age group than in any other group. Of the 82 parturients that were between 15 and 20 years old, 84% (69/82) were of parity 1 (FIGURE 2). There were more women of parity 2, 25/75 (33%) in the 21 to 25 years age group than in other age groups. Women of parity 6 or greater comprised 70% (12/17) of the 36 to 40 year old age group and as expected for Africa, parity increased with advancing age.

A total of 286 babies were born among which were 9 sets of twins. Of the 262 babies whose sex and weight were recorded on the maternity unit admission form, 137 (52.3%) were males and the remainder females (TABLE 3). The birthweight varied from 600 to 4720 grams for singletons and 1040 to 2960 grams for twins. The mean singleton birthweight

TABLE 1. Age frequency distribution of 277 parturients from Chiredzi District, Zimbabwe

Age	Freq	Percent	Cum
15	2	0.7%	0.7%
16	8	2.9%	3.6%
17	9	3.2%	6.9%
18	19	6.9%	13.7%
19	22	7.9%	21.7%
20	23	8.3%	30.0%
21	17	6.1%	36.1%
22	12	4.3%	40.4%
23	22	7.9%	48.4%
24	13	4.7%	53.1%
25	11	4.0%	57.0%
26	10	3.6%	60.6%
27	16	5.8%	66.4%
28	16	5.8%	72.2%
29	5	1.8%	74.0%
30	15	5.4%	79.4%
31	8	2.9%	82.3%
32	4	1.4%	83.8%
33	8	2.9%	86.6%
34	5	1.8%	88.4%
35	8	2.9%	91.3%
36	6	2.2%	93.5%
37	5	1.8%	95.3%
38	5	1.8%	97.1%
39	1	0.4%	97.5%
40	4	1.4%	98.9%
41	1	0.4%	99.3%
42	1	0.4%	99.6%
45	1	0.4%	100.0%
Total	277	100.0%	

Total	Mean	Std Dev	Mode
277	25.2	6.425	20

TABLE 2. Parity frequency distribution of 269 parturients from Chiredzi District, Zimbabwe

Parity	Freq	Percent	Cum
1	93	34.6%	34.6%
2	48	17.8%	52.4%
3	35	13.0%	65.4%
4	31	11.5%	77.0%
5	28	10.4%	87.4%
6	22	8.2%	95.5%
7	7	2.6%	98.1%
8	3	1.1%	99.3%
9	1	0.4%	99.6%
10	1	0.4%	100.0%
Total	269	100.0%	

Total	Sum	Mean	Std Dev
269	782	2.907	1.961

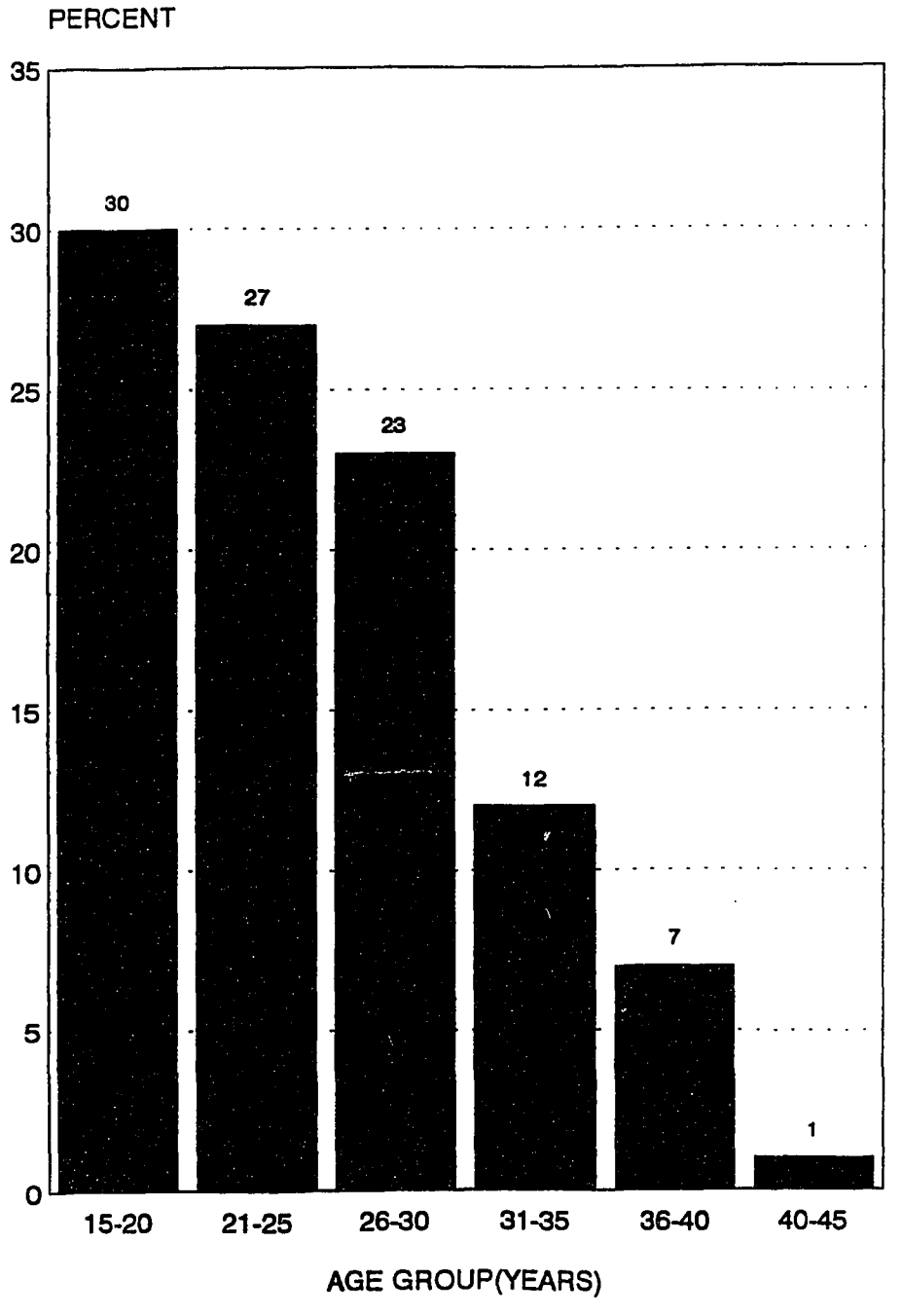


FIGURE 1. Age group frequency distribution of 277 parturients from Chiredzi District, Zimbabwe. The proportion (%) of parturients in each age group is shown above the bars.

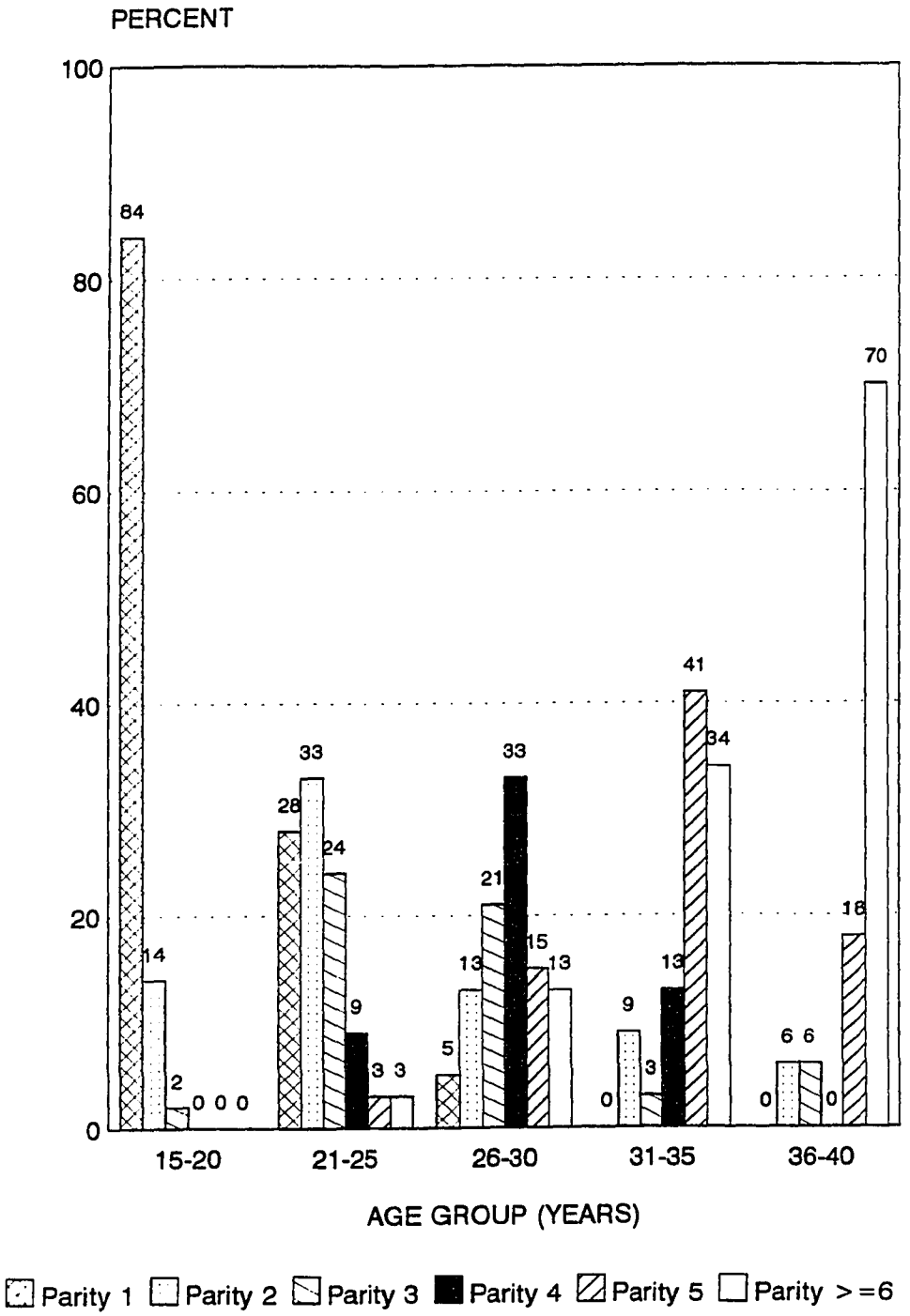


FIGURE 2. Parity distribution by age group of 267 parturients from Chiredzi District, Zimbabwe. The numbers shown above each bar are percentages of each parity in the various age groups.

TABLE 3. Comparison of birthweight and placental weight by sex of singleton babies born in Chiredzi, Zimbabwe

BIRTHWEIGHT BY SEX OF 262 SINGLETON BABIES

Number	Sex	Total *BW	Mean *BW	Std Dev
137	M	411350	3002.56	539.97
125	F	370890	2967.12	477.68

*BW=Birthweight in grams

Mann-Whitney or Wilcoxon Two-Sample Test (Kruskal-Wallis for two groups)

Kruskal-Wallis H (equivalent to Chi square) = 0.665
Degrees of freedom = 1
p-value = 0.414939

PLACENTAL WEIGHT BY SEX OF 261 SINGLETON BABIES

Number	Sex	Total *PW	Mean *PW	Std Dev
135	M	63453	470.03	96.27
126	F	59579	472.83	92.50

*PW=Placental weight in grams

Mann-Whitney or Wilcoxon Two-Sample Test (Kruskal-Wallis for two groups)

Kruskal-Wallis H (equivalent to Chi square) = 0.051
Degrees of freedom = 1
p-value = 0.821491

for males was 3002.56 grams and that of females, 2967.12 grams (TABLE 3). The placental weight varied from 197 to 763 grams for singletons, 186.5 to 470 grams for twins that were attached each to its own placenta and 710 to 1125.2 grams for twins that were both attached to the same placenta. The mean singleton placental weight was 470.03 grams for males and 472.8 grams for females (TABLE 3). There was no statistical difference in mean birthweight (χ^2 = 0.665, p-value = 0.415) and placental weight (χ^2 = 0.051, p-value = 0.821) between male and female babies.

Babies born to women of parities 1 and 2 had a mean birthweight of 2797.46 and 2915.75 grams respectively, whereas those born to women of higher parities weighed 2872.73 to 3115.88 grams (TABLE 4). Overall, there was a significant difference in birthweight between babies born to parturients of parities 1 through 6 or greater (χ^2 = 20.951, p-value = 0.001).

PARASITOLOGY

Out of a total of 277 matched maternal and cord blood as well as placental smears, only 3 pairs (1.08%) were positive for *P. falciparum* malaria. In all three pairs parasites were present in maternal blood, placental smears

TABLE 4. The effect of maternal parity on baby weight in paired 263 parturients and their neonates

Parity	Number	Mean *BW (grams)	Std Dev	Median *BW (grams)
1	91	2797.46	524.56	2900
2	47	2915.75	476.33	2820
3	34	3115.88	620.42	3100
4	30	3010.33	412.93	3000
5	28	3213.93	593.28	3110
≥6	33	2872.73	712.28	3100

*BW=Birthweight

Kruskal-Wallis One Way Of Analysis of Variance

Kruskal-Wallis H (equivalent to Chi square) = 20.951
Degrees of freedom = 5
p-value = 0.000827

and cord blood. In one of the pairs, *P. falciparum* gametocytes were found in all the three types of smears.

MALARIA SEROLOGY

i. *ELISA ANALYSES*

The seropositivity rates for *P. falciparum* IgG antibody in the 233 matched maternal and cord sera tested by ELISA are shown in TABLE 5. Of the 233 maternal sera tested, 117 (50.2%) were positive for *P. falciparum* IgG antibodies whereas only 58 (24.9%) of the same number of cord sera tested were positive. There was a significant difference in the prevalence rate of *P. falciparum* IgG antibody between maternal and cord sera ($z = 5.54$, p-value <0.001). *P. falciparum* IgG antibody was present in 47 (20.2%) of both maternal and cord sera of the 233 matched sera tested .

The *P. falciparum* IgG antibody ELISA positivity rates, by parity, for the 227 parturients tested are shown in TABLE 6. They were 35.4% (28/79) for parity 1, 52.6% (20/38) for parity 2, 51.7% (15/29) for parity 3, 60.7% (17/28) for parity 4, 69.6% (16/23) for parity 5 and 53.3% (16/30) for parity 6 or greater. There was no statistical difference in the prevalence rates of *P. falciparum* IgG antibody by parity when all groups were considered ($\chi^2 = 14.7$, p-value = 0.065). However when parity 1 was compared with parity 2 or

TABLE 5. Presence of *P. falciparum* IgG antibody in 233 matched maternal and cord sera by ELISA.

Maternal Serum IgG	Cord Serum IgG		
	Positive	Negative	TOTAL
Positive	47	70	117(50.2%)
Negative	11	105	116(49.8%)
TOTAL	58 (24.9%)	175 (75.1%)	233(100%)

Z-test for population proportions
z = 5.54
p-value = <0.001

greater there was a significant difference in the prevalence rates of *P. falciparum IgG* antibody (χ^2 = 8.53, p-value = 0.004). Increase in parity was associated with increased *P. falciparum* IgG antibody positive prevalence rates. With the exception of the parturients who were aged between 15 to 20 years, *P. falciparum* IgG antibody ELISA positivity rates increased with age for the parturients who were 21 to 25, 26 to 30, 31 to 35, and 36 to 40 years old (TABLE 7, r = 0.880, p-value >0.05). When all age groups were considered there was no significant difference in IgG antibody prevalence rates by age group.

TABLE 6. The distribution of *P. falciparum* IgG antibody (ELISA) by parity in 227 parturients

Maternal IgG	Parity					
	1	2	3	4	5	>=6
Positive	28	20	15	17	16	16
Negative	51	18	14	11	7	14
Total	79	38	29	28	23	30

For parity groups 1, 2, 3, 4 , 5, and >=6

Chi-square = 14.69
Degrees of freedom = 5
p-value = 0.06541763

For parity groups 1 and >=2

Chi-square = 8.53
Degrees of freedom = 1
p-value = 0.0035

TABLE 7. ELISA *P. falciparum* IgG antibody by age group in 225 parturients

Age Group (years)	Maternal IgG		
	Positive(%)	Negative(%)	Total
15-20	32(45.7%)	38(54.3%)	70
21-25	21(34.4%)	40(65.6%)	61
26-30	28(58.3%)	20(41.7%)	48
31-35	18(62.1%)	11(37.9%)	29
36-40	11(64.7%)	6(35.3%)	17

The Pearson product moment correlation was used for this test.

Correlation Coefficient (r) = 0.880
p-value = > 0.0500

Of the 228 maternal sera tested for *P. falciparum* IgM antibody by ELISA, 208 (91.2%) were positive. Only 34/228 (14.9%) cord sera were also positive for IgM (TABLE 8). *P. falciparum* IgM antibody was present in 29/228 (12.7%) matched maternal and cord sera. There was no association between maternal and cord sera *P. falciparum* IgM antibody prevalence rates ($z = 16.2$, p-value <0.001).

P. falciparum IgE antibody was present in 17/75 (22.7%) maternal sera and 2/75 (2.7%) cord sera tested by ELISA (TABLE 9). There was a significant difference in the *P. falciparum* IgE antibody prevalence rates between maternal

TABLE 8. Presence of *P. falciparum* IgM antibody in 228 matched maternal and cord sera by ELISA

	Cord Serum IgM		
Maternal Serum IgM	Positive	Negative	TOTAL
Positive	29	179	208(91.2%)
Negative	5	15	20(8.8%)
TOTAL	34 (14.9%)	194 (85.1%)	228(100%)

Z-test for population proportions
z = 16.2
p-value = 0.000

TABLE 9. The presence of P. falciparum IgE antibody in 75 matched maternal and cord sera by ELISA

	Cord Serum IgE		
Maternal Serum IgE	Positive	Negative	Total
Positive	1	16	17 (22.7%)
Negative	1	57	58 (77.3%)
Total	2 (2.7%)	73 (97.3%)	75 (100%)

Z - test for population proportions
z = 3.43
p-value = 0.00

and cord sera (z = 3.43, p-value <0.001).

ii. IMMUNOBLOTS ANALYSES

A subsample of 91 matched maternal and cord sera that were tested for *P. falciparum* IgG and IgM antibodies by ELISA were confirmed by immunoblot and the results are shown in TABLE 10. Of the 91 maternal sera tested for *P. falciparum* IgG antibody, 71 (78.1%) were reactive with *P. falciparum* antigen. Maternal sera reactivities with the parasite antigen varied from very strong (++) for 18/91 (19.8%), to strong (+) for 17/91 (18.7%), to weak(+-) for

TABLE 10. *P. falciparum* IgG and IgM antibody reactivities in 91 matched maternal and cord sera by immunoblot

	IgG antibody reactivity			
	++	+	+-	-
Maternal serum	18(19.8%)	17(18.7%)	36(39.6%)	20(21.9%)
Cord serum	16(17.6%)	18(19.8%)	32(35.1%)	25(27.5%)

	IgM antibody reactivity			
	++	+	+-	-
Maternal serum	9(9.9%)	12(13.2%)	15(16.5%)	55(60.4%)
Cord serum	2(2.2%)	4(4.4%)	7(7.7%)	78(85.7%)

++ = very strong positive
+ = strong positive
+- = weak positive
- = negative

36/91 (39.6%), to no reactivity (-) for 20/91 (21.9%). Sixty six (72.5%) of the 91 cord sera tested by immunoblot were also positive for *P. falciparum* IgG antibody. Of the cord sera tested, 16/91 (17.6%) gave very strong reactivities (++) with parasite antigen, 18/91 (19.8%) were strong reactive (+), 32/91 (35.1%) weak reactive (+-) and the rest were non reactive (-).

Thirty three matched maternal and cord pairs that gave very strong (++) to strong (+) *P. falciparum* IgG immunoblot reactivities with parasite antigen showed band patterns that were almost identical per pair, in terms of color intensity and the number of bands present on the immunoblot strips (left panel, lanes 3, 4, 5 and 6 in FIGURE 3). More than 20 high intensity bands of molecular weight mass from 220 to 21 kDa were produced on immunoblots with very strong reactivities (left panel, lanes 3, 4 and 9 in FIGURE 3). The most distinct bands for these paired sera were at the 220-, 195-, 170-, 91-, 83-, 75-, 69- and 48 kDa molecular weight mass positions (left panel, lanes 3 and 4 FIGURE 3).

In matched pairs in which maternal serum showed a very strong IgG response (++) and cord sera a strong IgG response (+) by immunoblot, band patterns were still almost identical but band color intensity was stronger for the former than the latter (lanes 9 and 10, FIGURE 3). Thirty seven P.

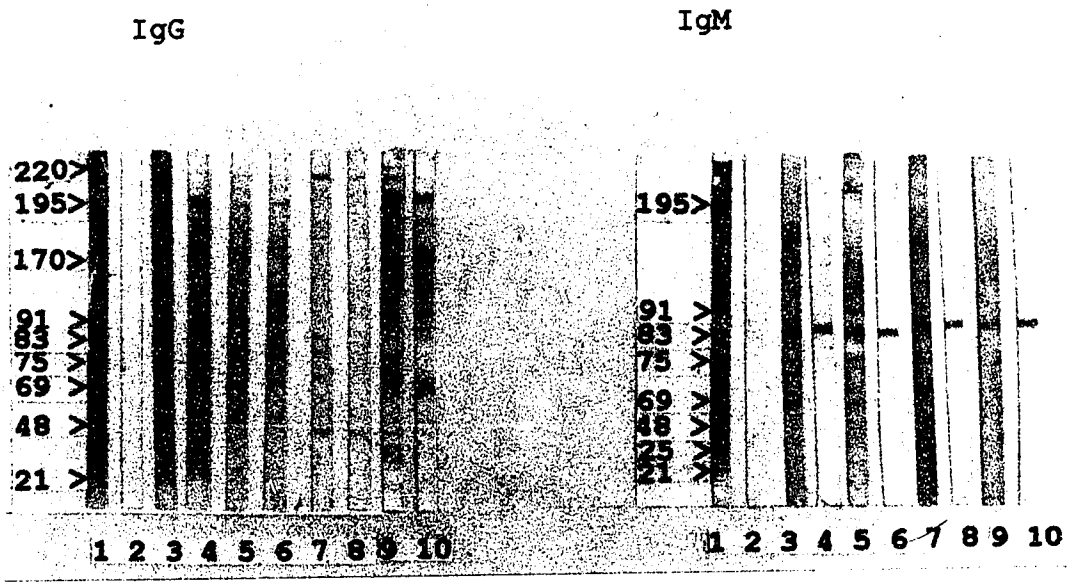


FIGURE 3. Representative immunoblots of IgG and IgM antibody responses to *P. falciparum* antigen, in four paired maternal and cord sera from Chiredzi District, Zimbabwe.
IgG (left panel). The maternal and cord sera in lanes 3 to 8 were ELISA positive for *P. falciparum* IgG antibody. Lanes 1 and 2 are IgG antibody positive and negative control sera. Lanes 3 and 4 are paired maternal and cord sera with ++ (very strong) IgG antibody response. Lanes 5 and 6 are maternal and cord sera, respectively, with + (strong) IgG antibody response. Lanes 7 and 8 are a pair with +- (weak) IgG response. Lanes 9 and 10 are maternal and cord sera respectively that were *P. falciparum* ELISA negative but with ++ (very strong) maternal and + (strong) cord sera responses.
IgM (right panel). The maternal sera in lanes 3, 5 and 7 were positive and that in lane 9 was negative by ELISA for *P. falciparum* IgG antibody whilst the cord sera in lanes 4, 6, 8 and 10 were negative. Lanes 1 and 2 are IgM positive and negative control sera, respectively. Lanes 3 and 4 are paired maternal and cord sera with ++ (very strong) maternal IgM and +- (weak) cord responses. Lanes 5 and 6 are a pair showing + (strong) maternal IgM and - (non-IgM reactivity) cord responses. Lanes 7 and 8 are paired maternal and cord sera showing +- (weak) maternal IgM and - (non-IgM reactivity) cord response. Lanes 9 and 10 are another pair which shows - (non-IgM reactivity) response.

falciparum IgG ELISA negative maternal and cord paired sera were included in the 91 pairs that were immunoblotted for *P. falciparum* IgG antibody and 28/37 (75.7%) were positive, giving band patterns that varied from very strong (++), strong (+), weak (+-) to non-reactivity (-). For these paired sera, the number of bands that were produced on immunoblots varied between 2 to 20, with the most distinct bands at 195-, 170-, 91-, 75-, 69- and 48 kDa positions (left panel, lanes 9 and 10, FIGURE 3).

Of the 91 maternal sera that were tested for *P. falciparum* IgM antibody, 9 (9.9%) showed very strong reactivities (++) with parasite antigen, 12 (13.2%) were strong reactive (+), 15 (16.5%) were weak reactive (+-) and the rest were non-reactive (-) (TABLE 10). Only 13/91 (14.3%) cord sera were positive for *P. falciparum* IgM antibody. Cord sera reactivities with parasite antigen also varied from very strong reactive (++) for 2/91 (2.2%), to strong reactive (+) for 4/91 (4.4%), to weak reactive (+-) for 7/91 (7.7%), to non-reactive (-) for 78/91 (85.7%).

The maternal sera had IgM antibody reactivities with parasite antigen of molecular weight masses varying from 180-, 170-, 91-, 83-, 75-, 69-, 48- and 21 kDa (right panel, lanes 3, 5 and 7 in FIGURE 3). The maternal sera in lane 9 was non-reactive with parasite antigen. Cord sera were

negative (-) for *P. falciparum* IgM (right panel, lanes 6, 8, and 10, Figure 3) except for that in lane 4 which was weakly reactive (+-).

HIV-1 SEROLOGY

i. *ELISA ANALYSES*

TABLE 11 shows the HIV-1 serostatus of the population study as determined by ELISA. Of the 277 maternal sera tested for HIV-1 IgG antibody by ELISA, 135 (48.7%) were positive and of the same number of cord sera tested, 130 (46.9%) were also positive. There was no statistical difference between cord and maternal HIV-1 IgG antibody rates ($z = 0.339$, $p = 0.735$). HIV-1 IgG antibody was present in 104/277 (37.6%) matched maternal and cord sera. The seropositivity rates by age group for the 135 maternal sera that were positive for HIV-1 IgG by ELISA were 50.6% for 15 to 20 years, 49.3% for 21 to 25 years, 45.2% for 26 to 30 years, 54.5% for 31 to 35 years, 42.9% for 36 to 40 years and 33.3% for 40 to 45 years (FIGURE 4).

ii. *IMMUNOBLOT ANALYSES*

Sixty three matched maternal and cord sera that included both ELISA positive and negative samples were further tested by immunoblot to confirm the presence of

TABLE 11. HIV-1 serostatus of 277 matched maternal and cord sera as determined by ELISA

Maternal Serum HIV Antibody	Cord Serum HIV antibody		
	Positive	Negative	TOTAL
Positive	104	31	135(48.7%)
Negative	26	116	142(51.3%)
TOTAL	130 (46.9%)	147 (53.1%)	277(100%)

Z-test for population proportions
z = 0.339
p-value = 0.735

HIV-1-specific IgG antibody. Of the 63 maternal sera that were immunoblotted (TABLE 12), 22 (34.9%) were seropositive, 32 (50.8%) negative and 9 (14.3%) indeterminate. Only 13/63 (20.6%) of the cord sera tested were positive, 33/63 (52.4%) negative and 17/63 (27.0%) indeterminate. There was no significant difference in the prevalence rate between maternal and cord sera ($z = 1.59$, $p = 0.111$).

FIGURE 5 shows representative immunoblots of three pairs of maternal and cord sera reactivities with HIV-1 antigen. A matched maternal and cord pair (lanes 4 and 5) that was highly positive for HIV-1-specific IgG antibody

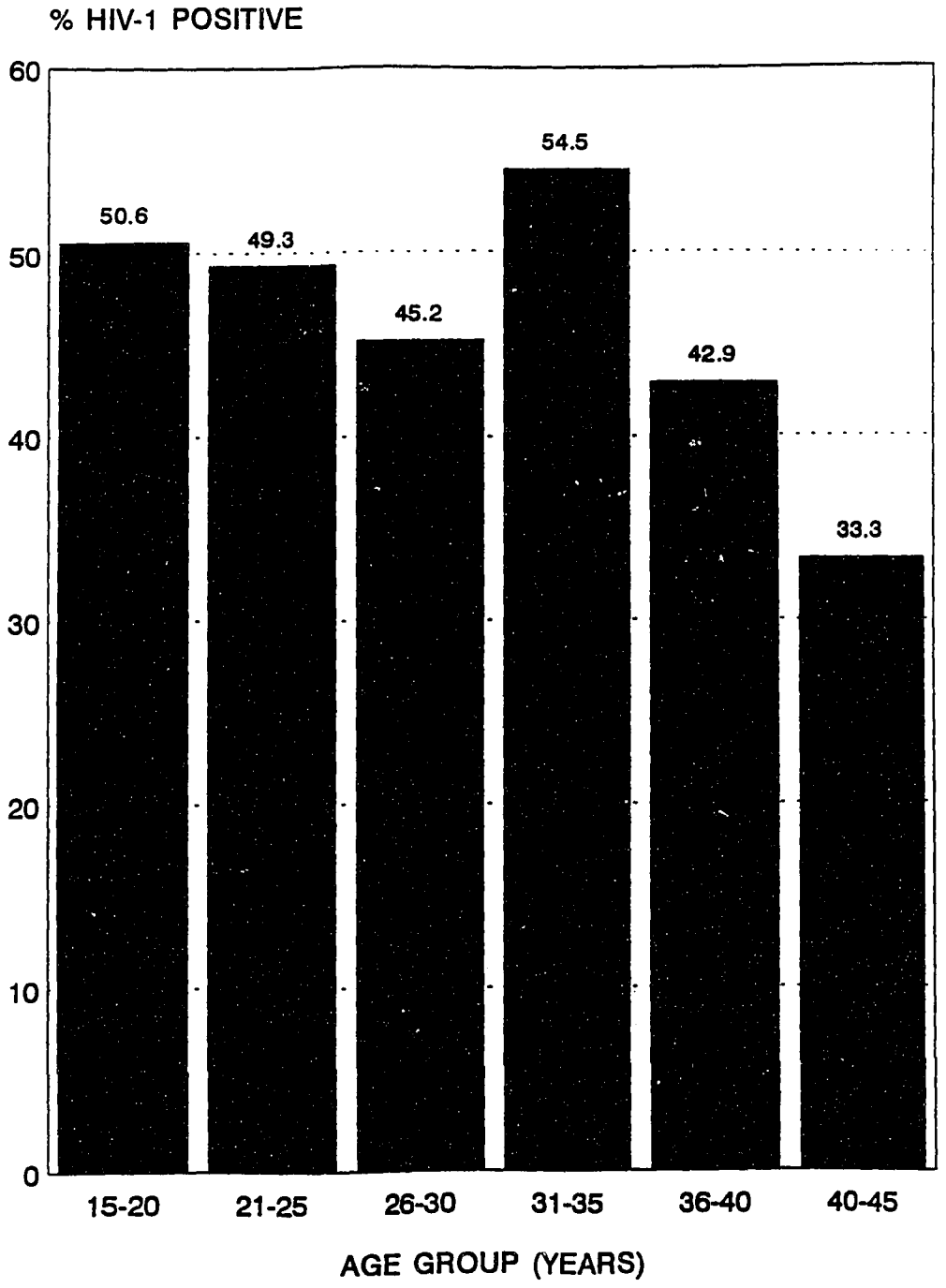


FIGURE 4. HIV-1 seropositivity distribution by age group of 135 HIV-1 ELISA positive parturients from Chiredzi District, Zimbabwe. The positivity rates (%) for each age group are shown above the bars.

TABLE 12. Seroprevalence of HIV-1 antibody in 63 matched maternal and cord sera determined by immunoblot

	Number tested	HIV positive	HIV negative	Indeterminate
Maternal serum	63	22(34.9%)	32(50.8%)	9(14.3%)
Cord serum	63	13(20.6%)	33(52.4%)	17(27.0%)

Z-test for population proportions
z = 1.59
p-value = 0.111

formed band patterns with the HIV-1 envelope protein precursor gp160, outer envelope protein gp120, the reverse transcriptases p65 and p51, transmembrane protein gp41, endonuclease p31, and core proteins p24 and p18. Another pair (lanes 6 and 7) whose maternal sera showed low positivity, gave reactivities with the HIV-1 proteins mentioned above except for gp41, p31 and p18. The cord sera was indeterminate showing reactivity with HIV-1 p24 only. Lanes 8 and 9 are another matched pair which was indeterminate showing reactivities with the HIV-1 proteins gp120 (very weak) and p24.

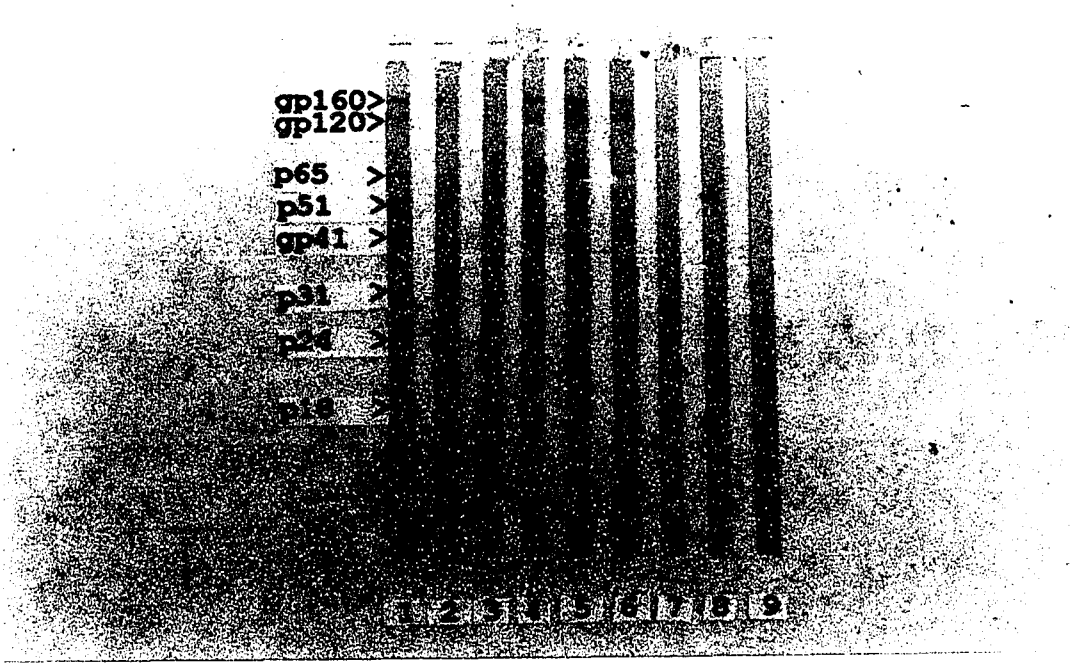


FIGURE 5. HIV-1-specific IgG antibody immunoblots of matched maternal and cord sera from Chiredzi District, Zimbabwe. Lanes 1, 2 and 3 are high positive, low positive and negative controls respectively. Lanes 4 and 5 are a matched pair that is positive for HIV-1. Lanes 6 and 7 are also a matched pair whose maternal sera is HIV-1 positive and the cord is indeterminate. Lanes 8 and 9 are a matched pair that is HIV-1 indeterminate.

HTLV-I AND II SEROLOGY

i. *ELISA ANALYSES*

Out of the 169 matched maternal and cord sera tested for HTLV-I antibody by ELISA, 147 (87%) were positive and 22 (13%) negative (TABLE 13). Cord sera were also tested for HTLV-I IgG antibody and 88/169 (52.1%) were positive and 81/169 (47.9%) negative (TABLE 13). HTLV-I IgG antibody was present in 76/169 (45%) matched maternal and cord sera. There was a significant statistical difference between the prevalence rates of maternal and cord HTLV-I-specific IgG antibody (Z = 6.5, p-value = 0.00).

TABLE 13. ELISA analyses of 169 matched maternal and cord sera for HTLV-I and HTLV-II

Maternal Serum HTLV antibody	Cord Serum HTLV Antibody		
	Positive	Negative	TOTAL
Positive	76	71	147(87%)
Negative	12	10	22(13%)
TOTAL	88 (52.1%)	81 (47.9%)	169(100%

Z-test for population proportions
z = 6.5
p-value = 0.00

ii. *WESTERN BLOT ANALYSES*

Forty eight matched maternal and cord sera that had been tested by ELISA were immunoblotted for HTLV-I and II-specific IgG antibody. These 48 paired sera included 6, 21 and 21 pairs that were maternal/cord negative, maternal/cord positive and maternal positive/cord negative for HTLV-I and II IgG antibody by ELISA respectively. The Western blot kit utilizes the same negative control sera for both HTLV-I and II. Of the 48 pairs tested, 21 (43.8%) were non-reactive with HTLV antigen and 27 (56.2%) were indeterminate. Of the 21 pairs that were negative for HTLV-I and II antibody by immunoblot, 9 (42.9%) were positive by ELISA.

FIGURE 6 shows HTLV-I and II immunoblot band patterns of 3 pairs of matched maternal and cord sera as well as their ELISA optical density readings. Lanes 4 and 5 are matched maternal and cord sera which were indeterminate but weakly reactive with gp46 envelope glycoprotein, P24 core protein and p21 env_r recombinant transmembrane envelope. Another matched pair in lanes 6 and 7 was non-reactive with the HTLV-I and II proteins. A pair shown in lanes 8 and 9 was also indeterminate and formed a band with p21 env_r only.

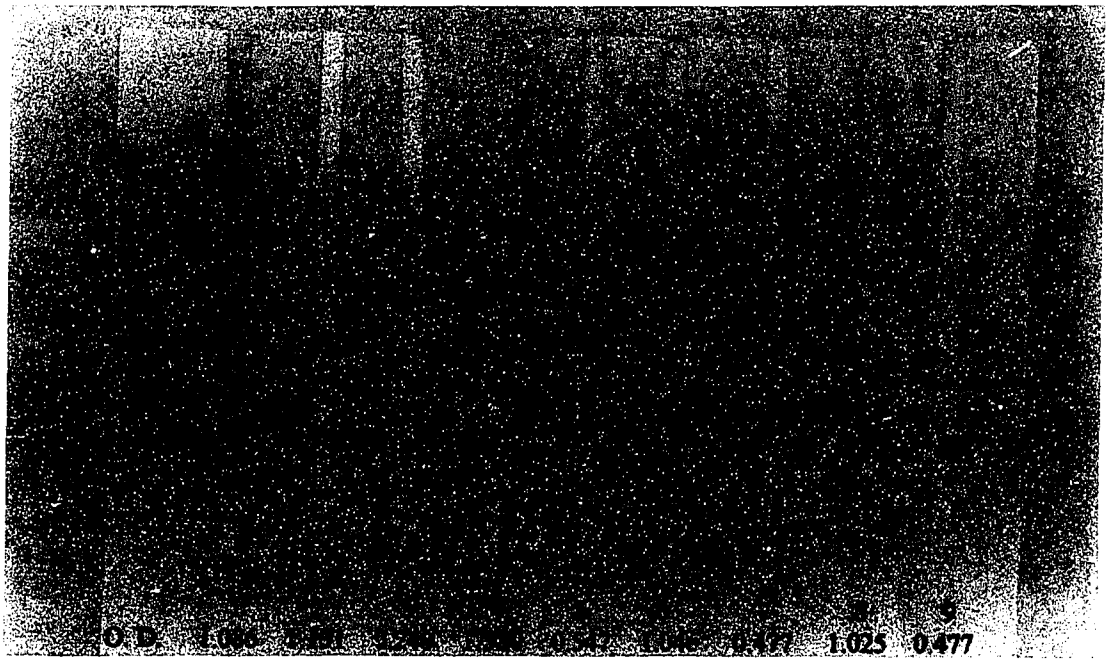


FIGURE 6. HTLV-I and II-specific IgG immunoblot analyses of matched maternal and cord sera. Lanes 1, 2 and 3 are HTLV-I, HTLV-II positive and negative controls respectively. Lanes 4 and 5 are matched maternal and cord sera showing very weak reactivities to gp46, p24 and p21 env_r. Lanes 6 and 7 are also a matched pair which are non-reactive with HTLV proteins. Lanes 8 and 9, another matched pair is indeterminate showing reactivity with p21 env_r only. The corresponding ELISA optical density readings are shown at the bottom of the immunoblots. The cut off value was 0.543.

THE RELATIONSHIP BETWEEN *P. FALCIPARUM* IgG ANTIBODY AND HIV-1 INFECTION

A total of 91 matched maternal and cord sera were tested for *P. falciparum* and HIV-1-specific IgG antibodies by immunoblot. Of the 55 pairs that were positive for HIV-1 IgG, 27 (49.1%) were also positive for *P. falciparum* IgG and 28 (50.9%) were negative (TABLE 14). Of the 36 pairs that were negative for HIV-1 IgG, 33 (91.7%) were positive for *P. falciparum* IgG and the rest were negative. Statistically, there was a significant difference in the prevalence

TABLE 14. Co-positivity of *P. falciparum* IgG and HIV-1-specific antibodies by immunoblot

	Maternal Serum *P. falciparum* IgG antibody		
Maternal HIV-1 Serostatus	Number tested	Number positive	Number negative
Maternal HIV-1 positive	55	27(49.1%)	28(50.9%)
Maternal HIV-1 negative	36	33(91.7%)	3(8.3%)
TOTAL	91	60(65.9%)	31(34.1%)

Z-test for population proportions
z = 3.97
p-value = 0.00

rates of *P. falciparum* IgG antibodies between the HIV-1 positive and HIV-1 negative parturients ($z = 3.97$, $p < 0.001$).

Some representative immunoblots of matched maternal and cord sera that were positive for *P. falciparum* IgG but either positive or negative for HIV-1-specific IgG antibodies are shown in FIGURE 7. HIV-1 IgG immunoblot negative pairs in the left panel show that all test sera in lanes 3 to 10 reacted with *P. falciparum* proteins to give up to 20 or more high intensity bands of molecular weight mass range 220 to 21 kDa. On the other hand, all the paired test sera in lanes 3 to 10 of the HIV-1 IgG positive group in the right panel were reactive with the parasite protein and gave up to 12 weak intensity bands of molecular weight mass range 195 to 21 kDa.

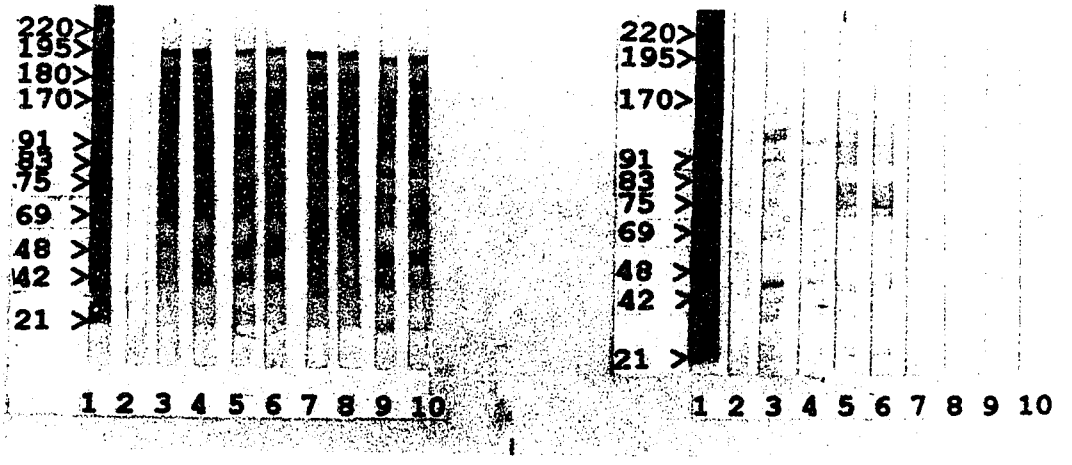


FIGURE 7. *P. falciparum*-IgG antibody immunoblot analyses of matched maternal and cord sera positive for HIV-1-specific IgG and negative for HIV-1-specific IgG antibody by immunoblot.
HIV-1 IgG immunoblot negative (left panel). Lanes 1 and 2 are *P. falciparum* IgG antibody positive and negative control sera respectively. Lanes 3 to 10 are matched maternal and cord sera (even and odd numbers are maternal and cord sera respectively).
HIV-1 IgG immunoblot positive (right panel). Lanes 1 and 2 are *P. falciparum* IgG antibody positive and negative control sera respectively. Lanes 3 to 10 are matched maternal and cord sera (even and odd numbers are maternal and cord sera respectively).

CHAPTER 4

CONCLUSION

SELECTED STUDY POPULATION STATISTICS

The mean age of the parturients in this study was 25.2 years and those who were 20 years of age contributed the largest proportion (8.3%) to the study population. There were more parturients in the youngest age group of 15 to 20 years than any other age group and 84% of the women in this age group were of parity 1. The population sampling bias for the women between 15 to 20 years of age and of parity 1 was because small health care centers such as Rupangwana, Chipiwa and Chilonga clinics advise and transfer primigravidae to deliver babies at referral hospitals such as Chiredzi district hospital, in case complications arise during parturition. Ninety five percent of the women in this study had their babies at Chiredzi district hospital. The multigravidae may choose to be delivered by a traditional midwife at home or by a midwife at a small local health center. Overall, parity increased with age as expected for many parts of Africa.

There was no difference in placental and birthweight between male (mean placental weight = 470.03 grams and mean birthweight = 3002.56 grams) and female (mean placental

weight = 472.83 grams and mean birthweight = 2967.12 grams) babies. In another study carried out in middle and lower economic groups in Harare the capital city of Zimbabwe (198) the mean birthweights were 3122 grams and 2992 grams for male and female respectively. These birthweights were slightly higher than for babies in this current study because the study population was mainly of lower economic class. Studies carried out in Papua New Guinea (199) and in Cameroon and the United States (200) showed that on average male babies are born heavier than females. The average birthweight for both male and female babies in the current study is comparable to that of other countries in Africa which is below 3000 grams with a range of 2730 to 2955 grams (201). In the current study, maternal parity was related to birthweight and babies of those delivering for the first and second time were lighter than the babies of women of parity 3 or more. There were 84% primiparae who were 15 to 20 years old and this age group was 30% of the study population. Dole and others (198), in their study mentioned above also found that birthweight rose with increasing parity. The young primigravidae, especially teenagers (and they contributed 21.7% of the study population in the current study), are known to have a higher risk of low birthweight babies (198, 199, 202, 203). This may be due to higher incidence rates of

preeclampsia, anemia, low socioeconomic status and not attending prenatal clinics for advice on pregnancy.

PARASITOLOGY

P. falciparum parasite rate of 1.08% was found amongst the parturients. During the time that the serum samples were collected, Zimbabwe had a drought and hence there were less breeding places for mosquitoes which in turn reduced malaria transmission. The main factors that affect the epidemiology of malaria in Zimbabwe are altitude and the associated temperature changes (183). Malaria transmission is seasonal in Chiredzi district with most transmission occurring between February to May annually. Season, temperature and altitude were shown to influence malaria transmission in Zimbabwe (186, 187) and hence affect the survival as well as the migration of the vector *Anopheles gambiae*. The vector populations are known to peak in March at the end of the rainy season followed by a peak in malaria at the end of March/beginning of April. The peak of slide submission and malaria positivity rates (26.2%) in Chiredzi for 1993 occurred in March.

MALARIA SEROLOGY

i. *ELISA*

P. falciparum IgG antibody was present in 50.2% of the maternal sera and only in 24.9% of the cord sera tested by ELISA. The presence of *P.falciparum* IgG antibody in the cord sera in this case was independent of maternal IgG antibody and hence the difference in IgG antibody positivity rates between the two types of sera. Some possible reasons for this observed difference are:-

a. The total quantity of IgG antibody transferred from the mother to the baby were not high enough to give a positive reading because the threshold was set at too high a level of optical density (O.D.) reading (0.080). Maternal serum IgG antibody is passively transferred across the placenta to the fetus and as such one would expect cord IgG antibody positivity rates to be related to maternal IgG antibody rates.

b. Maternal isotypes IgG2 and IgG3 antibodies are poorly transported across the placenta to the fetus (53, 58). In general all IgG subclasses may be present in maternal sera, however the isotype IgG4 may not be present in some immune adults (60) and also in pregnant women where it was detected in only 42% of the matched pairs (53).

c. False positives with maternal sera.

There was no difference in the prevalence of *P. falciparum* IgG antibody by parity when all parities 1 to 6 or greater in the parturients studied were considered. Therefore parity did not seem to influence the production of maternal *P.falciparum* IgG antibody in this case. Desowitz and others (58) also found that there was no correlation between parity and maternal IgG antibody positivity rates. When parity was grouped into parities 1 and 2 or greater, the parturients of parity 1 had a lower prevalence rate of *P. falciparum* IgG antibody than those of parity 2 or greater. Thus, in this case, parity influenced the production of maternal IgG antibody.

Immunity to malaria in pregnancy has been shown to increase with parity (13). It has been theorized that the newly formed uteroplacental tissue in primiparous women is immunologically "naive" and is thus more susceptible to colonization by the parasite (13, 21). Repeated exposure to malaria in subsequent pregnancies increases uteroplacental immunity and hence protects from severe infection (46).

P. falciparum IgG antibody positivity rates were not significantly related to age, however IgG antibody positivity rates increased as age rose for those who were 21 to 25, 26 to 30, 31 to 35 and 36 to 40 years old.

The ELISA positivity rates of *P. falciparum* IgM antibody for the maternal sera was higher (91.2%) than that of the cord sera (14.9%). Therefore cord IgM antibody prevalence as well as its production was not associated with maternal IgM antibody. Possible reasons for the observed difference in IgM antibody positivity rates are:-

a. Under normal circumstances where the placental barrier is intact, maternal IgM antibody is not transferred to the fetus. In this study, IgM was present in 12.7% of the matched maternal and cord sera tested, an indication that *P. falciparum* antigens may have crossed the placental barrier and induced a primary immune response in the fetus giving rise to IgM antibody production. This observation is similar to that made by Chizzolini and others in a study population in Gabon (53) Africa, who found that 11.9% of their paired maternal and cord sera were positive for *P. falciparum*-specific IgM antibody by indirect immunofluorescence assay. In similar studies looking at mothers and their neonates in Papua New Guinea, Desowitz and others (58) did not find *P. falciparum* IgM antibody in any of their cord sera.

b. Polyclonal activation of B cells giving false positive results.

c. Most healthy human beings are known to have natural IgM antibodies which are multispecific and react with self-related and non-self related antigens such as parasites. Studies have shown that many healthy humans have natural IgM antibodies that are reactive with the protozoan *Toxoplasma gondii* (204, 205). A survey, using the ELISA showed that almost everyone lacking IgG antibodies against this parasite did have such IgM antibodies (204) even though there was no accompanying active parasite infection. In other studies (206, 207), false positive results were obtained for 2 pregnant woman for *T. gondii* IgM antibodies during early gestation although no increased IgG antibody level was observed, an indication that they were not infected. *P. falciparum*, which is also a protozoan, may react with natural IgM antibodies in a similar manner to *T. gondii*. The level of natural antibodies varies from one individual to the next and different antigen molecules, for example *T. gondii*, *Leishmania*, *Trypanosoma cruzei*, are recognized by the natural antibodies in sera from various individuals (203, 208, 209). This study did not control for natural IgM antibodies in the population under study.

d. Rheumatoid factor, a multispecific natural autoantibody secreted by $CD5^+$ B cells, and antinuclear antibodies

may give false positive results with IgM antibody assays. Rheumatoid factor increases during pregnancy and may affect the results of IgM antibody assays (204). Rheumatoid factor and antinuclear antibodies were also not controlled for in the study.

e. Present in many sera are antibodies, especially of the IgM isotype, that react with *P. falciparum* antigen and are also specific to red blood cell (RBC) antigens (210, 211). The antigen used in this study was lysed crude *P. falciparum* antigen and no controls were included for the reactivity of each serum with RBC contaminants in the antigen preparation.

P. falciparum IgE antibody was present in only 1/75 of the matched maternal and cord sera pairs tested and there was no associated parasitemia in this pair. IgE antibody does not cross the placental barrier (212) but human fetuses can produce this antibody isotype during the 10th to 11th week after conception (64). Maeno and others (63) demonstrated the deposition of *P. falciparum* antigen in fetal blood vessels. This deposition was correlated with the deposition of IgE antibody and the inhibition of parasite growth. These authors hypothesized that the presence of antigen in the fetal vessels may have been related to prenatal immune priming to malaria (213). The production of

IgE in the studies by Maeno and others may have been by the fetuses. Studies conducted in Papua New Guinea showed that 15/45 serum samples from adults exposed to malaria were positive for *P. falciparum-specific* IgE by ELISA (61). In recent studies (58), it was found that *P. falciparum*-specific IgE antibody in paired maternal and cord sera were related to placental parasitemia rates, to parasitemia intensity and to parity. *P. falciparum* IgE antibody was present in 8.5% of the cord sera tested by ELISA. This current study shows the rarity of *P. falciparum* IgE antibody in matched maternal and cord sera from Zimbabwe. Some of the reasons why extremely low *P. falciparum* IgE antibody rates were observed in these paired sera are:-

a. At the time this study was conducted Zimbabwe was going through a drought and hence the possibility of low malaria transmission due to unfavorable conditions for mosquito breeding.
b. IgE antibody is more labile than IgG antibody and its activity may have been lost during storage or transportation from Zimbabwe to Hawaii.
c. Other investigators conducting similar studies with sera from Papua New Guinea (58, 61) and some parts of Africa (65) found *P falciparum* IgE antibody rates of up to 26% which increased as the parity order increased and 60%,

respectively in their sera. Zimbabwe is in a different geographical region from the areas mentioned above and the presence of IgE antibody may very well depend on the geographical location in which the study population resides. Different factors influence malaria transmission in various geographical regions and this may in turn affect the production of *P.falciparum* IgE antibody.

ii. *IMMUNOBLOTS*

Maternal *P. falciparum* IgG antibody positivity rates were 78.1% by immunoblot and 50.2% by ELISA. *P. falciparum* IgG antibody positivity rates for cord sera were 72.5% and 24.9% by immunoblot and ELISA respectively. Of the 37 ELISA negative paired sera that were included for testing by immunoblot, 28 (75.7%) were *P. falciparum* IgG antibody positive. The immunoblot was more sensitive in detecting parasite antigen/sera IgG antibody reactivities than the ELISA.

Maternal *P. falciparum* IgM antibody positivity rate was lower (39.6%) by immunoblot in comparison with 91.2% by ELISA. The immunoblot was more specific in detecting parasite antigen/maternal sera antibody reactivities than the ELISA which gave many false positive maternal IgM

antibody responses. The *P. falciparum* IgM antibody positivity rates for the cord sera by ELISA and immunoblot were almost the same (14.9% and 14.3%, respectively). The ELISA was as reliable as the immunoblot in *P. falciparum* antigen/cord sera IgM antibody assays and not in *P. falciparum* antigen/maternal sera IgM antibody assays.

These results may also indicate that the fetuses were producing IgM antibody which was specific to only the parasite antigen as a result of prenatal immune priming (213). These results agree with those of the studies from Gabon in Africa (53) in which 7/59 paired cord sera were positive for *P. falciparum* IgM antibody. Chizzolini and others suggested that the prenatal primary immune response in the fetuses with the consequent production of *P. falciparum* IgM antibody observed in their study might have been facilitated by parasitemia heavy enough to change the histopathology on the maternal side of the placenta in 6/7 of these cases.

In this Zimbabwean study, only 3/277 placenta were parasitized and it was beyond the scope of the study to do histopathological tests for malaria. Congenital malaria exists in Zimbabwe but its prevalence is unknown. Although parasites were not evident by microscopy in all but 3/277 of the paired samples, subpatent parasitemia may have been

present hence the *P. falciparum* IgM antibody positive reactivities in cord sera. Many pregnant woman residing in malarious areas of Zimbabwe are given chloroquine prophylaxis at prenatal clinics but compliance with prophylaxis is a big problem. Chloroquine, which is the drug of choice for treatment in Zimbabwe, is very bitter and malaria patients do not often complete their course. Partial compliance with malaria chemoprophylaxis and non completion of antimalarial drug courses may encourage subpatent parasitemia and this may explain the presence of IgM antibody in cord sera by immunoblot. There were only 3 matched maternal and cord pairs whose placental, cord and maternal smears were *P. falciparum* positive. This observation confirms the fact that congenital malaria occurs in Zimbabwe but the prevalence rate at which it occurs is not known.

Immunoblot *P. falciparum* IgG and IgM antibody reactivities with *P. falciparum* antigen were categorized into very strong (++), strong (+), weak (+-) and non-reactive (-) responses. All 33/91 matched pairs that gave very strong to strong *P falciparum* IgG antibody responses by immunoblot, showed near homology per pair, giving band patterns that were almost identical in terms of band intensity and number of bands present on the immunoblot

strips. Matched pairs whose maternal sera gave very strong and cord sera strong *P. falciparum* IgG antibody responses also showed near homology in the band patterns with regard the number of bands that were present per pair but band intensity was weaker for the latter than the former. Very similar banding patterns between all positive sera were formed on immunoblots. In this study, the most frequent and strong antigen complexes in the immunoblots of IgG antibody positive sera were 195- to 170-, 91- to 75- and 69- to 48-kDa. In a similar study conducted in Papua New Guinea (58), it was observed that the most strongly and prevalent antigen complexes in IgG antibody positive immunoblots were 48- to 51-kDa. In another study conducted using sera from volunteers in several families in Burkina Faso, Africa (214), the two major antigens associated with high IgG antibody levels were of molecular mass weight 115 and 103 kDa.

In this current study the serum samples whose maternal sera were categorized as very strong (++) and cord sera as weak (+-) positive for *P. falciparum* IgM antibody showed no homology per pair, in terms of band patterns and intensity. This confirms that there was no leakage of maternal IgM antibody into the fetal circulation and that this antibody was produced by the fetus and not the mother. The

immunoblots showed heterogeneity in *P. falciparum* IgM antibody reactivities with parasite antigen between the sera tested. The antigen complexes that were strongly recognized by the sera and were most persistent were 91- to 75-kDa and 21 kDa. Thelu and others, (214) found that the 115-kDa was strongly recognized by IgM antibody in their serum samples, on the other hand Desowitz and others (58) showed that strong IgM bands were at 127-, 46-, and 34-kDa in their IgM immunoblots. All 33/91 but 3 maternal sera in the current study, that were positive for IgM antibody by immunoblot also had an accompanying IgG antibody positive response by immunoblot.

HIV-1 SEROLOGY

i. *ELISA*

The HIV-1 infection rate in the parturient women in Chiredzi was 48.7% by ELISA and that of matched maternal and cord sera was 37.6%. There was an association between maternal and cord HIV-1-specific IgG antibody seropositivity rates, the reason for this being that maternal HIV-1-specific IgG antibody can cross the placental barrier to the fetus. The highest rates of HIV-1 infection were found in sera that were collected from parturients 31 to 35 years

old. Those who were between 15 to 20 years old had the second highest rates of infection.

ii. *SEROIMMUNOBLOTS*

The seropositivity rate of HIV-1 infection in the parturients by immunoblot was 34.9% and lower than that by ELISA (48.7%). Cord sera HIV-1 seropositivity rates by ELISA and immunoblot were 46.7% and 20.6% respectively. The explanation for this is that all the maternal (14.3%) and cord (27%) sera that gave indeterminate reactivities with HIV-1 antigen were positive by ELISA. These indeterminate sera were mainly reactive with the core protein p24 gag only or to a lesser extent in combination with gp120 to give very faint bands. These reactivities were interpreted as an early HIV-1 infection since in HIV infection an IgM antibody response precedes the formation of IgG antibodies. The passive transfer of maternal HIV antibody to the fetus in utero makes it difficult to determine whether the HIV-1 IgG antibody reactivities observed in cord sera were of fetal or maternal origin. Results from other laboratories show that this may not be the case (215, 216). Parity was a risk factor for an indeterminate HIV-1 Western blot in the Chiredzi study since indeterminate reactivity increased with increasing parity.

The diagnosis of HIV infection in Zimbabwe is based on two commercial ELISA kits and as seen above HIV-1 ELISA seropositive samples include those that are otherwise indeterminate by immunoblot. A study carried out in Mutoko, another district in Zimbabwe, showed 25% HIV infection rates in pregnant woman (193). The highest positivity rates were found in women of 25 to 30 years of age. In a survey looking at HIV-1 infection and perinatal mortality in Zimbabwe (194), the HIV-1 seropositivity rates in neonatal deaths and stillbirths were 23.6% and 15.4% respectively. HIV perinatal transmission rates of 25 to 48% have been reported from many African countries (69, 109-111).

HTLV-I AND II SEROLOGY

i. ELISA

The HTLV-I IgG antibody ELISA positivity rates in mothers and their newborn babies were 87% and 52.1% respectively. There was no relationship between maternal and cord HTLV-I IgG antibody. Since this isotype is passively transferred transplacentally from mother to fetus, one expected maternal and cord HTLV antibody positivity rates to be related. Some possible reasons for this observed difference are:-

a. The total quantities of IgG antibody transferred from the mother to the fetus were not high enough to give a positive optical density reading.
b. Maternal isotypes IgG2 and IgG3 antibodies are poorly transferred across the placenta to the fetus (53).
c. False positives with maternal sera.

ii. SEROIMMUNOBLOTS

All the matched pairs that were positive by ELISA were either non-reactive or indeterminate giving very weak reactivities with either p19 gag, p21 env_r, p24 gag, p28, p38, gp46 envelope glycoprotein or recombinant transmembrane p21 env_r by immunoblot. It was thus impossible to confirm the presence of HTLV-I and II antibody in the parturients from Chiredzi using the Western blot kits provided. Such indeterminate reactivities by immunoblot are common (160, 217).

In the current study, indeterminate HTLV-I/II reactivities were interpreted as an indication of early infections to HTLV infection because HTLV exists in Zimbabwe. Although immunoblot confirmation of HTLV-I and II IgG antibody in the matched maternal and cord sera tested was not possible, a seroprevalence of 0.32% was found among blood donors in Zimbabwe (195). In South Africa which shares

its northern border with Zimbabwe, seroprevalence rates of 0 to 5.2% have been reported (168).

INDETERMINATE REACTIVITIES BETWEEN *P. FALCIPARUM*, HIV-1 and HTLV-I/II

Risk factors for HIV-1 indeterminate Western immunoblots among the nonseroconverter in a study by Celum and others (218) were parity and autoantibodies, suggesting cross-reactive antibody. The investigators suggested that parity could be a reflection of alloimmunization during pregnancy, producing antibody to cellular proteins that comigrate with HIV proteins on Western immunoblots. Indeterminate HIV and HTLV Western immunoblots may result from antibody production against viral core antigens or loss of core antibodies late in infection (219-221). It may also result from cross-reactive autoantibodies and alloantibodies (220, 222, 223). Serologic cross-reactivity has also been reported between *P.falciparum* and HIV-1 (224, 225), *P. falciparum*, HIV-1 and HTLV-I (Desowitz personal communication) and HIV-1/2 and HTLV-I/II (226). These serologic cross-reactivities are likely to complicate the epidemiology and pathogenesis associated with *P. falciparum*, HIV-1, HIV-2, HTLV-I, and HTLV-II in areas such as Chiredzi where all 5 infections may co-exist.

THE RELATIONSHIP BETWEEN *P. FALCIPARUM* IgG ANTIBODY AND HIV-1 INFECTION

There was a significant difference in the proportions of the parturients that were *P. falciparum* IgG positive/HIV-1 IgG positive (49.1%) and those that were P. falciparum-specific IgG positive/HIV-1 IgG negative (91.7%) by immunoblot. Representative immunoblots of matched maternal and cord sera by HIV-1 immunoblot serostatus showed that HIV-1 infection suppressed *P. falciparum* IgG antibody reactivity. Suppression of *P. falciparum* IgG antibody in the parturients that were HIV-1 IgG antibody positive was observed at the 195-, 180-, 170- and 83-kDa molecular mass weight levels. The clinical status of the HIV-1 seropositive parturients in terms of AIDS was unknown.

Wabwire-Mengen and others (227), showed that there was evidence of a possible relationship between a host's immunologic response to malaria antigenic stimuli and the clinical stage of HIV-1 infection. In their study, they looked at hospitalized HIV-1 seropositive patients with AIDS as well as trauma patients without clinical AIDS and found that the mean optical densities produced against synthetic peptides of *P. falciparum* were consistently lower in the former group than the latter.

However a study of normal healthy Africans from Zaire (228) demonstrated that antibody against HTLV-I, HTLV-II and HTLV-III (HIV-1) by ELISA correlated strongly with the level of antibodies against *P. falciparum* malaria. In another study which investigated children (137), there was no significant difference between the HIV-1 seropositivity rates in children with *P. falciparum* malaria and healthy controls. Simooya and others (176) also did not find any relationship between HIV-1 seropositivity and the presence of malaria antibody. A study which investigated cerebral malaria patients (229) did not show an association between HIV and the presence or degree of malaria parasitemia.

The *P. falciparum* major merozoite protein and candidate human vaccine against malaria, gp195, has a molecular weight of 180-200 kDa (230). It is processed into smaller polypeptides of which the 83-, 42-, and 19-kDa fragments are found on the surface of the merozoite and are recognized by antibodies in human sera (230). Several studies have shown that anti-gp195 antibodies exist in regions of both low and high malaria transmission (231-234). In the current study the suppression of *P. falciparum* IgG antibody reactivities with the 195-, 180-, and 83-kDa parasite proteins in HIV-1 seropositive parturients suggests that a gp195 polypeptide based vaccine may not protect HIV-1 infected individuals

against *P. falciparum* malaria. Acquired immunity in malaria is mainly dependent on IgG antibodies as demonstrated by the classic passive transfer of adult IgG antibodies (6). Both malaria and HIV-1 infections cause functional abnormalities of cell mediated immunity with a lowering of the CD4/CD8 lymphocyte ratio (171,172) which in turn affect antibody generation by B-cells.

In the current study the difference observed in the *P. falciparum* IgG antibody prevalence rates between HIV-1 positive and HIV-1 negative parturients may reflect B-cell dysfunction which is associated with HIV-1 infection in addition to poor T-cell help. HIV-1 is known to reduce the efficiency of antigen presentation and consequently this interfers with the production of antibody.

Although this study suggests that there is an association between *P. falciparum* IgG antibody and HIV-1 infection, further seroepidemiologic studies should be conducted to elucidate this association in all age groups who have either malaria or HIV-1 or both infections. Such studies should to put special emphasis on *P. falciparum* IgG antibody production in parasitemic and aparasitemic HIV-1 seropositive as well as seronegative population groups.

THE MAIN CONCLUSIONS DEDUCTED FROM THIS STUDY

This is the first study to address the seroepidemiology of *P. falciparum*, HIV-1 and HTLV-I/II in mothers and their neonates in Zimbabwe. The study also determined the possible co-existence of *P. falciparum* malaria and HIV-1 infections in the same population groups. Matched blood samples from parturients and their neonates were screened for malaria parasites and analyzed for *P. falciparum* IgG, IgM, IgE, HIV-1 and HTLV-I/II.

The mean age of the 277 parturients was 25.2 years and those who were 20 years old contributed to the largest proportion of the parturients. There was no difference in birthweight between male and female babies and the higher the maternal parity order the bigger the baby. There was a parasite rate of 1.08% amongst the parturients.

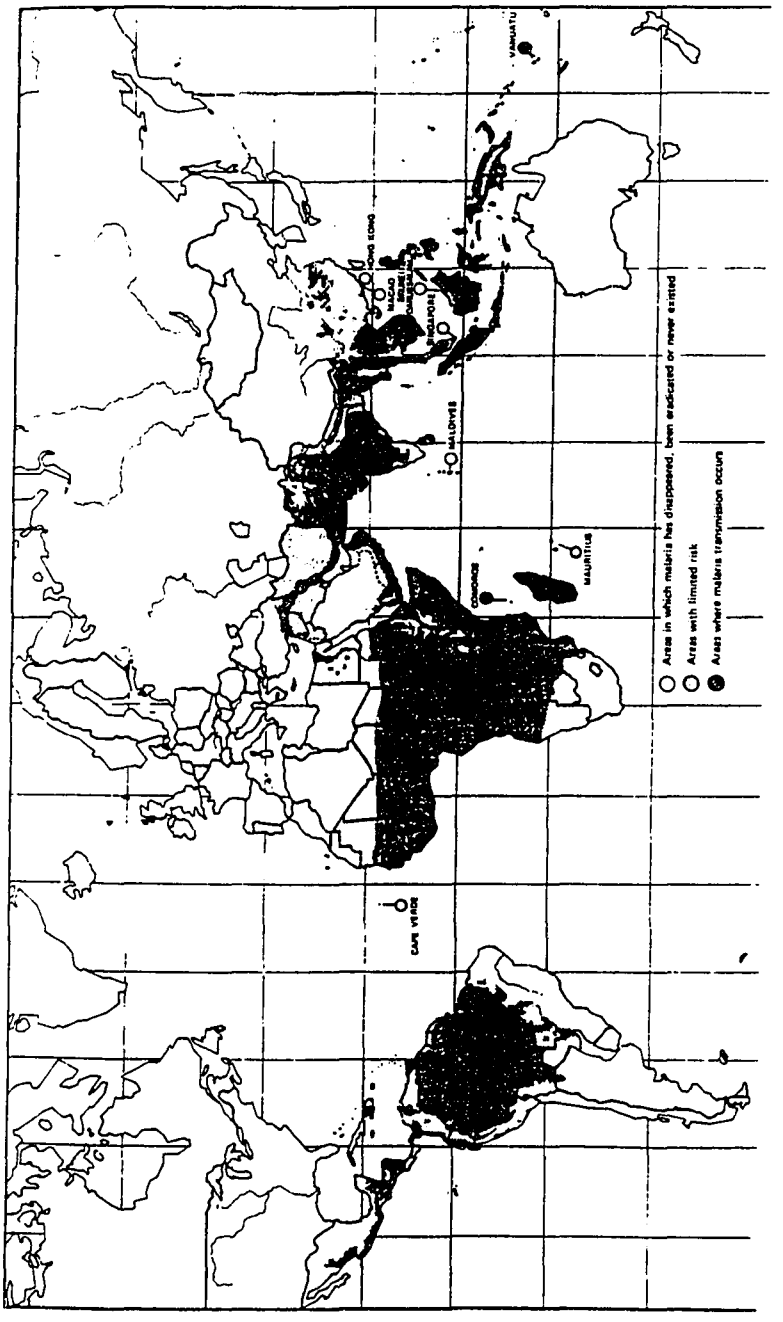
The presence of *P. falciparum* IgG antibody, by ELISA, in the cord was independent of maternal IgG antibody. The immunoblot, however, picked up more IgG antibody positive matched pairs than the ELISA demonstrating that it was more sensitive than the latter in IgG antibody assays. The most prevalent and strong *P. falciparum* antigen/IgG antibody complexes formed on immunoblots of matched maternal and cord sera were 195- to 170-, 91- to 75- and 69- to 48-kDa. Parity did not seem to have a significant influence on the

production of maternal IgG antibody. *P. falciparum* IgG antibody positivity rates were also not age related when all age groups were considered.

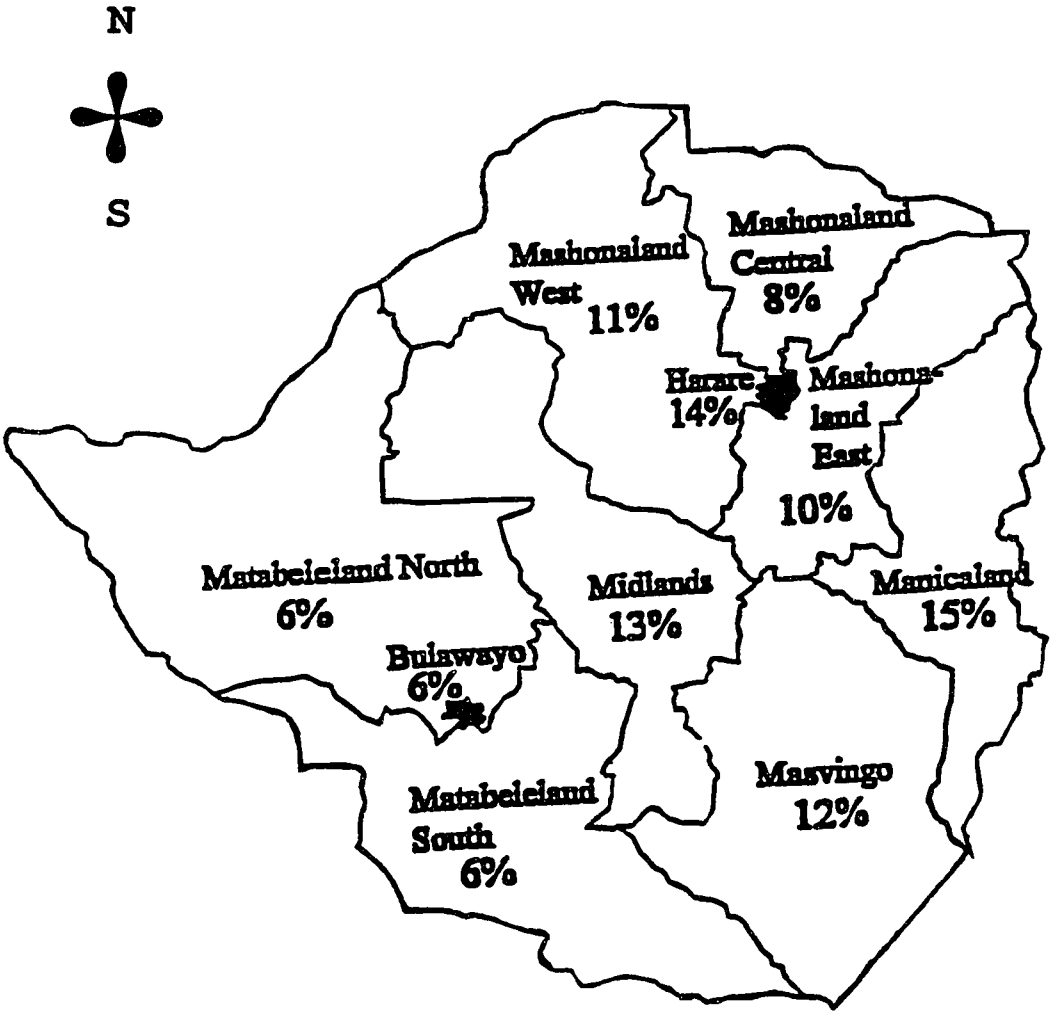
Cord *P. falciparum* IgM antibody prevalence rates by ELISA were not associated with maternal IgM antibody prevalence rates. The maternal IgM antibody positivity rate was reduced from 91.2% (ELISA) to 39.6% by immunoblot hence demonstrating that the immunoblot was more specific than the ELISA in IgM assays. The most prevalent and strongly recognized *P. falciparum* antigen/IgM antibody complexes on immunoblots of matched pairs were 91- to 75- and 21-kDa. *P. falciparum* IgE antibody was rare in the parturients and their neonates studied.

Western immunoblot confirmed HIV-1 seropositivity rate in mothers was 34.9% and those between 31 to 35 years old had the highest rate of infection. It was not possible to confirm by Western immunoblot if HTLV-I and II were also prevalent amongst the parturients. However, maternal HIV-1 infection was found to suppress *P. falciparum* IgG antibody reactivities on immunoblots, suggesting that there was a possible association between malaria and HIV-1 infections.

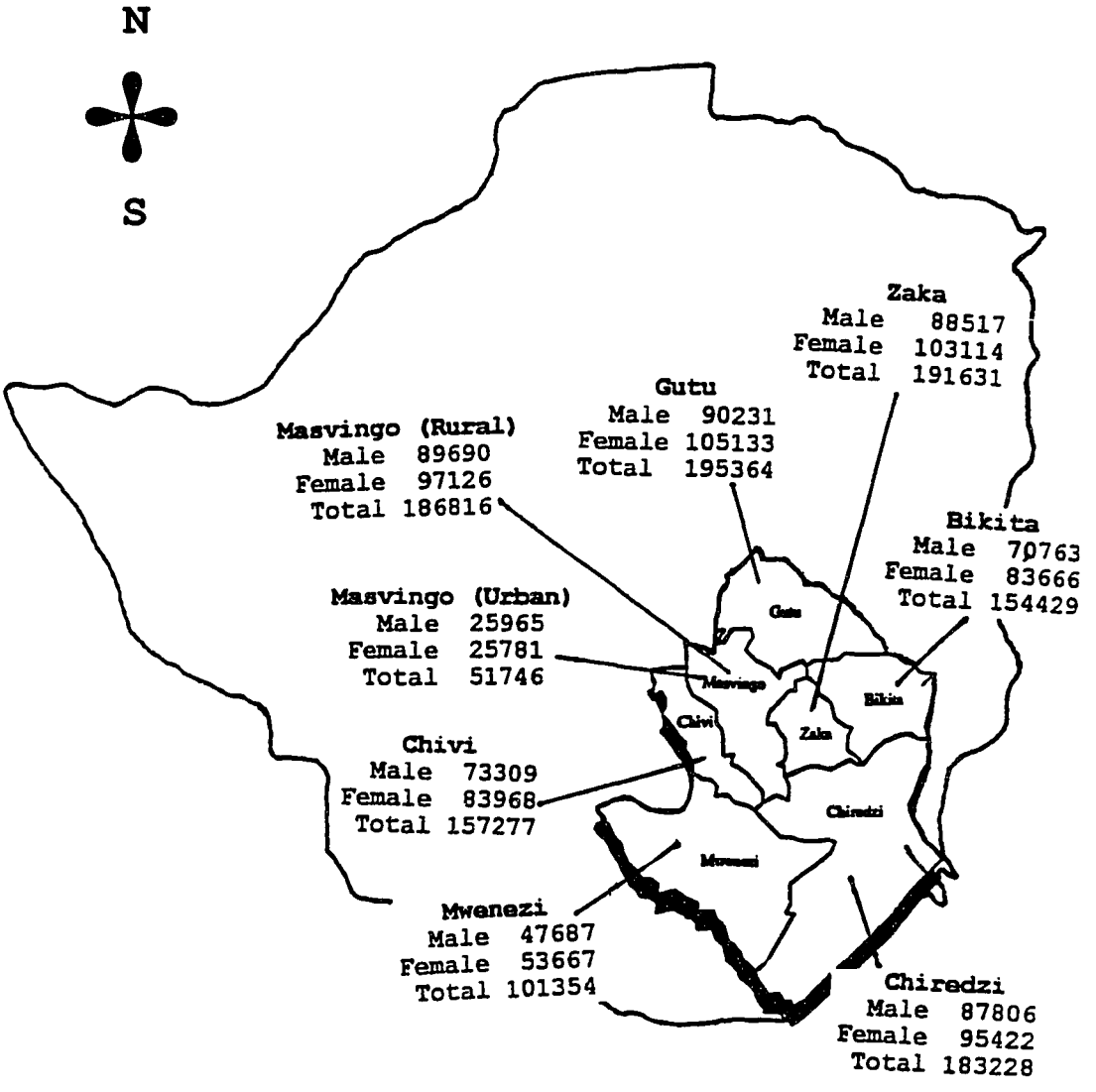
APPENDIX A. Epidemiological assessment of the status of malaria, 1988



APPENDIX B. The population of Zimbabwe by province, 1992



APPENDIX C. The location of Chiredzi district and its population by sex in relation to other districts in Masvingo Province, 1992



APPENDIX D. Chiredzi District - Total population by sex, number of households and average household size in each ward, 1992

Ward No.	Ward Name	Population Males	Females	Total	Household Number	Average Size
00	Chambuta Refugee Camp	8530	10475	19005	4070	4.7
01	Xini	1983	2671	4654	828	5.6
02	Sengwe	2060	2756	4816	784	6.1
03	Maose	1924	2502	4426	763	5.8
04	Chiba Vahlengwe	3016	3978	6996	1059	6.6
05	Chikombedzi	3007	3594	6601	1107	6.0
06	Mukuwini	2086	2484	4570	751	6.1
07	Makambe	1604	1888	3492	605	5.8
08	Batanai	2091	2406	4497	707	6.4
09	Twananani	1989	2296	4285	714	6.0
10	Chechingwe	2853	3314	6167	984	6.3
11	Chibwedziwa	2819	3584	6403	1103	5.8
12	Dzidzela	2724	3082	5806	961	6.0
13	Chitsa	1849	2192	4041	762	5.3
14	Mpinga	2087	2676	4763	890	5.4
15	Tshovani	1675	2184	3859	698	5.5
16	Mkwasine	2288	2907	5195	935	5.6
17	Dikitiki	1802	2106	3908	647	6.0
18	Chizvirizvi	1138	1301	2439	459	5.4
19	Gonakudzingwa	277	238	515	75	6.9
20	Gonarezhou	308	195	503	129	3.9
22	Sabi Valley I.C.A	795	761	1556	367	4.2
23	Triangle	2423	2277	4700	1224	3.8
24	Mkwasine	5795	5120	10915	2695	4.1
25	Ruware	1064	1157	2221	454	4.9
26	Hippo Valley	11687	9983	21670	5884	3.7
27	Chiredzi	1224	1138	2362	554	4.3
28	Eaglemond	451	404	855	229	3.7
29	Triangle Estates	5894	4933	10827	2467	4.4
30	Tshovani	9053	9498	18551	4962	3.7
31	Nyangambe	1063	1112	2175	320	6.8
32	Lundi/Tokwe	247	210	457	117	3.9
District Total		87806	95422	183228	38294	4.8

APPENDIX E. Comparison of demographic indicators of Chiredzi and Masvingo Province based on the 1992 population census of Zimbabwe

	CHIREDZI DISTRICT	MASVINGO PROVINCE
Population	183228	1222581
Rate of natural increase (rate of natural growth per 1000 population)	25	22.2
Crude birth rate (direct) per 1000	36.72	32.99
*Infant mortality rate (infant deaths per 1000 live births)	87	75
*Child mortality rate (child deaths per 1000 children aged 1-4 years)	40	32
*Life expectancy at birth	57	59
*Crude death rate (deaths per 1000 population)	11.7	10.8
Maternal mortality rate (death from maternal causes per 100,000 live births	460	374
Average number of children per woman (total fertility rate	6.55	6.7

* Rates for 1990

APPENDIX F. Age group distribution in Chiredzi District and Masvingo Province, 1992

AGE GROUP (YEARS)	CHIREDZI DISTRICT	MASVINGO PROVINCE
Women 15-49	24.22	22.82
Infants 0-1	3.42	3.07
Children 0-5	16.6	15.25
Children 5-14	30.97	33.45
Under 15	47.58	48.7
Over 15	52.42	51.3
Adults 15-49 (sexually active)	48.89	47.47
Elderly over 59	3.54	5.82

APPENDIX G. Malaria hospital admissions in 1988 and 1989 as a proportion of other parasitic and infectious diseases in Zimbabwe

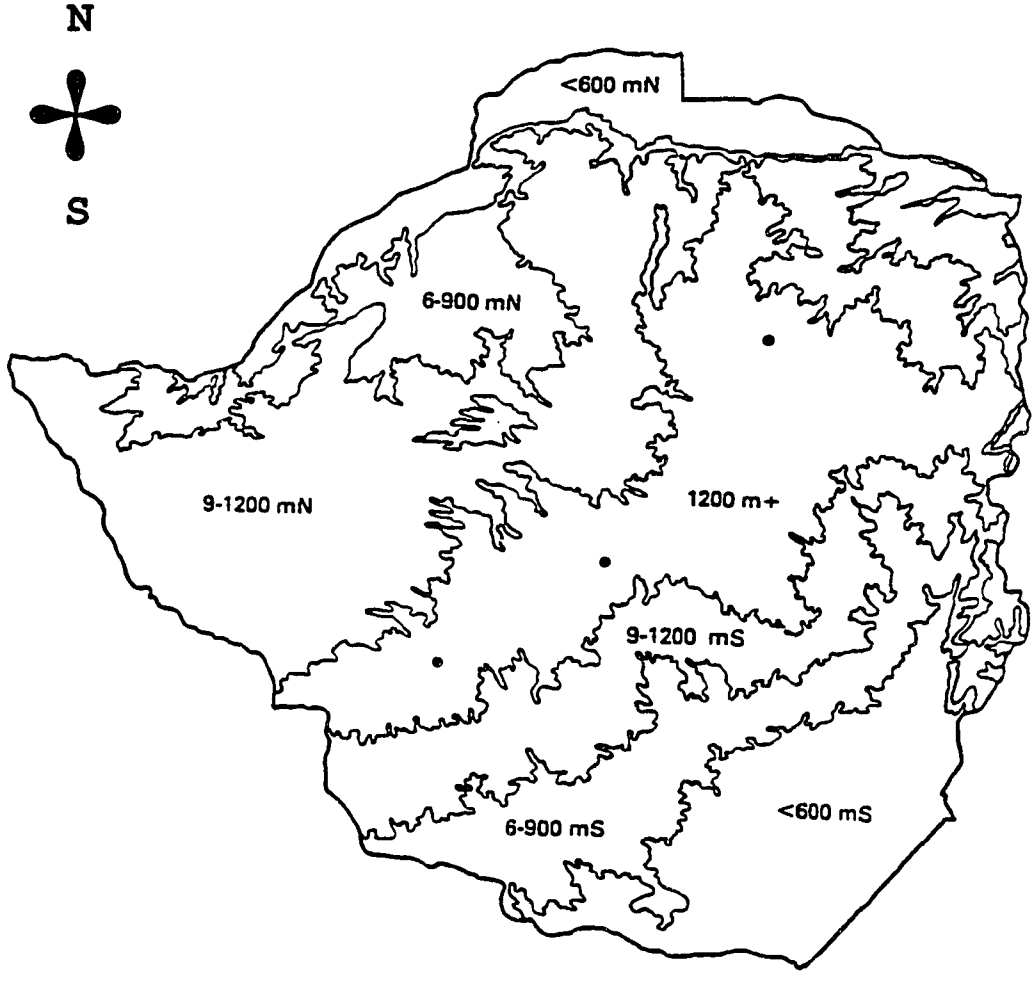
YEAR	1988	1989
Malaria admissions	51929	24048
Other admissions	26478	17050
Total admissions	78407	41098
Proportion of malaria	66.23005	58.51379

APPENDIX H. Malaria mortality as a proportion of all deaths registered between 1980-1987 in Zimbabwe

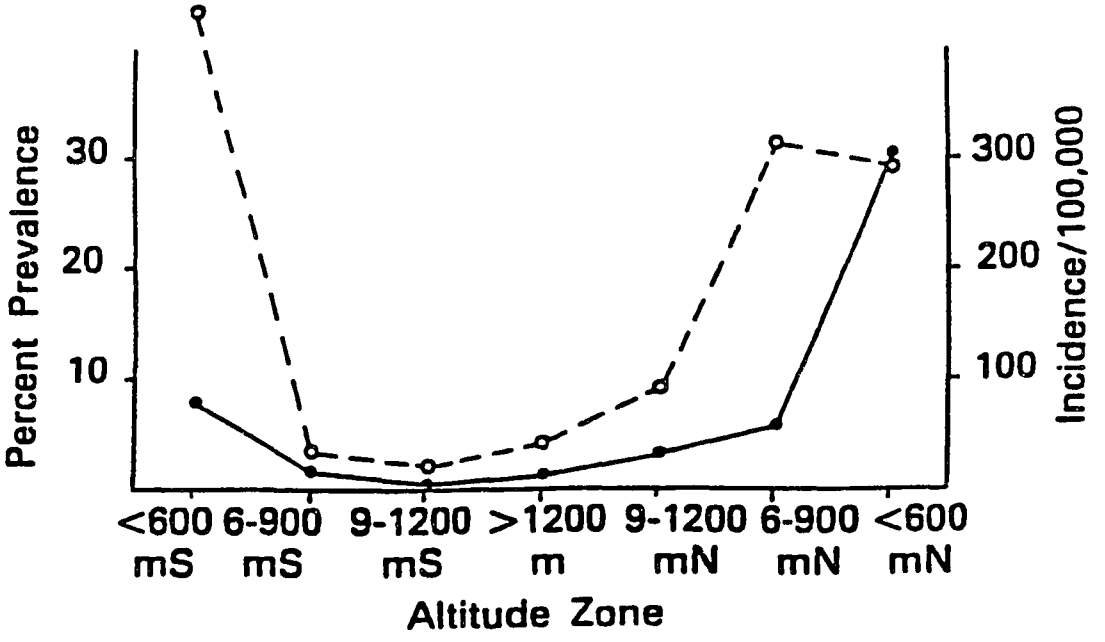
YEAR	NUMBER OF MALARIA DEATHS	NUMBER OF ALL DEATHS	PERCENTAGE OF MALARIA DEATHS
1980	357	7939	4.496788
1981	338	19943	1.69483
1982	245	20042	1.22243
1983	243	23651	1.02744
1984	289	21875	1.32114
1985	424	20688	2.05433
1986	NR*	NR*	NR*
1987	297	18962	1.56629

NR* = no record

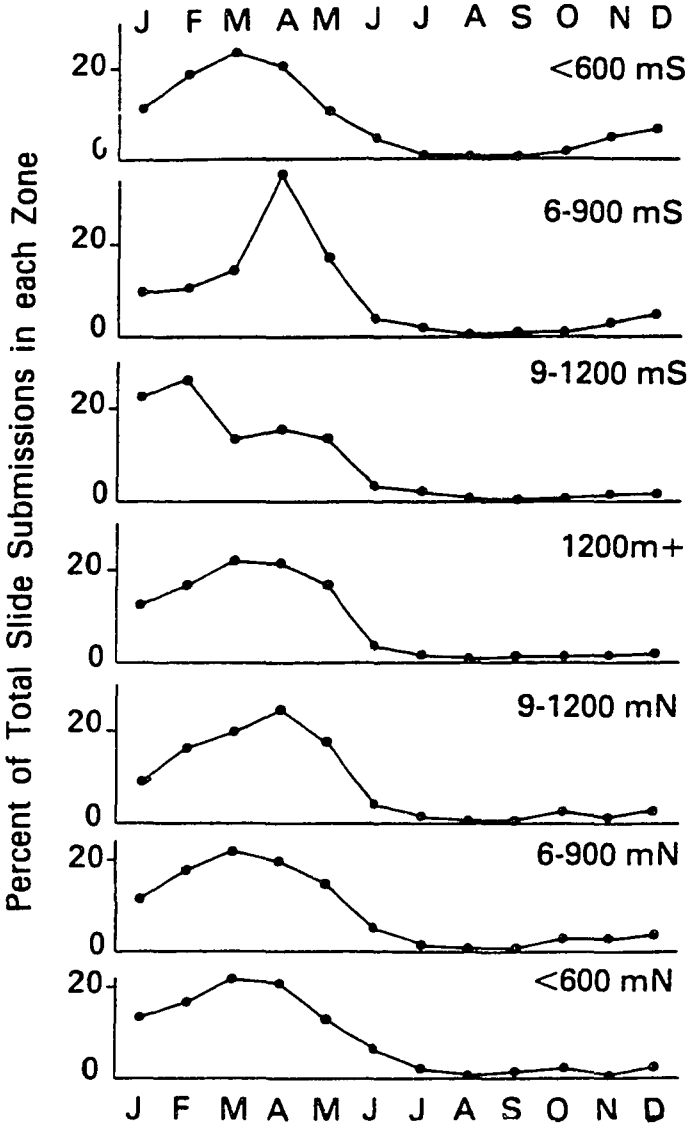
APPENDIX I. Altitudinal classification of Zimbabwe



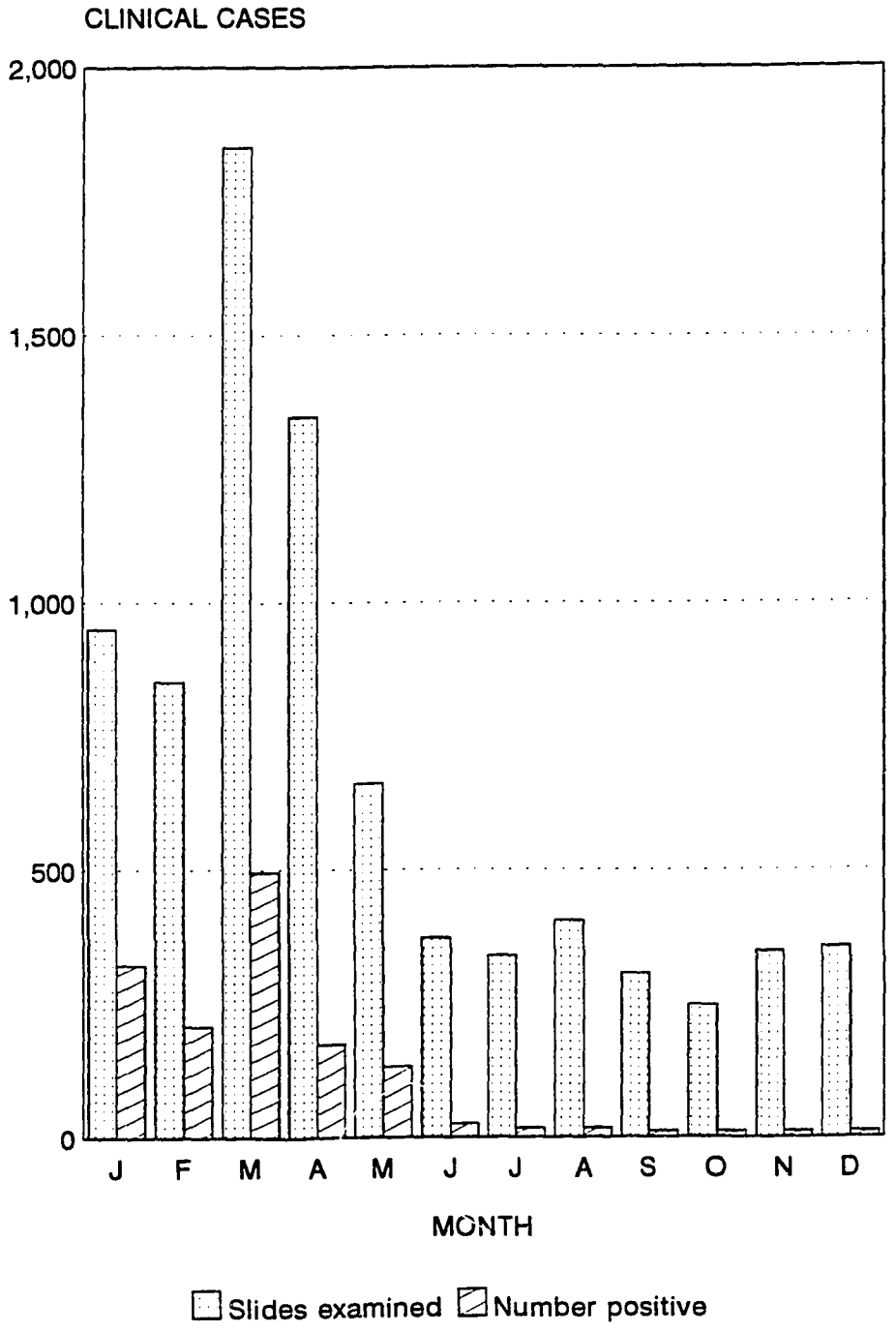
APPENDIX J. Average annual incidence/100000 population (dashed line) of malaria by altitude zone for 1972-1981 and average prevalence of malaria (solid line) by altitude zone for 1969-1981 - Zimbabwe



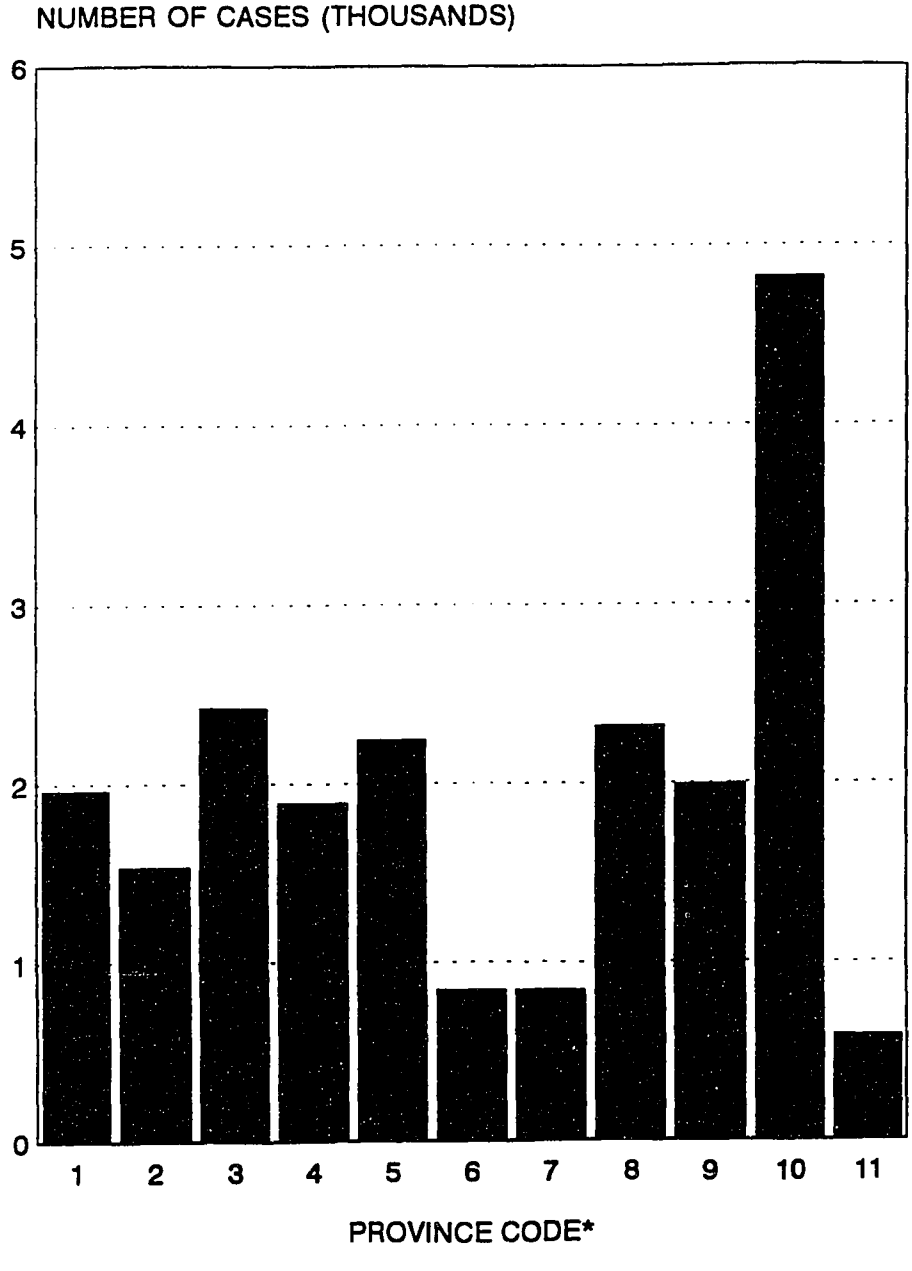
APPENDIX K. Seasonal pattern of positive blood slide submission by altitude zone between 1972 and 1981 - Zimbabwe



APPENDIX L. Malaria blood slide submission and examination in Chiredzi District, Zimbabwe, 1993

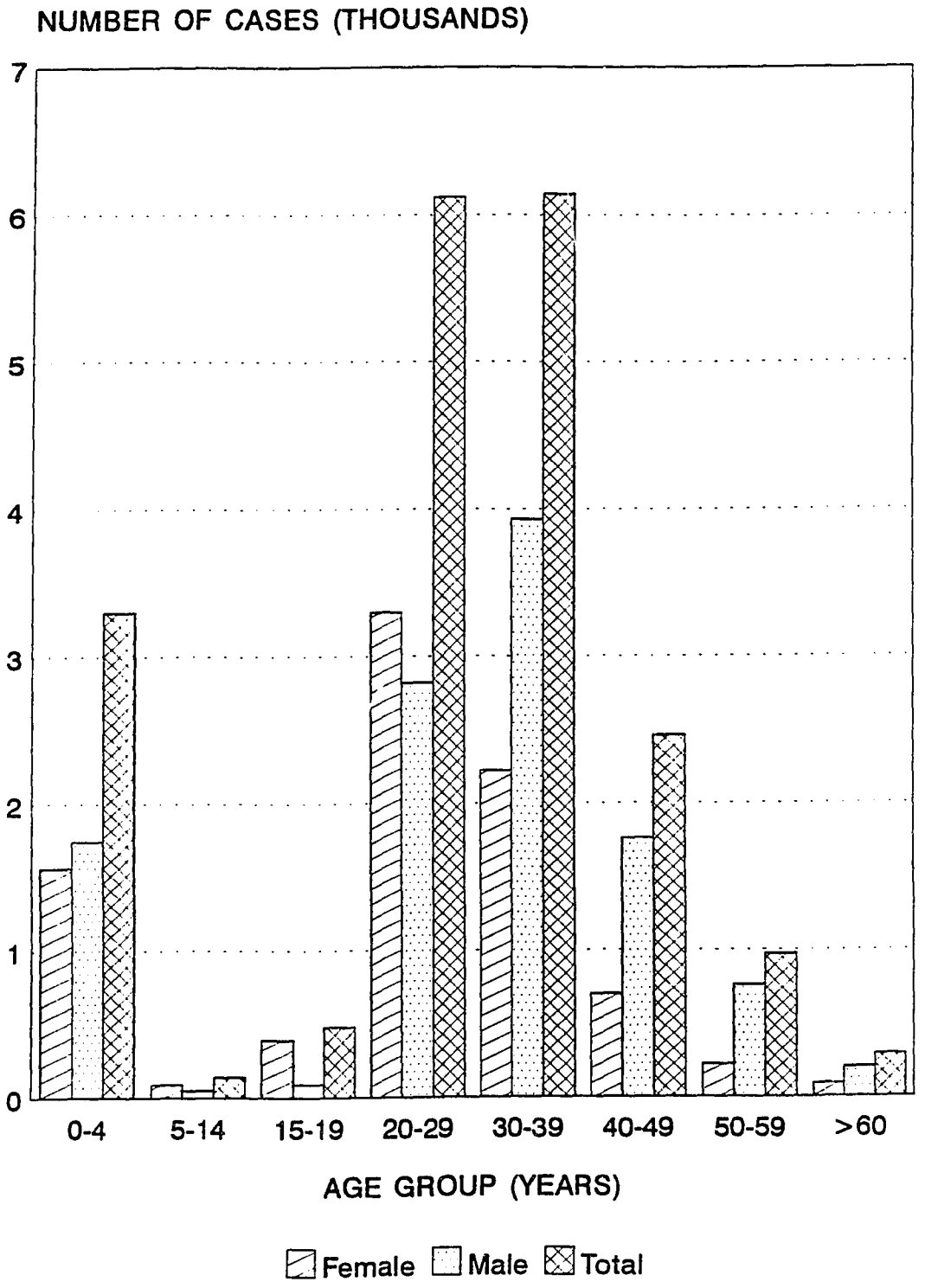


APPENDIX M. Cumulative AIDS cases by province in Zimbabwe from 1987-1993

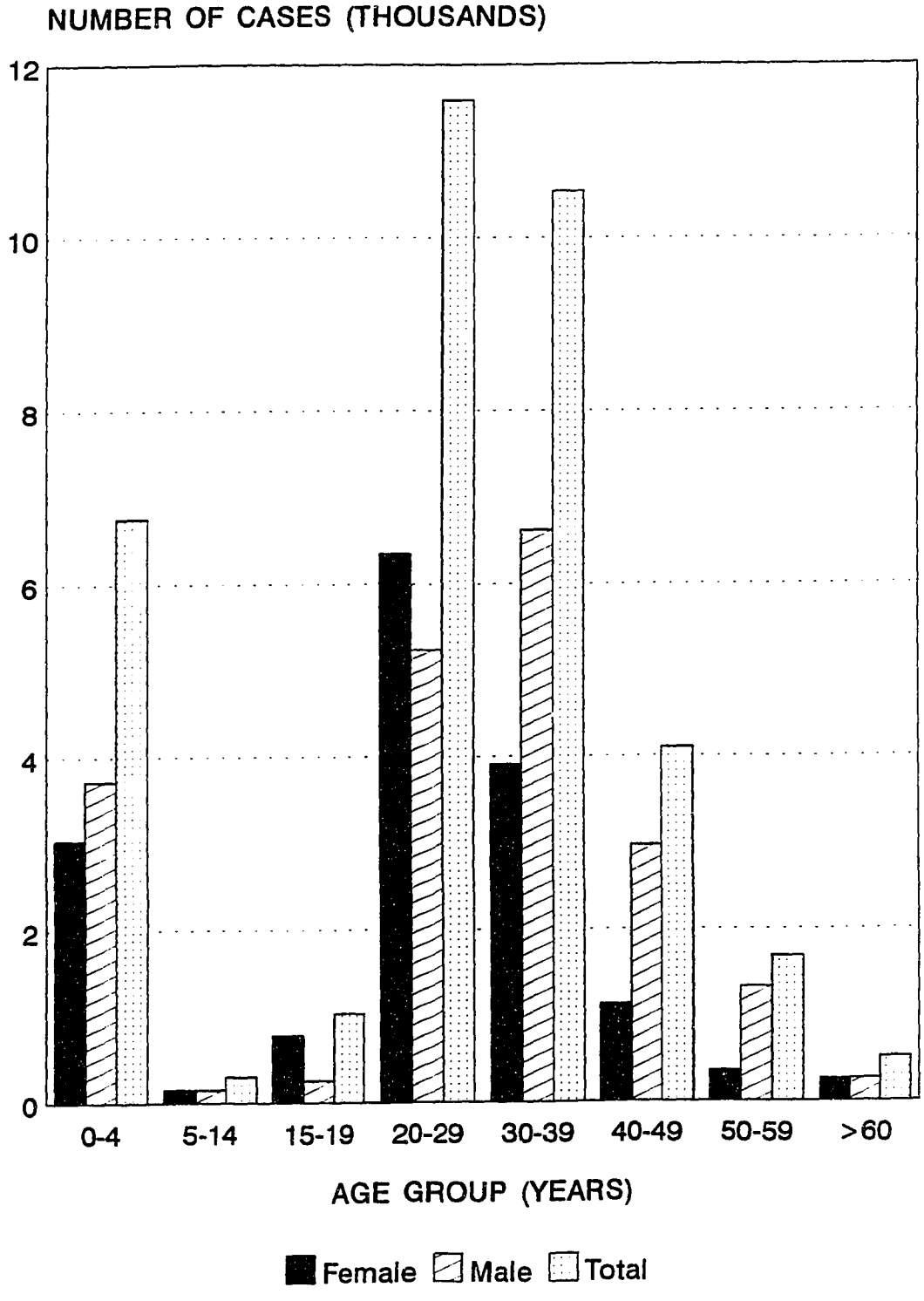


* 1. Manicaland 2. Mashonaland Central 3. Mashonaland East 4. Mashonaland West 5. Masvingo 6. Matabeleland North 7. Matabeleland South 8. Midlands 9. Bulawayo 10. Harare 11. Unspecified

APPENDIX N. Cumulative AIDS cases by age group and sex in Zimbabwe from 1978-1993



APPENDIX O. Cumulative AIDS related cases by age group and sex in Zimbabwe from 1991- 1993



APPENDIX P. Sexually transmitted diseases treated at health care centers by province in Zimbabwe during 1992

PROVINCE	FIRST QUARTER	SECOND QUARTER	THIRD QUARTER	FOURTH QUARTER	1992 TOTAL
Manicaland	31284	30650	27146	26564	115644
Mashonaland Central	16222	16636	19705	18516	71079
Mashonaland East	16703	16342	17178	18793	69016
Mashonaland West	23412	20571	20906	23044	87933
Masvingo	31515	28265	27850	31151	118781
Matabeleland North	9308	8012	8738	5678	31736
Matabeleland South	9010	8206	7983	8680	33879
Midlands	29652	25819	22379	22379	100753
Bulawayo	19901	17239	15658	16236	69034
Chitungwiza	6888	8509	6426	3108	24931
Harare	55260	46918	36112	20850	159140
TOTAL	249155	227167	210081	195523	881926

APPENDIX Q. The distribution of tuberculosis infection diagnosed quarterly in the provinces of Zimbabwe in 1992

PROVINCE	FIRST QUARTER	SECOND QUARTER	THIRD QUARTER	FOURTH QUARTER	1992 TOTAL
Manicaland	545	365	467	659	2036
Mashonaland Central	235	244	282	254	1015
Mashonaland East	49	717	847	1039	2652
Mashonaland West	416	376	563	437	1792
Masvingo	361	472	500	407	1740
Matabeleland North	222	208	411	199	1040
Matabeleland South	260	259	356	295	1170
Midlands	438	424	388	556	1806
Bulawayo	308	204	-	-	512
Harare	364	-	-	-	364
TOTAL	3198	3269	3814	3846	14127

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