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Prevalence of infectious risk factors during pregnancy: An infectiological snapshot of 180 pregnant women in a rural setting of Western Ghana

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Abbreviations

AVT	Antiviral therapy
CRS	Congenital rubella syndrome
CVS	Congenital varicella syndrome
HCC	Hepatocellular carcinoma
HIC	High-income countries
IARC	World Health Organization International Agency for Research on Cancer
ICD-10	International Statistical Classification of Disease and Related Health Problems (10^{th} Revision)
Ig	Immunoglobulin
IPTp	Intermittent preventive treatment during pregnancy
IUGR	Intra-uterine growth retardation
IST	Intermittent screening and treatment
LBW	Low birth weight
LIC	Low-income countries
MDG	Millennium development goals
MMR	Maternal mortality rate
PID	Pelvic inflammatory disease
PROM	Premature rupture of the membrane
RKI	Robert Koch Institut
qPCR	Quantitative PCR
SMPH	St. Martin de Porres Hospital
SPR	Solid phase receptacle
STI	Sexually transmissible infection
UMG	Universitätsmedizin Göttingen
WHO	World Health Organization

1 Introduction

In the last decades economic, social and health issues in developing countries attract more and more attention to the world public. This trend is accompanied by an increasing engagement of private, non-governmental, political and scientific organizations. In 2000 the millennium development goals (MDG), eight international aims to improve living conditions in developing countries, were postulated by the 189 member countries of the United Nations (UN). This development declaration expresses three main objectives for the health sector: i) and ii) Reduction of maternal and under-five child mortality, and iii) Combat against HIV/Aids, malaria and other major diseases.

Current data illustrate the importance but also the massive challenge of this development plan. Due to medical, infrastructural, political and cultural reasons, the number of maternal deaths (death of a woman during pregnancy or within the following 42 days from any cause related or aggravated by the pregnancy, see International Statistical Classification of Disease and Related Health Problems, ICD-10) varies strikingly between developed and developing countries. The same applies to the child mortality rate. Data show that the lifetime risk of a women dying as a direct or indirect result of pregnancy or delivery is about 1:14 in the poorest parts of Africa and Asia compared with 1:11.000 in central Europe (WHO 2010). Consequently, in some parts of the world for every fourteenth women, one dies due to maternal complications. Reasons for this striking difference are multifarious. The main medical causes for maternal death are hemorrhage, hypertensive disorders and infections which go along with a high mortality rate due to weak health systems in low-income countries (LIC). Between 2000 and 2008 the maternal mortality rate (MMR) in Sub-Saharan Africa decreased from 1.000 maternal deaths per 100.000 live births to 640 per 100.000 (Ronsmans and Graham 2006). Conversely, MMR is considerably lower with 14 per 100.000 live births in high-income countries (HIC) (WHO 2010).

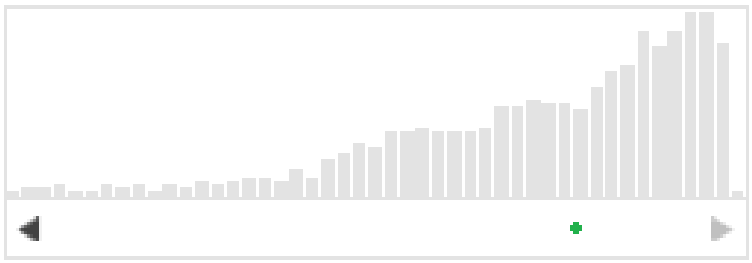
Maternal infections, the third most common cause of maternal death, are an intensive field of research. Current studies focus mainly on single infections, like malaria and HIV infections irrespective of a possible broader spectrum of infectious risk factors and their potential interdependencies (Duda et al. 2005; Apea-Kubi et al. 2006). To fill this

research gap, we wanted to determine infectious risk factors that have an effect on mother and child morbidity/mortality rate in a rural setting of Western Ghana. We wanted to focus hence on maternal infections that either jeopardize directly maternal health or have a potentially negative effect on the newborn by maternofetal transmission. Thanks to the existence of bacteriological laboratories in some Ghanaian regional hospitals, so in Eikwe, we have sufficient local infrastructure for this scientific work.

By using a battery of microbiological and serological tests we determined the prevalence of potentially dangerous infections during pregnancy and possible interdependencies.

2 Literature review

Over the past years, health issues in Africa turned more and more into the focus of national and international research activities. Figure 1 clarifies this trend, on the basis of published scientific papers with the keywords pregnancy, infection and Africa.



Source: PubMed.org 2013

Figure 1 *Number of publications with the keywords "pregnancy, infection, and Africa" from 1963 2011(green point: MDG in 2000). The y-axis shows the number of papers, whereas the x-axis represents the years 1968-2013*

The number of scientific papers containing the keywords pregnancy, infection and Africa has more than doubled between 2000 (140) and 2012 (292). The main catalyst of this development was the declaration of the MDG in 2000 by the UN (see figure 1: green point). On the basis of this publication pool, this literature review was created.

Hence, the following subsection presents a comprehensive literature review on infections which are of medical relevance for mother and child health during pregnancy. In this context the disease patterns of each infection are clarified. For better understanding of our

results, the latest studies on the prevalence of these infections are presented. Apriori vaginal pathogens are illustrated, then non-vaginal agents.

2.1 Vaginal pathogens

This subsection describes vaginal pathogens that may affect mother and child health. In general, these primarily bacterial agents cause none or only mild infections of the urinary and/or genital tract. During pregnancy some specific complications may be associated with these infections. Furthermore, a particular health risk exists due to maternofetal transmission. All listed pathogens are detectable by microbiological testing of vaginal swabs.

Group B streptococci (GBS)

These pathogens can cause neonatal infections like predominantly sepsis (early/late onset), pneumonia or meningitis. Basis for neonatal infections by GBS is a maternal colonization of the genital tract. Maternofetal transmission occurs mainly during delivery. Transmission from mother to newborn is with 11.2 % relatively frequent (Kunze et al. 2011). Current data number the incidence of neonatal GBS infections with 1 per 1.000 live births in industrialized countries (Yu et al. 2011). Furthermore there is evidence on a direct relation between GBS colonization/infection during pregnancy and premature rupture of the membranes (PROM) and preterm birth (Breckwoldt et al. 2008).

In industrialized countries, incidences consistently decreased during the past decades due to antibiotic prophylaxis during pregnancy at pregnant women with risk factors (Speer and Gahr 2013). Recent studies show an incidence of neonatal sepsis caused by GBS in Sub-Saharan Africa comparable to those in industrialized countries. In Malawi incidences diversify between 0.92 of 1.000 newborns with early onset infection with GBS and 0.89 of 1.000 newborns with late onset sepsis (Gray et al. 2007). On the contrary, the mortality rate is much higher with up to 33% in Sub-Saharan Africa (Gray et al. 2007) than 7.6 % in the US (Watson et al. 2003). This spread in the mortality rate is caused by poor medical supply in Sub-Saharan Africa. The maternal carrier rate is also similar between industrialized and developing countries and lies between 5-40% (Halle et al. 1988; Citernesi et al. 1996; Kieran et al. 1998). Current data from Eastern Ghana back up these findings. Enweronu-Laryea et al. (2011) describe a prevalence of 19 % among

pregnant women. Literature on the role and prevalence of GBS in rural areas of Western Ghana is lacking.

Listeria

Listeriosis is a rare and mostly foodborne infection, but more frequent in pregnant women, particularly in the last trimester of pregnancy. Maternal infection could be asymptomatic or causes flu-like symptoms with fever, but is in the majority of cases a mild process. However, transplacental or perinatal transmission may provoke fetal or neonatal listeriosis (Halle et al. 2000). These infections may be severe and involve sepsis, pneumonia and meningitis with mortality rates around 20-30% in the case of adequate medical management (Janakiraman 2008). Current incidence of listeriosis in the general population of Germany is 0.4 per 100.000 inhabitants. Neonatal infections are more frequent with an incidence of 3.7 per 100.000 newborns (RKI 2010b) Due to pregnancy-related immunomodulation leading to TH2 bias, the incidence of listeriosis in pregnancy is higher with 12 per 100.000 (Janakiraman 2008). In addition, case reports hypothesize a relation between maternal listeriosis and repeated abortion (Rappaport et al. 1960). However, this relation could never be confirmed in more recent studies (Manganiello and Yearke 1991). The rate of vaginal carriage among pregnant women lies between 0.2% (Stepanovic et al. 2007) and 2.0% (Lamont and Postlethwaite 1986) in Europe.

As shown in the preceding passage scientific data is available especially on incidences of maternal and neonatal infections in industrialized countries. Unlike in developing countries: here data on listeriosis during pregnancy, neonatal infections and vaginal colonization of women in Sub-Saharan Africa does practically not exist. Consequently, the role of *Listeria* stays unknown.

Neisseria gonorrhoeae

This gram-negative intracellular diplococcic pathogen causes infection and disease in the lower urinary and vaginal tract. Typical clinical manifestations are urethritis in men and endometritis or cervicitis in women. However, a high percentage (approx. 50%) of gonococcal infections is asymptomatic. Undetected and untreated infection can cause complications. In women possible sequelae are pelvic inflammatory disease (PID) or infertility due to tubal blockage after salpingitis. During delivery, infected infants may contract an uncomplicated conjunctivitis. Transmission path in adolescents and adults is

sexual contact and less frequent contact of the infant with the infected birth canal during delivery (Goering et al. 2013).

In Germany, a valid database on the prevalence of infections with *Neisseria gonorrhoeae* does not exist since the obligation to notify the authorities was suspended. In Africa, a number of studies investigated the prevalence among the general population and the relation between gonococcal infection and ectopic pregnancy.

The prevalence of gonococcal infection in Ghana seems to be low with 0.9 percent in women at risk of acquiring sexually transmitted infections (STIs) (Opoku and Sarkodie 2010). Surprisingly, one study found a higher prevalence among the general population of 3.4% (Bentsi et al. 1985). This result could not be confirmed by Pepin et al. (2004), who could not detect any cases of an infection with *Neisseria gonorrhoeae* in 199 Ghanaian women). In 2004, Apea-Kubi et al. (2004) were able to determine a prevalence of 0.4% among pregnant women from the urban area of Accra. In other African countries, prevalences vary from 0.5-14% (Latif et al. 1999; Gray et al. 2001; Marai 2001). Prevalences of infections with *Neisseria gonorrhoeae* during pregnancy in Western Ghana are not yet investigated.

Chlamydia trachomatis

Genital *Chlamydia trachomatis* infections (serotypes D-K) are one of the most frequent STIs worldwide. About 90 percent of these infections are asymptomatic. Common symptomatic manifestations are cervicitis, endometritis, salpingitis, PID and secondary tubal factor infertility (Kiechle 2011). During pregnancy, *Chlamydia trachomatis* infection may cause obstetric pathologies. A significantly higher incidence of PROM or preterm delivery could be shown (Blas et al. 2007). There is limited evidence for an association of maternal *Chlamydia trachomatis* infection with general infant health pathologies like low birth weight and infant death (Blas et al. 2007). But perinatal transmission by a colonized birth canal is frequent with up to 60-70% (Chojnacka et al. 2012). This transmission may provoke localized infections like acute conjunctivitis, and less common pneumonia (Schachter and Grossman 1981). A multiplicity of studies does show a relation between Chlamydia infection and tubal infertility. But valid evidence on the risk of infertility after Chlamydia infection is missing (Wallace et al. 2008).

Epidemiological data show high prevalences in industrialized countries. The Robert Koch institute (RKI) estimates the prevalence in the general female population on 6 % and up to 20% in women at risk of acquiring sexually transmitted infections in Germany (RKI 2010a). Prevalences during pregnancy should be established in the upper range. Data from African countries number the prevalences between 5.3% and 31% (Latif et al. 1999; Fonck et al. 2000; Marai 2001). Recent studies conducted in Ghana found comparable low prevalences. Opoku and Sarkodie (2010) detected *Chlamydia trachomatis* in 4.8 % of tested women. Another study numbered the prevalence on 3.0 % (Apea-Kubi et al. 2004). Prevalences of *Chlamydia trachomatis* during pregnancy in Western Ghana are not yet investigated.

Herpes simplex virus (HSV-1/-2)

Herpes genitalis is the most frequent viral infection of the genital region in industrialized countries (Halle et al. 2000). The causing pathogen is in the majority of cases HSV-2, less often HSV-1. In many cases, primary infections lead to skin efflorescence which consists of painful vesicles on an erythematous base. These skin lesions are attended by inguinal lymphadenopathy. The clinical spectrum ranges from subclinical to fulminant infections especially in patients with immunosuppression. Herpes genitalis does have an outstanding medical importance during pregnancy. Perinatal vertical transmission from mother to the newborn may result in disseminated herpes with neurological involvement and a high rate of mortality (Halle et al. 2000). The incidence ranges strongly from 1 case/3.200 to 1 case/200.000 live births (Anzivino et al. 2009).

Epidemiological data about the vaginal carriage of HSV-1/HSV-2 is limited. There is no current data from Ghana available.

Human papillomavirus (HPV)

This small DNA virus is associated with benign (genital warts) and malignant neoplasm of the cervix, penis, vulva, vagina, anus and oropharynx (Groß 2013). According to its carcinogen potential, the World Health Organization International Agency for Research on Cancer (IARC) classifies the different HPV genotypes as follows: definite carcinogens (group 1), HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59; probable carcinogens (group 2a), HPV 68; possible carcinogens (group 2b), HPV 26, 53, 66, 67, 69, 70, 73, 82,

85, 97; and questionable carcinogens (group 3), HPV 6, 11, 42, 44, 54, 62, 72, 81, 83, 84, 90, and 91 (Schiffman et al. 2009).

Current data indicate a high prevalence of HPV infections worldwide. In women without cervical pathologies, the average HPV rate is around 11 % with higher rates in low-income countries of Sub-Saharan Africa (24%) and Latin America (16%). At the pathogenic level, HPV 16 (3.2%) and HPV 18 (1.4%) are determined as the most prevalent HPV types worldwide. These high prevalences are directly related to a high prevalence of HPV induced cancers. It is estimated that 14.2 % of tumors are linked to an HPV infection in Sub-Saharan countries, compared to less than 1.6% in Northern America (Forman et al. 2012). Up to now, no primary (HPV vaccination) and secondary prevention (HPV screening) has been installed nationwide in Ghana (Louie et al. 2009).

Table 1 illustrates the impact of the mentioned vaginal pathogens on mother and child health. Table 2 summarizes the current data situation for the most important vaginal pathogens.

Table 1: Medical impact on mother and child health

Pathogen	**Medical impact**	
	Mother	Child
GBS	Asymptomatic PROM	Preterm birth Neonatal sepsis
Listeria spp.	Asymptomatic Fever	Neonatal infection
N. gonorrhoeae	Vaginal infertility	Conjunctivitis
Chlamydia trachomatis	Infertility PROM	Preterm birth Conjunctivitis Pneumonia
HSV-1/-2	Herpes genitalis	Herpes neonatorum
HPV	Cervical cancer	

Source: own depiction

Table 2: Maternal prevalence of vaginal pathogens

Pathogen	**Prevalence of vaginal carriage**		
	High-income countries	Low-income countries	Ghana
GBS	5-40% (Halle et al. 1988) 25.6% (Kieran et al. 1998) 6.6% (Citernesi et al. 1996)	4-25% (Marai 2001) 23% (Joachim et al. 2009)	19% (Enweronu-Laryea et al. 2011)
Listeria spp.	2.0% (Lamont and Postlethwaite 1986) 0.1% (Stepanovic et al. 2007)	No data	No data
N. gonorrhoeae	2.6% (Patel et al. 2008)	1.7 % (Gray et al. 2007) 3.9 % (Latif et al. 1999) 0.5 -14 % (Marai 2001)	0.4 % (Apea-Kubi et al. 2004)
Chlamydia trachomatis	11.4% (Patel et al. 2008)	5.3% (Latif et al. 1999) 9.0% (Fonck et al. 2000) 7-31% (Marai 2001)	3.4 % (Apea-Kubi et al. 2004)
HSV-1/-2	No data	0.9 % (Latif et al. 1999)	No data
HPV	11-12% (Forman et al. 2012)	24% (Forman et al. 2012) 37% (Akarolo-Anthony et al. 2014)	10.7% (Domfeh et al. 2008)

Source: own depiction

2.2 Non-vaginal pathogens

This chapter lists parasitic, viral and bacterial infections that may threaten maternal or/and fetal health.

2.2.1 Parasitic pathogens

Infections by the genus *Plasmodium* may directly threaten maternal health and the course of pregnancy. In recent years therapies, prophylaxis and prevention have been ameliorated the medical situation in developing countries, so that the mortality rates are plummeting. In the immunocompetent pregnant woman, *Toxoplasma gondii* causes a mild maternal infection but may cross the placental barrier and infect the fetus. This may lead to congenital toxoplasmosis with severe malformations.

Plasmodium spp.

The World Malaria Report 2012 states that approximately 32 million pregnant women live in endemic areas of *Plasmodium falciparum* (WHO 2013). Consequently, mothers, their fetus and respectively the infant, are at high risk of *Plasmodium falciparum* infection. A broad spectrum of studies has proven the negative effects of malaria during pregnancy for mother and child health. The clinical course of malaria infection during pregnancy depends on the pre-existing immunity and may even take a subclinical course without fewer. Apart from that, severe courses of disease are possible. Precise data on the maternal mortality rate (MMR) of malaria are scarce. The MMR lies between 0.5% and 23.0% in Sub-Saharan Africa (Desai et al. 2007). The prevalences of severe anemia (hemoglobin <7g/l) in Africa range from 5 to 10% among pregnant women. Approximately one third of cases are associated with an infection by *Plasmodium falciparum* (Shulman et al. 2002). In addition, malaria may affect the health of the developing fetus: perinatal death or low birth weight (< 2.500g) are a result of preterm birth or intrauterine growth retardation (IUGR) induced by the parasite (Guyatt and Snow 2004). The Mangochi Malaria Research Project (Slutsker et al. 1996) showed that a low birth weight increases the likelihood by 9 to die in the first month of life.

The burden of malaria may be reduced by an intermittent preventive treatment during pregnancy (IPTp), which is now common policy in many Sub-Saharan countries including Ghana. The initiation of IPTp (usually Sulphadoxine-pyrimethamine thrice during pregnancy) has led to a considerable decrease in maternal anemia (reduction by 33%) and an increase in birth weight by approximately 130 g (Hommerich et al. 2007).

Prevalences of malaria during pregnancy in Africa are high and vary from 10.4% in Benin (Newman et al. 2003) to 15.2% in Sudan (Ouedraogo et al. 2012). In Ghana, the prevalence of malaria-infected women decreased from 34.9% without IPTp in 2000 to 15.0% after the introduction of IPTp in 2006 (Hommerich et al. 2007). Furthermore, there is evidence that a coinfection with HIV provokes negative effects (Nkhoma et al. 2012). The rate of coinfections (malaria/HIV) in Ghana is largely unknown.

Toxoplasma gondii

This protozoan parasite causes a mild and benign infection among immunocompetent children and adults. *Toxoplasma gondii* is transmitted by close contact to cats (definitive host) and raw/uncooked contaminated food. Primary infection during pregnancy may lead to the transplacental transmission of the parasite to the fetus (Kiechle 2011). The risk of transplacental transmission increases during the course of pregnancy with a mean transmission rate of 50%. Congenital toxoplasmosis is most severe if transmission occurs during the first trimenon (Jones et al. 2001). One tenth of infected fetuses develop severe ophthalmologic and neurologic impairment. Worldwide, the frequency of congenital toxoplasmosis ranges between 3 and 6 cases of 1.000 live births (Speer and Gahr 2013) with country-specific variations.

The prevalence of acute toxoplasmosis during pregnancy is lower in high-income countries, as shown by Roos et al. (1993) with a rate of acute toxoplasmosis of 0.6% among 2104 pregnant women. A Danish study showed an IgG seropositivity rate of 27.4% with a seroconversion rate of 1.16% per year and an incidence of primary *Toxoplasma* infection during pregnancy of 0.64%. (Lebech et al. 1993). Another study determined a seroprevalence of 43.8% among pregnant women in France (Berger et al. 2009). Concerning low-income countries, a Ghanaian study outlines a relatively high seroprevalence of 88.7% in pregnant women (Ayi et al. 2009). *Table 3 and 4* summarize the medical impact and the current data situation of malaria and toxoplasmosis.

Table 3: Medical impact of malaria and toxoplasmosis on mother and child health

Pathogen	**Medical impact**	
	Mother	Child
Plasmodium	Asymptomatic Fever	Perinatal death LBW Neonatal malaria
T.gondii	Mild infection	Stillbirth Congenital toxoplasmosis

Source: own depiction

Table 4: Maternal seroprevalence of parasitemia/specific antibodies

Pathogen	**Prevalence during pregnancy**		
	High-income countries	Low-income countries	Ghana
Plasmodium	No incidences	10.4% (Newman et al. 2003) 15.2% (Ouedraogo et al. 2012)	34.9% (Hommerich et al. 2007) 15.0% *****(Hommerich et al. 2007)
T. gondii.	27.4% (Lebech et al. 1993) 43.8% (Berger et al. 2009)	75.4% (Onadeko et al. 1996) 20.3% (Linguissi et al. 2012) 6.4% (Kistiah et al. 2012)	88.7% (Ayi et al. 2009)

Source: own depiction, ***** + IPTp

2.2.2 Viral pathogens

Most viral infections acquired during pregnancy are clinically unapparent, mild, or associated with a typical skin rash in the woman (rubella virus, parvovirus B19, and cytomegalovirus), but pose an even life-threatening hazard to the fetus. Depending on the stage of pregnancy, the above mentioned viral pathogens may cross the placenta and infect the developing fetus (vertical transmission). This may result in stillbirth and partly severe malformations. Due to limited and complex management of intrauterine viral infections, the focus lies on prevention, which mainly consists of vaccination and protective measures and rules of conduct.

Other viral infections, such as some hepatotropic viruses and the human immunodeficiency virus, affect both mother and child health. All hepatotropic viruses (amongst others HBV, HCV, HEV) may cause hepatitis and maternofetal transmission is possible. Particular HEV is able to provoke fulminant hepatitis during pregnancy with high maternal mortality.

Hepatitis B virus (HBV)

Hepatitis B virus belongs to the family of hepadnaviruses. The acute infection may result in clinical symptoms of a liver damage (fever, nausea, loss of appetite, jaundice). Acute infection of adults results in a chronic course of disease in 10% (Goering et al. 2013). Current data indicate that 15% to 40% of chronically-infected patients develop cirrhosis and/or hepatocellular carcinoma (HCC) (Lok and McMahon 2009). Incidence of HCC in West Africa is approximately 20 cases/100.000 inhabitants (El-Serag and Rudolph 2007).

Both vertical (mother to child) and horizontal (sexual, parenteral) transmission are common. Depending on the maternal virus load, the rate of maternofetal transmission lies between 3.3% and 55.5% (Candotti et al. 2007). Especially perinatal transmission without treatment/vaccination of the affected newborn may result in up to 90% in a chronic progression. In contrast, maternal acute and chronic HBV infections do most likely not influence maternal mortality rates during pregnancy. But there is some evidence that prevalences of low birth weight and preterm birth are slightly higher (Jonas 2009).

Low prevalence rates are recorded in industrialized countries. As an example, in 1998 the seroprevalence rate of HBV infection (HBs carrier) in the German general population was found to be 0.6% (RKI 2013a). In contrast, an accumulation of HBV infections has been shown in middle- and low-income countries: Among pregnant women in Africa, the seroprevalence is close to 10% (Olokoba et al. 2011; Ramos et al. 2011; MacLean et al. 2012). Two studies determined an even higher maternal HBs carrier rate in the Eastern Region of Ghana ranging between 10.6% (Cho et al. 2012) and 16% (Candotti et al. 2007).

Hepatitis C virus (HCV)

HCV causes an acute infection of the liver that processes up to 75% of cases subclinically. Nine out of ten patients develop a chronic infection with cirrhosis. According to the WHO (2013), about 150 million people worldwide are chronically infected with HCV (Piper 2013). Mother-to-child transmission occurs in 5-10% of the cases. This mode of infection is the most significant cause for pediatric HCV infection (Le Campion et al. 2012). Prevalences vary strongly between high- and low-income countries: The seroprevalence among pregnant women in Europe is about 1-2% (Martyn

et al. 2011), and ranges in Sub-Saharan Africa from 2.1% in Burkina Faso (Ugbebor et al. 2011) to 5.2% in central Ghana (Apea-Kubi et al. 2006).

Hepatitis E virus (HEV)

Probable transmission of HEV is by close contact to animals and consumption of swine, wild boar or game (Clayson et al. 1995; Tei et al. 2003; Tamada et al. 2004). Also bad hygienic conditions and surface water are suspected sources of infection (Martin-Latil et al. 2012). Endemic outbreaks are described in Africa and Southeast Asia in context with flooding and successive contamination of drinking water (Tsega et al. 1993; Guthmann et al. 2006; Nelson et al. 2011).

Infections with this RNA virus tend to cause a mild hepatitis. A high number of infections probably does have a subclinical course (Piper 2013), but several studies document that fulminant HEV infection during pregnancy may frequently lead to liver failure with a fatality rate of 7-40% (Howard et al. 2010; Labrique et al. 2012).

Currently there is only limited data about prevalences of HEV infections among pregnant women in high- and low-income countries. Nevertheless, some research groups focus on HEV infections in endemic areas of Africa and Asia. Adijei et al. (2009) showed a high seroprevalence of HEV (28.7%) in 157 pregnant women from Ghana. Until now there are only 3 reported and analyzed cases of hepatic failure due to HEV infection in Ghana. In one of these cases, mother to child transmission was confirmed (Bonney et al. 2012).

Cytomegalovirus (CMV)

CMV is one of the most common viral infections during pregnancy. Seroconversion indicates an acute primary infection and takes place in about 1 to 4% of pregnant women. The average prevalence of congenital CMV infection is estimated on 0.64% of all births (Johnson et al. 2012). Congenital CMV infection may lead in approximately one third of cases to severe neurologic defects like hear and vision loss. Less frequently is fetal or neonatal death (Adler 2011). Prevention of primary maternal infection is difficult, since infected contact persons are frequently asymptomatic. Currently neither a licensed vaccine nor a postexposure prophylaxis is available (Johnson et al. 2012).

Previous studies show a high seroprevalence worldwide. Prevalences tend to be slightly lower in industrialized countries. Picone et al. (2009) showed that 53.2% of 4.287 tested

pregnant women in France were IgG negative. In middle-and low-income countries, the median seroprevalence of CMV-specific IgG antibodies was 95.7% (Velu et al. 2011). Single studies in Africa showed seroprevalences that ranged from 72.2% in Sudan (Hamdan et al. 2011) to 97.2% in Benin (Rodier et al. 1995). Consequently, only a minority of people living in middle- and low-income countries are seronegative and thus susceptible for a primary CMV infection. There is no data available on the CMV status among pregnant women in Ghana.

Rubella virus

Primary infection with the rubella virus during pregnancy may cause a mild maternal infection with typical skin rash but severe embryopathy. This negative effect on the pregnancy outcome was firstly described by Norman Gregg. The Australian ophthalmologist made in 1941 the connection between a "German measles" epidemic and an unusual accumulation of congenital cataract, hearing loss and cardiac malformations among infants (Gregg 1991). Besides these malformations, there is a risk of 50% of spontaneous abortion (Cutts et al. 1997). Nowadays, the prevalence of congenital rubella in industrialized countries is marginal. In Europe, the prevalence fluctuates between 0 and 3 cases per year (Massa Calles et al. 2015). Intensive rubella vaccination programs in industrialized countries have led to this low infection rate. In contrast, similar vaccination programs do exist in only 28% of developing countries (Lawn et al. 2000). Ghana does not have a nationwide vaccination program against rubella. Statistic estimations number the incidence of Congenital Rubella Syndrome (CRS) to 1 case per 1.000 births in developing countries without vaccination programs (Cutts and Vynnycky 1999).

An important parameter to evaluate a possible threat by rubella virus infections is the seroprevalence of IgG positive pregnant women; IgG positive women gained immunity before pregnancy and are consequently not susceptible for an infection by the rubella virus. IgG seropositivity is over 90% in Sub-Saharan Africa (Lawn et al. 2000; Corcoran and Hardie 2005; Barreto et al. 2006). Seroprevalences in Europe range from 85.5% (Calimeri et al. 2012) to 95% (Hernandez Diaz et al. 2011).

Human immunodeficiency virus (HIV)

HIV infection and Aids is still a major health issue in Sub-Saharan Africa. According to the UNAIDS report, 1.6 million people died in 2012 due to HIV infection and AIDS. In the same year, 2.3 million people were newly infected. Besides horizontal transmission, mother-to-child transmission of HIV is the major infectious path. Depending on the maternal CD4 count, probabilities of transmission are estimated to be 30% in the prenatal period (pregnancy and delivery) and 28% in the postnatal period (breast feeding). Antiviral therapy (AVT) may significantly decrease the transmission rate to 2% and 0.2%, respectively (Rollins et al. 2012). Transmission rates illustrate the importance of sophisticated medical care during pregnancy (HIV screening, AVT). By improving the coverage of AVT during pregnancy in developing countries, United Nations plan to eliminate pediatric HIV infections until 2015 (UNAIDS 2012a).

Whereas the seroprevalence rate among pregnant women is around 0.5 % in the U.S. (Nicoll et al. 1998) prevalence rates can be found in Africa and Asia. According to the HIV Sentinel Surveillance Report by UNAIDS, the HIV prevalence in the general population of Ghana was found to be 2.1%. The regional HIV prevalence in the Western Region of Ghana is lower with 1.9% (UNAIDS 2012b). Among pregnant women, slightly higher infection rates were recorded and range from 3.1% (Duda et al. 2005) to 6% (Apea-Kubi et al. 2006). However, both studies were conducted in the urban region of Accra; the prevalence of HIV infection in rural areas of Western Ghana is unknown.

Varicella-zoster virus (VZV)

Varicella-zoster virus (VZV) belongs to the family of herpesviridae and causes varicella that usually occurs during childhood and varicella zoster (shingles) that occurs after reactivation. Varicella is one of the exanthematous viral diseases that may have severe negative impact on mother and infant during pregnancy. Consequently, primary infection acquired during pregnancy increases the risk for maternal, fetal, and neonatal morbidity and mortality (Daley et al. 2008). In 10%, varicella progresses to pneumonia with a case fatality rate of 10%. Fetuses bear a risk to acquire congenital varicella syndrome (CVS), particular if maternofetal transmission occurs during the first trimenon. Clinical manifestations of this syndrome are dermatomal scarring (70%), ocular deformations (60%), neurologic impairment (30%), low birth weight (80%), and mental retardation

(50%) (Müllegger and Glatz 2010). Perinatal maternofetal transmission (5 days prior to 2 days after) results in 17-30% of cases to neonatal varicella with a fatality rate of 30% (Daley et al. 2008). Incidence rates of neonatal varicella are 6 cases/100.000 life births in industrialized countries (Müllegger and Glatz 2010). It is important to emphasize that only VZV-seronegative pregnant women are susceptible for a primary infection. Seroprevalences are higher in industrialized countries (Karunajeewa et al. 2001; Alanen et al. 2005) than in middle- and low income countries (Hannachi et al. 2011).

Parvovirus B 19 (PB19)

Human PB19 is a single-stranded DNA virus. The main risk factor for a PB19 infection is close contact to children (Jensen et al. 2000). Typical clinical manifestation in childhood is a mild infection with an exanthematous skin lesion, erythema infectiosum, also named "fifth disease". Further symptoms or pathologies caused by PB19 are arthralgia in adults, especially among women, and transient aplastic crisis. Severe courses of disease may occur in patients with chronic anemia, common in sickle cell disease and other hemolytic conditions (Goering et al. 2013). Infection during pregnancy is clearly associated with intrauterine fetal death and hydrops fetalis (Miller et al. 1998; Tolfvenstam et al. 2001). A prospective evaluation of 1.018 pregnant women with acute PB19 infection showed a fetal death rate of 6.3 %. In 3.9 % of the pregnancies hydrops fetalis was recorded. Due to poorer medial supply, fetal mortality is significantly higher in developing countries (Enders et al. 2004). Latest studies in industrialized countries suggest a high prevalence of previous but low prevalence of acute PB19 infection among pregnant women. Enders et al. (2007) tested around 6.000 asymptomatic pregnant women and showed an IgG and IgM prevalence of 69.2% and 0.7%, respectively. A Nigerian study determined an IgG prevalence of 40.7% among pregnant women. Whereas IgM antibodies were detected in 13.2 %, IgG antibodies were found in 27.5% of the examined women (Emiasegen et al. 2011). So far, there are no data on the prevalence of PB19 infection in Ghana. Table 5 and 6 summarize the medical impact and the current data situation of the above mentioned viral infections.

Table 5: Medical impact of viral infections on mother and child health

Pathogen	**Medical impact**	
	Mother	Child
HBV	Hepatitis	Preterm birth
	Cirrhosis	LBW
	HCC	Chronic infection
HCV	Hepatitis	Chronic infection
	Cirrhosis	
HEV	Fulminant hepatitis	Abortion
		Still birth
		Preterm birth
CMV	Mild infection	Fetal death
		Neonatal death
		CNS damage
Rubella virus	Mild infection	Abortion
		CRS
HIV	AIDS	Transmission
VZV	Pneumonia	Stillbirth
		CVS
		Neonatal varicella
HSV -1/-2	Herpes genitalis	Herpes neonatorum
PB19	Mild infection	IUFD
		Hydrops fetalis

Source: own depiction

Table 6: Seroprevalences of viral infections

Pathogen	Seroprevalence during pregnancy		
	High-income countries	Low-income countries	Ghana
HBV	0.6% (RKI 2013a) 0.1% (Salleras et al. 2009)	8.0% (MacLean et al. 2012) 8.2% (Olokoba et al. 2011)	16% (Candotti et al. 2007)
HCV	0.4% (RKI 2013b) 1.4% (Martyn et al. 2011)	6.4% (Zahran et al. 2010) 3.6% (Ugbebor et al. 2011)	5.2% (Apea-Kubi et al. 2006)
HEV	16.8% * (Faber et al. 2012)	14.1% (Caron and Kazanji 2008)	18.5 (Adjei et al. 2009)
CMV	46.8% (Picone et al. 2009) 56.3% (Alanen et al. 2005)	72.2% (Hamdan et al. 2011) 87% (Bello and Whittle 1991) 97.2% (Rodier et al. 1995)	No data
Rubella virus	85.8% (Calimeri et al. 2012) 95% (Hernandez Diaz et al. 2011)	95,3% (Barreto et al. 2006)	92.6% (Lawn et al. 2000)
HIV	0.025–0.19% (Nicoll et al. 1998)	2.1% (UNAIDS 2012b)	6% (Apea-Kubi et al. 2006) 3.1% (Duda et al. 2005)
VZV	96.2% (Alanen et al. 2005)	80.9 (Hannachi et al. 2011)	No data
HSV-1/-2	54.3% (Alanen et al. 2005) 82.7% (Kucera et al. 2012)	No data	No data
PB19	58.6% (Alanen et al. 2005) 66% (Jensen et al. 2000)	27.5 % (Emiasegen et al. 2011) 61% (Elnifro et al. 2009) 24.9% (Schoub et al. 1993)	No data

Source: own depiction, * adult population

2.2.3 Bacterial pathogens

The following section focuses on the non-vaginal bacterial pathogens *Treponema pallidum*, the causative agent of syphilis, and *Brucella spp.*, a germ that may provoke a febrile zoonosis in humans. Both pathogens may have a negative effect on the pregnancy-outcome by maternofetal transmission.

Brucella

Brucellosis is a worldwide occurring zoonosis that is caused by bacteria of the genus *Brucella*. Sources for human infection are farm animals like sheep, goat (*B. melitensis*), swine (*B. suis*), and cattle (*B. abortus*). The infection occurs after close contact with body fluids (skin lesions) or oral uptake of contaminated milk products. A high percentage of infections are subclinical, but may have a negative impact on the pregnancy outcome.

Several studies confirm a likely correlation between maternal brucellosis and a significant accumulation of abortion, intrauterine fetal death (IUFD) and preterm birth (Khan et al. 2001; Elshamy and Ahmed 2008).

Data on prevalence rates of maternal brucellosis are scare. Sufficient data are only available from studies performed in the Middle East indicating a prevalence among pregnant women of 12.2% in Egypt (Elshamy and Ahmed 2008). Epidemiological data from Europa and Sub-Saharan Africa are very rare.

Treponema pallidum

Syphilis, a worldwide occurring STI caused by *Treponema pallidum* is known as one of the major pregnancy-related infections. The disease can be phased in three subsequently occurring stages of primary, secondary and tertial (latent) syphilis. Primary syphilis is characterized by a localized so-called chancre, a papular lesion, which is typically situated in the genital region, but may also be found at extragenital locations (rectal, oral). Without treatment, secondary syphilis, a systemic manifestation, follows 2-6 weeks after spontaneous disappearance of the chancre. This second phase often involves influenza-like symptoms with generalized lymphadenopathy, disseminated maculopapular rash, condyloma latum and mild hepatitis. If left untreated, approximately 35% of the patients develop tertiary syphilis with cardiovascular and central nervous manifestations (Hahn et al. 2009).

The stage of secondary syphilis during pregnancy has a severe impact on the fetus, because through its hematogenous dissemination the bacteria may cross the placenta resulting in spontaneous abortion, IUGR, preterm delivery, stillbirth, or congenital syphilis.

Furthermore, this transmission may result in congenital syphilis during the postnatal period. Congenital syphilis is traditionally divided into an early and late syndrome. Clinical characteristics of the early form (appearance within two years postnatal) involve a wide spectrum of clinical symptoms. Bone abnormalities, hepatosplenomegaly, skin lesions and anemia are the most frequent features. The late form (appearance after two years postnatal) is generally described by the term traditionally known as Hutchinson's triad, a clinical complex of dental dystrophies, interstitial keratitis and eight nerve deafness (Genc and Ledger 2000).

In high-income countries, syphilis is less frequent than in low-income countries. Nevertheless, rising incidence rates of syphilis are recorded in European countries. Epidemiological data from Germany confirm this increasing trend. In 2012, an incidence rate of 5.4/100.000 inhabitants was reported, reflecting an increase of 20% compared to 2011 (RKI 2013c). The seroprevalence of maternal syphilis in other high-income countries is also very low: Towards the end of the 20th century, several studies from Norway and the United States show a seropositivity rate between 0.02% and 4.5% in pregnant women (Genc and Ledger 2000). In contrast with these data, prevalence rates of maternal syphilis in Africa are very high. Different studies amount the prevalence during pregnancy to 3.3% in Uganda (Gray et al. 2001), 3.8% in Zimbabwe (Latif et al. 1999), and of 7% in Kenya (Fonck et al. 2000). The only existing study suggests a similar prevalence rate in Ghana. This study reports a seroprevalence rate of 7.1% among 294 pregnant women (Apea-Kubi et al. 2004).

Table 7 and 8 summarize the medical impact and the current data situation of syphilis and brucellosis.

Table 7: Medical impact on mother and child health

Pathogen	**Medical impact**	
	Mother	Child
Brucella	Mild infection	Abortion
		IUFD
		Preterm birth
Treponema pallidum	Syphilis	Abortion
		IUGR
		Preterm birth
		Congenital syphilis

Source: own depiction

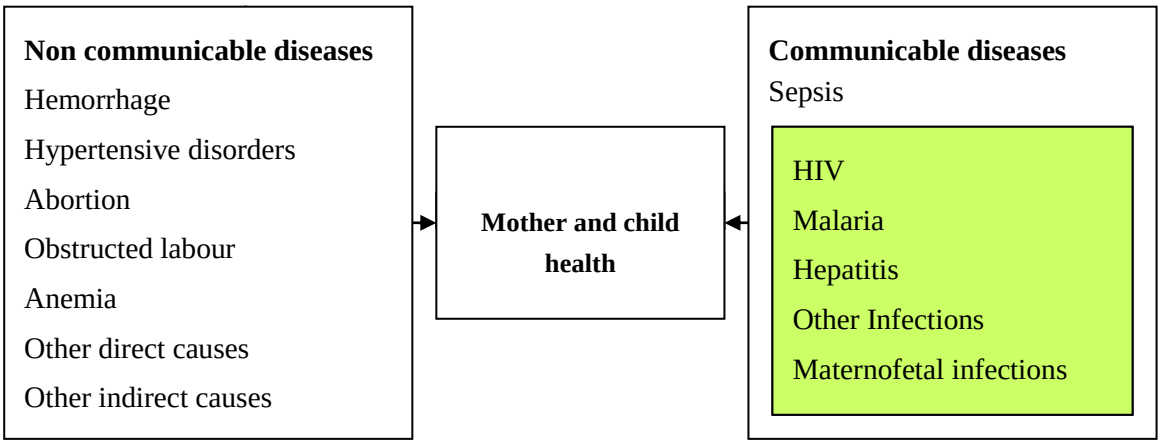
Table 8: Seroprevalences of syphilis and brucellosis

Pathogen	**Seroprevalence during pregnancy**		
	High-income countries	Low-income countries	Ghana
Brucella	No data	12.2% (Elshamy and Ahmed 2008)	No data
Treponema pallidum	0.4% (RKI 2013c) 1.4% (Martyn et al. 2011)	0.04% (Meyer Sauteur et al. 2012) 4.5% (Genc and Ledger 2000)	7.1% (Apea-Kubi et al. 2004)

Source: own depiction

3 Rationale and objectives of the study

In general, pregnancy-related risk factors for mother and child health can be classified in non-communicable (non-infectious) and communicable (infections) diseases (see figure 2).



Source: Khan et al. (2006)

Figure 2 Risk factors for mother and child health

Previous studies offer a good insight into causes, prevalence rates, and regional variations of non-communicable diseases. Based on a meta-analysis of 34 datasets and a total of 35.197 pregnant women, Khan et al. (2006) outline the major non-communicable and communicable risk factors during pregnancy. Ronsmans et al. (2006) confirm that hemorrhage and hypertensive disorders are the two most common reasons for maternal death, followed by infections and sepsis.

Amazingly the causes of infections, the third most frequent cause of maternal death, are rarely specified on the pathogenic level in low-income countries. In addition, the majority of research groups focus on single infections or single pathogens than considering possible coinfections or monitoring a broad spectrum of pathogens with known relevance for mother and child health. Prevalence rates of several pregnancy-related infectious diseases and the role of coinfections are hence unclear. Only *Plasmodium* and HIV (co-) infections are sometimes in the focus of research, at least in urban areas of low-income countries (Brentlinger et al. 2006; Nkhoma et al. 2012).

Based on this existing limitation in knowledge, the aim of the following study is to determine the prevalence rates of those communicable diseases, which are known to be of relevance for mother and child health during pregnancy and thereafter by conducting an infectiological screening on a broad spectrum of bacterial, viral and parasitic pathogens. In other words, this study should create an infectiological snapshot of a study population in a rural setting of Sub-Saharan Africa. Our research objectives are:

(i) To determine the prevalence rate of pregnancy-related infections in a rural setting of Ghana
(ii) To determine the prevalence of coinfections

Based on the literature review (Chapter 2), pregnant women are screened for pathogens causing infections that fulfil at least one of the following criteria (see table 9):

(i) Infection may cause serious damage to maternal health or cause maternal death
(ii) Infection may damage fetal development or health of the newborn or cause fetal death

Table 9: Selection of pathogens fulfilling criteria i. and ii.

Bacterial pathogens	**Maternal health (i.)**	**Child health (ii.)**
Group B streptococci		•
Listeria		•
Neisseria gonorrhoeae	•	•
Chlamydia trachomatis	•	•
Treponema pallidum spp. pallidum	•	•
Brucella spp.	•	•
Viral pathogens	**Maternal health (i.)**	**Child health (ii.)**
HIV	•	•
Hepatitis B virus	**(•)**	•
Hepatitis C virus	**(•)**	•
Hepatitis E virus	•	•
Cytomegalovirus		•
Varicella zoster virus	•	•
Rubella virus	**(•)**	•
Herpes simplex virus 1	**(•)**	•
Herpes simplex virus 2	**(•)**	•
Parvovirus B 19	**(•)**	•
Parasitic pathogens	**Maternal health (i.)**	**Child health (ii.)**
Plasmodium spp.	•	•
Toxoplasma gondii	•	•

Source: own depiction

In addition, we aim to evaluate the influence of socioeconomic factors, such as access to medical services, age, origin and education on the prevalence of certain infectious diseases. In this context, we expect higher rates in areas with limited access to medical services and a lower socioeconomic standard.

Due to the insufficient data in non-urban areas of Africa, we selected a rural area in Western Ghana and performed this study in the regional hospital St. Martin de Porres Hospital in Eikwe.

4 Patients, Materials and Methods

4.1 Patients

The following chapter describes the geographic area of research and data origin. Essential content of this description is the selection and recruitment of the study population. In addition, type and implementation of the sampling is clarified.

4.1.1 Study design

This cross-sectional study was conducted within a three-months period from October 2011 to January 2012 at the Department of Obstetrics and Gynecology at St. Martin de Porres Hospital (SMPH), situated in Eikwe, 310 km west of Ghana's capital Accra. Eikwe has a population of about 5.000 people. SMPH is the largest health provider in the western areas (Ellembelle, Nzema East, Jomoro districts) of the Western Region and ensures health care for a total population of 100.000 people. The hospital, which has a capacity of 175 beds, is mainly specialized in obstetrics and gynecology but also provides medical services in general surgery, internal medicine, pediatrics and preventive care services. Medical staff consists of two specialists for obstetrics and gynecology and four medical assistants. The department of obstetrics supplies approximately 150 deliveries each month.

4.1.2 Study population and sampling

The study population consisted of 180 pregnant women attending medical care services at SMPH. The calculated number of 450 deliveries throughout the course of study could not be reached in practice due to staff limitations. Women who met the inclusion criteria (gestational age $\geq$ 39 weeks or delivery within $\leq$ 1 week) were introduced to aim and procedure of the study and asked for participation. Education and informed consent were translated by the hospital staff into the two most common languages, Nzema and Akan. For data evaluation, we analyzed the participating women for accessibility of SMPH. Thus two sub-groups, one with relatively good access (origin: Ellembelle district) and one with limited access (origin: other districts), were created.

A standardized questionnaire, consisting of 80 questions, was used to collect general (origin, journey time), demographic (age, marriage) and obstetric (gravidity, obstetric history) data. Specific antenatal medical care services (screening on pregnancy-related infections), performed in SMPH were recorded. Subsequently, a vaginal swab and a venous blood sample of 6 ml were taken.

4.2 Methods

The analyzes of the vaginal and the blood specimen were performed in two stages. Basic microbiological and parasitic investigations were done in the laboratory of SMPH. In contrast, a broad serological testing and the confirmation of the bacteriological results from the local laboratory were performed at the Institute for Medical Microbiology in Göttingen. Table 10 illustrates all analyses made at SMPH and UMG.

Table 10: Summary of the analyses carried out in Ghana and Germany

Specimen	St. Martin de Porres Hospital	Medical Microbiology UMG
Vaginal swab	Culture (blood/chocolate agar)	MALDI-TOF
	Differentiation - macroscopic	Real time PCR
	- microscopic	
	- biochemical	
	- agglutination	
Blood/Serum	Malaria slide (field's stain)	Serological screening (ELISA/EIA/MEIA)

Source: own depiction

The bacteriology of SMPH was established in cooperation with Prof. Uwe Groß (UMG) and the Medical Mission Institute, Würzburg in 2000. Since then, the facility performs basic bacteriologic analyses, such as blood cultures and antibacterial drug susceptibility testing. Furthermore, SMPH participates successfully in regular external quality control programs.

All general needed equipment (incubator, Bunsen burner, loops, microscope, and plastic petri dishes, agar) are available in the local laboratory. Reagents for gram/field's stains and solutions for catalase (hydrogen peroxide), oxidase (TMPD), and coagulase (serum) tests are also available. In addition, a rapid latex agglutination test (Oxoid, UK) for identifying Lancefield streptococcal groups, and Amies medium transport swabs (Copan, Italy) were imported.

4.2.1 Vaginal swab: Procedure and bacteriological testing

Low vaginal swabs were taken of all 180 study participants. After consulting the pregnant women, all specimens were collected by the investigator or therefore trained nurses. For examination, a combined swab/transport medium system was used. The swab was realized approximately 2cm above the introitus vaginae without using a speculum and then immediately stored in the Amies transport medium. The swabs were used to screen for *Streptococcus agalactiae* (GBS), *Listeria spp.*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and Herpes simplex virus (HSV) 1 and 2. To detect GBS, *Listeria spp.*, and *N. gonorrhoeae*, the vaginal swab was analyzed by classical microbiological methods. The presence of all others pathogens was determined by Real-Time PCR.

4.2.1.1 Cultivation of bacteria

Media for cultivation of bacteria

Blood and chocolate agar were produced at the laboratory of SMPH. Agar was prepared out of Columbia agar base (Liofilchem, Italy) with the following formula (g/l): Peptospecial 23.0, Starch 1.0, sodium chloride 5.0, Agar 14.0. At first, 43.0 g of the agar base were completely dissolved in one liter of distilled water by heating slightly. Then, the solution was autoclaved. Following cooling down to 50°C, 50 ml sheep or horse blood was usually added, resulting in 5% blood agar. However, since animal blood was rarely available in St. Martin de Porres Hospital, expired citrated donor blood was used. Consequently, this procedure may limit the growth of some bacteria if inhibitory substances (e.g. antibiotics) have been present in the human donor blood. After adding blood, the agar was cooled down to room temperature by continuous agitation and then poured into petri dishes. The blood agar plates were then incubated overnight at 37°C to exclude contamination during manufacture. For chocolate agar, blood was added to the agar base at temperatures well above 50°C. The name chocolate agar is derived from the fact that erythrocytes are lysed at a temperature of 80°C, what creates a chocolate-like, brownish color. Intracellular metabolites like NAD or hemin are discharged by lysis of the erythrocytes and allow the growth of demanding bacteria, such as *N. gonorrhoeae* (Ochei and Kolhatkar 2000).

Inoculation and incubation

After sampling, vaginal swabs were briefly stored for a maximum of 2 hours in Amies transport medium, before performing fractionated inoculation on blood and chocolate agar. Inoculated blood agar plates were then incubated at 37°C under atmospheric conditions, chocolate agar plates at 37°C under 5-10% CO_2 conditions using a candle extinction jar. After 12, 24, 36 and 48 hours, the plates were monitored for bacterial growth including determination of colony color, shape and hemolysis reaction (Kayser et al. 2005).

4.2.1.2 Identification of bacteria

We focused on the identification of the pregnancy-related bacteria GBS, *Listeria spp.*, and *N. gonorhoeae* based on morphological characteristics and biochemical tests (Table 11). Latexagglutination was used to confirm GBS. Species identification of all bacteria identified in the Eikwe laboratory was subsequently confirmed by MALDI-TOF in Göttingen.

Macroscopic and microscopic morphology

GBS form bright, small colonies with a clear but small ß-hemolysis zone on blood agar (Kayser et al. 2005). *Listeria* appears as small grey-whitish colonies with small hemolysis on blood agar. Smooth, greyish and small (0.5 – 1 mm) colonies on chocolate agar are specific for *N. gonorhoeae* (Halle et al. 2000). Microscopy was used to further confirm the initial macroscopic judgment. Basis for microscopy of bacteria is the gram stain. This old but still essential technique was performed in the following way. A bacterial colony was transferred to a glass slide and mixed with a drop of NaCl solution. After air drying and heat fixation, the slide is step by step treated with crystal violet, iodine, ethanol, and safranin.

Gram-positive bacteria (blue) have a multilayered cell wall with a thick peptidoglycan layer which prevents loosing off crystal violet by ethanol, whereas gram-negative bacteria (red) loose this dye due to their thinner layer of peptidoglycan (Groß 2013). This decolorization of gram-negative bacteria is compensated by counterstaining with the red safranin (Shimeld and Rodgers 1999).

Biochemical testing

The expression of the bacterial enzymes catalase and oxidase were determined allowing a first classification in different genera. The catalase test is based on the capability of certain genera to disproportionate and thus neutralizes hydrogen peroxide into water and oxygen. This reaction is mediated by the enzyme catalase (Wheelis 2008).

In St. Martin de Porres Hospital, the catalase test was preceded by using the slide method. A drop of hydrogen peroxide was added on a microscope slide and mixed with a colony of bacteria to be determined. Bubbles of gas indicate the production of oxygen and therefore a positive catalase test. Hereby, gram-positive cocci can be separated in *Streptococci*/*Enterococci* (catalase negative) and *Staphylococci* (catalase positive).

The oxidase test was applied to determine the expression of cytochrome c oxidase in bacteria. This reducing enzyme is produced by the genera of *Neisseria* and *Pseudomonas*. The test is based on the fact that cytochrome c oxidizes an artificial electron acceptor like tetramethyl p-phenylenediamine dihydrochloride to a dark purple product, indophenol. In the laboratory of SMPH, the so-called spot oxidase test was performed. A drop of reagent was placed on a microscopy slide. Then a bacterial colony was added and mixed. If there is a color change to dark purple, the tested species is positive of cytochrome c (Shimeld and Rodgers 1999).

Immunoagglutination

A rapid latex agglutination test (Oxoid, UK) was used to further characterize ß-hemolytic *streptococci* via Lancefield groups. Here, group-specific rabbit immunoglobulins sensitized by non-soluble latex particles bind to polysaccharide antigens on the bacterial surface. This antigen-antibody reaction follows the formation of a net structure at the molecular level which is macroscopically visualized by agglutination of the latex particles (Groß 2013). In practice and to identify group B streptococci (GBS), several colonies of gram-positive, catalase negative, ß-hemolytic cocci in pure cultivation were mixed with the agglutination reagent Lancefield type B. If agglutination was visible within five minutes, the bacteria were identified as *Streptococci agalactiae* (GBS).

MALDI-TOF mass spectrometry

This method was used to identify bacterial species due to different ribosomal protein spectral fingerprints. Bacterial samples were recultivated on their adapted medium. Blood

and chocolate agar plates were incubated for 24 hours and then reanalyzed by using MALDI-TOF (Matrix-assisted laser desorption ionization time-of-flight) mass spectrometry (Bruker Daltronics). This technique is based on the measurement of the mass-to-charge ratio (m/z) of organic chemical bonds. Resultant chemical structures (i.e. proteins) provide a specific spectrum. Recent studies showed high rates of positive identifications for more than 95 % at the genus level and for 85 % at the species level (van Veen et al. 2010).

Table 11: Summary of the morphologic and biochemical characteristics of the cultured bacteria

Species	Morphology	Microscopy	Biochemical testing
Streptococcus agalacticae	small, white ß-hemolysis	gram+ cocci	catalase neg.
Listeria	small , grey	gram+ rods	catalase pos.
Neisseria gonorrhoeae	small, smooth, grayish	gram- diplococci	catalase pos. oxidase pos.

Source: own depiction

4.2.2 Procedure and analysis of blood/serum samples

Venous blood specimens were analyzed for the presence of *Plasmodium spp.* parasites and antibodies against the bacterial, viral and parasitic pathogens as listed in chapter 2. Several different methods were used for this broad screening. Malaria parasites were detected by peripheral blood smear and light microscopy. In addition, serum samples were screened for antibodies against viral pathogens such as VZV, HSV-1/-2, PB19, CMV, HEV and against *Brucella* by an Enzyme-linked immunosorbent assay (ELISA). Furthermore, the microparticle enzyme immunoassay (MEIA) was used to detect antibodies against HBV (HBsAg), HCV (Anti-HCV) and HIV (antigen/antibodies). In order to determine the seroprevalence of IgM/IgG antibodies against *Toxoplasma gondii*, an enzyme-linked fluorescent assay (ELFA) was performed. The screening for antibodies present in patients suffering from syphilis was done using the *Treponema pallidum* particle agglutination (TPPA) and venous disease research laboratory (VDRL) test.

Peripheral blood smear

For the detection of malaria parasites, the thick blood smear had been used as method of choice. This fast and cheap technique has the highest accurateness under the premise that testing is performed by well-trained and experienced investigators.

Several different staining techniques exist. Besides the Giemsa staining method, Field's stain technique is a widely used technique for staining thick films. In the laboratory of SMPH Field's stain technique was applied.

This technique enables to stain malaria slides quickly and stably by achieving particularly good results with fresh thick blood films. Field's stain consists of two solutions, A and B. Field's stain solution A is a mixture of methylene blue, azure I, and phosphate salts as buffer, while solution B contains eosin, an acid agent, and phosphate salts. After staining and drying, the smear may be examined: in the microscope 100 power fields were reviewed and stained parasites were counted. Depending on the quantity of parasites, infections were categorized into four levels of parasitemia (see table 12).

Table 12: Severity of a malaria infection on the basis of parasitemia

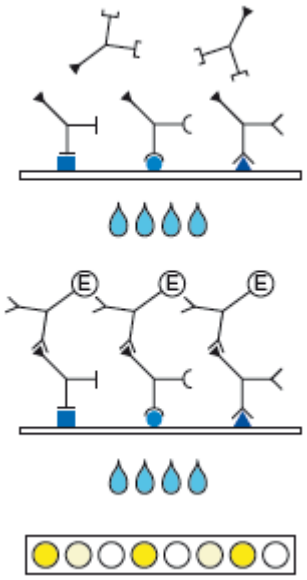
Number of parasites	Level of parasitemia
1-10 parasites per 100 power fields	+
11-100 parasites per 100 power fields	++
1-10 in every power field	+++
>10 in every power field	++++

Enzyme-linked immunosorbent assay (ELISA)

In order to determine the seroprevalence of antibodies against VZV, CMV, HEV, PB19, HSV-1/-2 and *Brucella*, all serum specimens were tested on pathogen-specific IgM and IgG antibodies by indirect ELISA.

Essentially, this method is based on the detection of a specific antigen-antibody reaction. Recombinant antigens (antibodies in case of HIV antigen detection) were fixed to the wells of a microtiter plate. Then diluted serum or plasma was added and incubated in the wells. Existing antibodies or antigens bind to the fixed antigens or antibodies, respectively (1). Subsequently, unbounded antibodies or antigens were washed away (2). To determine pathogen-specific antigen-antibody complexes, anti-human IgG/IgM

antibodies, which are conjugated with an enzyme that enables a color reaction (e.g. horseradish peroxidase), were added and incubated (3). Washing was repeated (4). Then the substrate of the conjugated enzyme (e.g. TMB = 3, 3, 3‘, 5‘-Tetramethylbenzidindihydrochlorid) was added. During incubation, the substrate was enzymatically converted and the product provoked a color reaction. This color reaction is proportionate to the quantity of bound IgM/IgG antibodies or bound antigens, respectively. Intensity was measured by photometer and converted to IgM/IgG or antigen concentrations (5) (see figure 3). Table 13 shows the used commercial test systems.



(1) Formation of antigen-antibody complexes

(2) Washing

(3) Anti-human IgG/IgM antibodes + enzyme bind

(4) Washing

(5) Measurement of the color reaction -> c (IgM/IgG)

Source: Mikrogen Diagnostik 2013

Figure 3: Principle of the indirect sandwich-ELISA to detect antibodies

Table 13: Pathogen and ELISA test systems that have been used

Screening parameter	Test system	Producer
Anti-HEV-IgM/IgG	recomWell HEV IgG/IgM	Mikrogen Diagnostik, Germany
Anti-VZV-IgA/IgG	Varicella-zoster virus (IgA/IgG)	Serion ELISA classic, Germany
Anti-CMV-IgM/IgG	Cytomegalovirus IgM/IgG	Serion ELISA classic, Germany
Anti-HSV-IgM/IgG	Herpes simplex virus 1+2 IgM/IgG	Serion ELISA classic, Germany
Anti-PB19-IgM/IgG	Parvovirus B19 IgM/IgG (Test A)	Serion ELISA classic, Germany
Anti-PB19-IgM/IgG	Parvovirus B19 IgM/IgG (Test B)	Mikrogen recomWell, Germany

Source: own depiction

Microparticle enzyme immunoassay (MEIA)

This test is a variation of the enzyme immunoassay (EIA). As in EIA, specific antibodies in the patients' serum (anti-HCV) bind to recombinant viral antigens. Likewise, specific antigens (e.g. HBs antigen) may be detected by binding to recombinant antibodies. The difference to EIA is the location of the antigen-antibody reaction: In EIAs the reaction takes place in solid phase, in MEIAs antigens or antibodies are located on the surface of microparticles which are in soluble phase. After binding, the antigen-antibody complex is transferred to the matrix cell. There, the microparticles bind irreversibly to a glass fiber matrix. Then the matrix is washed to delete unbound antibodies or antigens, respectively.

As in EIA, antihuman IgM or IgG antibodies combined with an enzyme (alkaline phosphatase) is added and incubated. During incubation, anti-IgM/IgG antibodies bind to the pathogen-specific antigen-antibody complex. In some MEIAs (HBs antigen, HIV Ag/Ab), antigen-antibody complexes are labeled by recombinant antigens or antibodies which are conjugated with biotin. These complexes are then detected by anti-biotin alkaline phosphatase conjugates.

After washing, 4-methylumbelliferyl phosphate is added and converted to 4-methylumbelliferone. This reaction is catalyzed by the alkaline phosphatase bound to the antigen-antibody complex. The fluorescent product is then measured by photometer and the value compared to the cutoff rate (Abbott HCV version 3.0). Table 14 shows the commercial test systems that have been used in this study.

Table 14: Pathogen and MEIA test systems that have been used

Screening parameter	Test system	Producer
Anti-HCV-IgM	HCV version 3.0 Anti-HCV	Abbott, Germany
HBsAg (HBV)	HBsAg(v2)	Abbott, Germany
HBsAg (HBS)	HBsAg EIA Test Kit	Ascon, USA
HIV Ag/Ab	HIV Ag/Ab Combo	Abbott, Germany

Source: own depiction

Enzyme linked fluorescent assay (ELFA)

This method was conducted using IgM and IgG ELFA kits and the miniVIDAS system (bioMérieux, France). This assay combines the principle of ELISA with a concluding detection of a fluorescent product.

In ELFA IgM, a solid phase receptacle (SPR) which is coated by anti-human IgM antibodies fixates IgM antibodies of the sample. In this pool of IgM antibodies *Toxoplasma gondii*-specific IgM antibodies are detected by recombinant *Toxoplasma* antigen SAG1/P30. Subsequently, these antigen-antibody complexes are bound by murine monoclonal anti-P30 antibodies, which are conjugated by the alkaline phosphatase. Finally, the substrate 4-methyl-umbelliferon-phosphate is added and the conjugated enzyme catalyzes the creation of the fluorescent product 4-methyl-umbelliferon. The absorbance of this product at 450 nm is proportionate to the concentration of P30-specific IgM antibodies in the sample. Specimens are classified as positive if the test value that is generated out of the photometric measurement and the standard value is ≥ 0.65.

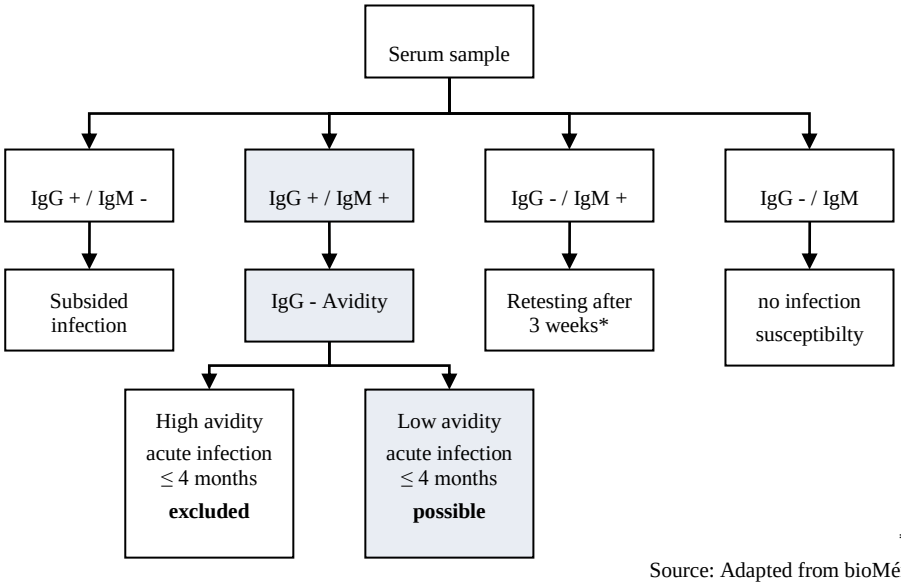
In ELFA IgG, the SPR is coated with cytoplasmic and membrane *Toxoplasma* antigens. The detection of the bound IgG antibodies is analogous to the detection of IgM antibodies. The specimen is positive, if the test value is $\geq$ 8IU/ml. To exclude a recently acquired infection, IgG avidity (bioMérieux, France) was tested in IgM- and IgG positive samples. The background of this method is maturation (increase of avidity) of IgG antibodies after initial immune response by antigen-driven B-cell selection. This enforced binding of the antigen-antibody complex can be measured. Titration curves of urea-treated and untreated samples determine the affinity/avidity of existing antigen-antibody complexes. Samples with a high IgG avidity exclude an infection within the last three to five months (Montoya et al. 2002). Our approach of toxoplasmosis screening among the

study participants is illustrated in figure 4. Table 15 shows the commercial test systems that have been used.

Table 15: Pathogen and ELFA test systems that have been used

Screening parameter	Test system	Producer
Toxo-IgM	VIDAS Toxo-IgM ELFA	bioMérieux, France
Toxo-IgG	VIDAS Toxo-IgG ELFA	bioMerieux, France
Toxo-IgG Avidity	VIDAS Toxo-IgG avidity ELFA	bioMérieux, France

Source: own depiction



Source: Adapted from bioMérieux 2002

Figure 4: Procedure of the screening for maternal toxoplasmosis

Serological tests for infection with Treponema pallidum

T. pallidum-specific or indicative antibodies can be detected dependently from the stage of infection. This is why serological methods are an important tool to diagnose syphilis. Our screening was based on three tests: *Treponema pallidum* particle agglutination assay (TPPA), the detection of specific antibodies by fluorescent treponemal antibody absorption (FTA-ABS) and the Veneral Disease Research Laboratory (VDRL) test. All serum samples were initially tested by TPPA to determine the baseline serological status. Afterwards, all TPPA-positive samples were retested for the putative presence of specific

treponemal antibodies to specify the presence of syphilis. Finally, all samples were screened again by VDRL to eventually identify false-positive TPPA results. These three methods are now explicitly described as follows.

TPPA detects *Treponema*-specific antibodies of different immunoglobulin (Ig) classes. The key to determine specific antibodies are colored gelatine particles coated with *T. pallidum* (Nichols strain) antigens. Serum or plasma is filled in microtiter plate wells. Then conjugated gelatine particles are added and the contents of the plate are mixed. This preparation is incubated for 2 hours at room temperature. If present, *Treponema*-specific antibodies bind to antigens on the particles' surface. This antigen-antibody reaction provokes an agglutination of the colored and coated particles, visible on the base of the microtiter plate wells. This visible agglutination is evaluated as a positive TPPA, and consequently as successful detection of *Treponema*-specific IgM- or/and IgG antibodies. (Serodia, TPPA). Due to long persistency of IgG antibodies, TPPA may produce false-positive results in case of treated and cured syphilis infection. Another limitation is cross-reactivity with other *Treponema pallidum* species, like *Treponema spp. pallidum pertenue*, the infectious agent of yaws (Groß 2013).

FTS-ABS was performed to detect specific antibodies against *T. pallidum*. In this test, the patient's serum is initially treated with a species of non-pathogenic *Treponema* (*T. phagedenis*) to bind all group-specific antibodies. In a second step, the serum is incubated with *T. pallidum* antigens. If the patient's sera contain *T. pallidum*-specific antibodies, an antigen-antibody complex will be formed. Finally, this complex is marked by a fluorescent anti-human immunoglobulin. The result of this antigen-antibody reaction can be evaluated under a fluorescence microscope (Parija 2009).

VDRL is another screening test for the identification of *T. pallidum* infection and is also part of the group of unspecific non-treponemal tests. The principle of this method is a reaction of non-specific but indicative anti-lipoid antibodies produced by infected patients with the cardiolipin test antigen (Tille 2014). Table 16 shows the commercial test systems that have been used.

Table 16: Screening parameter and commercial test systems that have been used

Screening parameter	Test system	Producer
Treponema-Ig	Serodia TPPA	Fujirebio, Japan
T.pallidum-Ig	FTA-Abs	Sekisui Diagnostics, Japan
Cardiolipin-Ig	VDRL	Omega Diagnostics, Germany

Source: own depiction

4.2.2.1 Real-Time PCR

Besides bacteriological and serological methods, we were using the polymerase chain reaction (PCR), one of the fundamental methods of molecular biology. PCR allows a rapid replication of specific DNA or RNA regions. Using these gene segments, the reliable detection and differentiation of bacterial, viral and parasitic pathogens is possible. In this project, we applied the real-time PCR for the direct detection of *Chlamydia trachomatis*, Herpes simplex virus 1/2 and human papillomavirus in the vaginal swabs and for Parvovirus B19 in the serum sample.

The detection of pathogens by PCR is based on an amplification of specific nucleic acids regions of the pathogen genome. In real-time PCR, the amplified nucleic acids are marked by fluorescent dyes. These dyes are linked to oligonucleotides which specifically bind to the replicated nucleic acids. By measuring the fluorescence intensity during the PCR, the accumulation of the amplified pathogen-specific nucleic acids may be detected directly (Qiagen, 2007). Table 17 shows the commercial test systems that have been used.

Table 17: Pathogen and real-time PCR test systems

Screening parameter	Test system	Producer
Chlamydia trachomatis	In house	UMG, Göttingen
HSV-1/-2	Artus HSV-1/-2 LC PCR Kit	Qiagen diagnostics, the Netherlands
Parvovirus B19	In house (Liefeldt et al. 2005)	German Consulting Laboratory, Regensburg
HPV	In house	German Consulting Laboratory, Köln

Source: own depiction

5 Results

This chapter illustrates the results of our study. First, the study population is described by considering socioeconomic characteristics. Furthermore, the antenatal period is analyzed from an infectiological point of view. Therefore, we investigate the frequency of antenatal screenings on pregnancy-relevant infections and preventive measures during pregnancy. Subsequent to this introductory part, the results of the testing on parasitic, viral and bacterial infections are specified. In a first step, we list prevalences of single pathogens and their potential risk factors. In a second step, we describe the frequency of co-infections in our study sample.

5.1 Characteristics of the study population

All socioeconomic, general and obstetric variables are captured in the structured questionnaire. A total of 180 pregnant women were enrolled into the study. Since there is no information on the origin of 9 women, the final sample consists of 171 observations. These are then divided into 2 subgroups derived from the distance of living to the hospital. Consequently, distinctive feature was the accessibility of the hospital. This subdivision was done to detect possible differences among infectiological parameters between pregnant women with good and limited access to the hospital. The St. Martin de Porres Hospital is located in the Ellembelle district. This district (subgroup 1) was the origin district of 88 pregnant women; 83 women travelled from the surrounding districts, mainly Jomoro district, to the hospital (subgroup 2). Patients with origin from Ellembelle had a mean journey time of 144 min to reach the hospital. Individuals of subgroup 2 had to travel 229 min on average. Mode of transport was, besides boat or by foot, mainly shared taxi.

General characteristics of all pregnant women who participated are shown in table 18. The mean age was 26.3 years with a range from 14 years to 48 years. Another feature was the number of pregnancies (gravidity) and the number of live births (parity). The averaged study participant has their third pregnancy and less than two children. Rate of illiteracy was high with up to 32 %.

Table 18: Descriptive statistics of study subjects

Variable	Mean	Standard deviation
Age (years)	26.3	.52
Education (at least primary degree) (0/1)	.68	
Origin: Ellembelle (0/1)	.51	
Journey time (min.)	186.8	26.73
Household size (number of persons)	6.3	.26
Household: children < 5yrs (number of persons)	.9	.01
Gravidity (number of pregnancies)	3.0	.16
Parity (number of live births)	1.9	.16

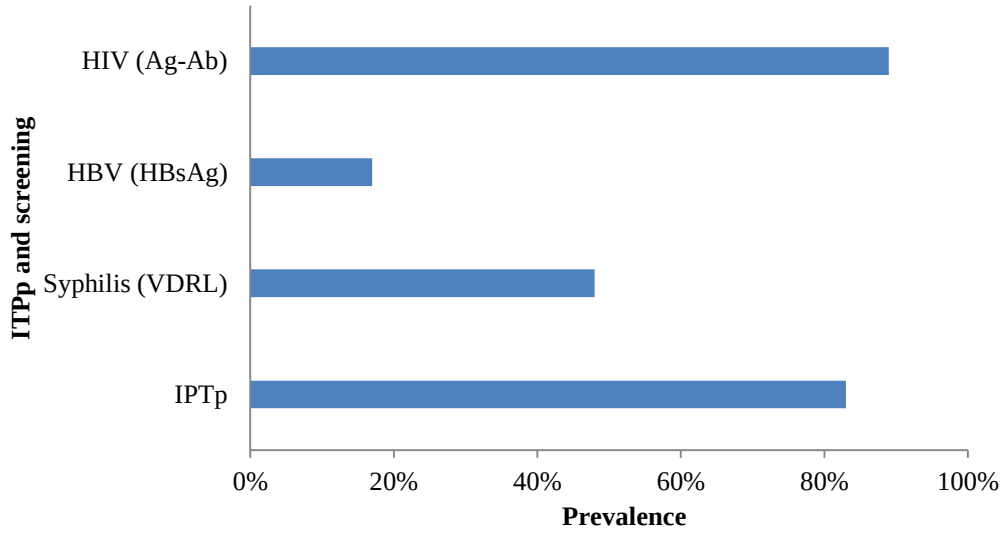
Source: own depiction

5.1.1 Infectiological screening and preventive medical measures

Out of our sample of 180 women, we collected data of antenatal medical services of 174 (n=6 missing observations) study participants. Source of data was the maternal health record book. On average, an antenatal clinic was visited 7.8 times during the current pregnancy. Nationwide guidelines in Ghana recommend a screening on syphilis, hepatitis B and HIV in the course of pregnancy. A further nationwide medical measure to improve the outcome of pregnancy for mother and child is an intermittent preventive treatment during pregnancy against malaria (IPTp). This prophylaxis with sulphadoxine-pyrimethamine (Fansidar®) was implemented in Ghana in 2004. Hommerich et al. (2007) show a substantial decline of placental malaria and maternal anemia with a concurrent increase of the birth weight in the consecutively years and attribute this effect to the introduction of IPTp. Nowadays this malaria prophylaxis is well accepted among Ghanaian physicians. While 83% of the surveyed pregnant women indeed receive an IPTp, only around one third (34%) of those get the complete treatment of three doses. Thus, a large share of about two thirds (66 %) are only preventively treated once or twice, what limits the effectiveness of the IPTp.

What concerns the screening for HBV, *T. pallidum* and HIV, no significant differences between our two subpopulations (Ellembelle 0/1) were found. In our study sample, only 48% were screened for an infection with *T. pallidum* (syphilis). Hence, more than one half delivers without having been tested on syphilis causing medical risks for mother and

child. Although a relevant nationwide program exists, only 17.2% of the study sample was tested for the presence of HBsAg, the appropriate screening parameter for hepatitis B. In those cases, a rapid serological test (ACON, San Diego, USA) was applied. The accuracy of this test is 100%, as we were able to show by direct comparison to the MEIA (Abbott). Thus, the majority (82.8%) passes through pregnancy without the diagnosis of a potential HBV infection. The coverage of the antenatal HIV screening is relatively high: 89% of the pregnant women were tested on HIV (see figure 5). This result confirms a consequent antenatal screening for HIV in our study area. Another study from Ghana offers much poorer results: In 2007, nationwide only 12% of pregnant women were tested on HIV, while in Wa municipality (Upper West Region) two thirds of women (60%) received a routine screening during pregnancy (Nyuzaghl et al. 2011).



Source: own depiction

Figure 5: Percentage of participants that receive screening and IPTp

5.2 Prevalences of pregnancy-relevant infections

We tested all study participants on a broad spectrum of infectious diseases that may interfere with mother and child health.

5.2.1 Prevalences of parasitic infections

Within the group of parasitic infections, we investigated the prevalence of *Plasmodium spp.*, the causative agent of malaria and *Toxoplasma gondii* causing toxoplasmosis.

Plasmodium spp.

In 180 blood samples of our study population, *Plasmodium spp.* parasites were detected in 10.6 % of the samples. Table 19 depicts the parasitemia level for the respective pregnant women. Parasitemia ranged from low to high levels. High levels (+++/++++) are an indicator for severe malaria. The majority (58%) of the detected Plasmodium infections had a low parasitemia. However, a low concentration of parasites does principally not exclude a severe clinical course of disease.

Table 19: Parasitemia of Plasmodium-positive pregnant women (N=19)

Level of parasitemia	Prevalence
+	58% (N=11)
++	16% (N=3)
+++	21% (N=4)
++++	5.3% (N=1)

Source: own depiction

Results on the relation between infectiological screening and preventive medical measures, access to medical services and the prevalence of malaria are provided in table 20. We estimated a logistic regression with malaria as the dependent variable. IPTp did lower the prevalence of malaria infection in our study sample. However, this positive effect was not statistically significant. In almost the same manner, general education did not have a significant effect on the malaria prevalence. However, our data indicate a lower prevalence of malaria in Ellembelle district (subgroup 1) compared to the surrounding districts (6.8% vs. 14.5%). According to this, pregnant women from subgroup 2 had a 3-fold higher risk of acquiring a *Plasmodium* infection in the last trimester of pregnancy. This value was significant at ten percent (significance at $p<0.1$). Furthermore, age had an important influence on a positive malaria diagnosis with, again, a significance level of 10 percent. Particularly, the risk of acquiring malaria decreased with every year of age. This suggests the older the women are the less likely it is that they will get malaria in the future compared to younger women.

According to this model both age and origin influence significantly the possibility of being infected with *Plasmodium spp.* parasites. Low age and bad accessibility of the hospital increase the risk for a malaria infection.

Table 20: Results of the logit model with malaria as the dependent variable

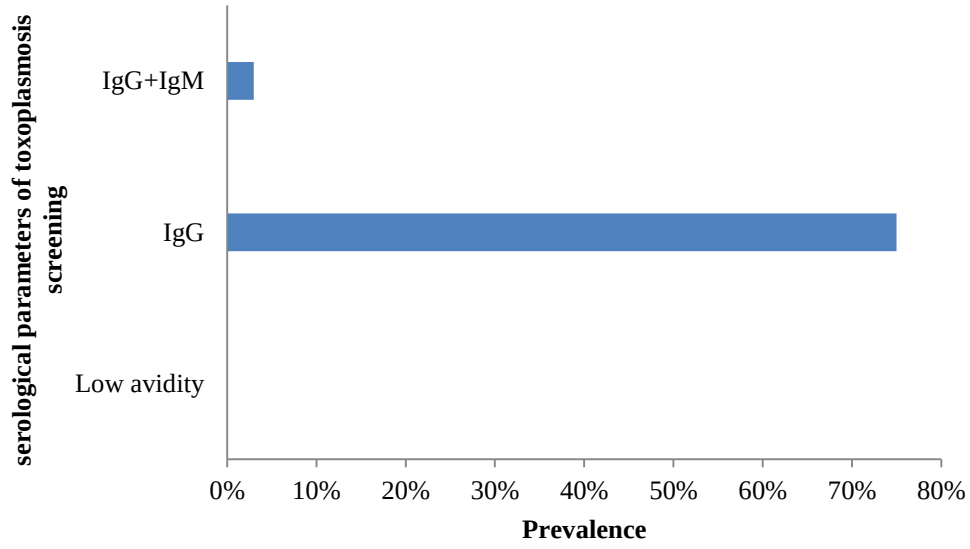
Independent variable	Observations	Number of Malaria +	Prevalence (%)	Odds ratio (standard errors)
Age (years)	/	/	/	.9034* (.04976)
Origin (0/1)				
Ellembelle	88	6	6.8	2.956* (1.923)
Other	83	12	14.5	
Education (0/1)				
No	44	3	6.8	1.236 (.8963)
Educational degree	90	11	12.2	
IPTp (0/1)				
No	114	14	12.3	.3923 (.3221)
Yes	60	4	6.7	

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Toxoplasma gondii

Serological results indicate a high seroprevalence of IgG antibodies specific for *Toxoplasma gondii*, outlined in figure 6. Hence, 73.2% (N=123) of the study sample (N=168, N=12 missing observations) had been in contact with *Toxoplasma gondii*. Acute infections were excluded in nearly all cases. Although 1.8% (N=3) of the women had elevated levels of specific IgM antibodies, all of these presented also with high IgG avidity, suggesting an undergone infection that occurred at least four months ago. Hence, these rather recently acquired infections may have hit our study participants during the first five months of pregnancy or even earlier. Furthermore, our study showed that younger women had an increasing risk of being IgM-positive (OR: .5643, p<0.1). To be precise, the odds ratio decreased per unit of year with 0.5643. All other independent variables (origin, education, gravidity) were not statistically significant.



Source: own depiction

Figure 6: Prevalence of serological parameters of screening for toxoplasmosis

5.2.2 Prevalences of viral infections

In this chapter, we focus on viral infections that may affect maternal health, the outcome of pregnancy or the newborn's health. We used serological methods (see 4.2.2) to detect pathogen-specific antibodies in the serum of our study population. In addition to this, HSV-1, HSV-2 and HPV were also detected in vaginal swabs by PCR. In the following, we describe the seroprevalences of these pregnancy-relevant viral infections and the prevalence of HSV-1, HSV-2 and HPV.

Hepatitis B virus (HBV)

Individual plasma specimens from our sample of 180 (N=174, N=6 missing observations) pregnant women were screened for HBsAg by MEIA (Abbott). We found HBsAg in 29 specimens resulting in a prevalence of 16.7%. In contrast, only two women knew about their HBV infection. Table 21 shows that none of the socioeconomic factors (age, gravidity, origin, education) were significantly associated with the prevalence of seropositivity for HBs antigen in our study population. We estimated a logit regression with HBsAg seroprevalence as the dependent variable.

All HBsAg seropositive mothers were tested negative for HIV. HBsAg-positive pregnant women did not receive antiviral treatment. By comparing the two geographical subgroups, the prevalence of hepatitis B was considerably higher in Ellembelle district.

Based on a chi square test, this difference was statistically significant ($p<0.05$). However, when including other socioeconomic and obstetric factors in a multivariate logit model, the district can not significantly explain the higher hepatitis B prevalence. Furthermore, we could show the accuracy (100%) of the HBsAg rapid test kit (Ascon), which is used in SMPH, by direct comparison to the MEIA (Abbott).

Table 21: Results of the logit model with HBs antigenemia as dependent variable

Independent variable	Observations	Number of HBsAg +	Prevalence (%)	Odds ratio (standard error)
Age (years)	/	/	/	1.015 (.05137)
Origin (0/1)				
Ellembelle	87	19	21.8	.4701 (.2798)
Other	71	8	11.2	
Education (0/1)				
No	47	8	17.0	.9017 (.5423)
Educational degree	98	14	14.3	
Gravidity (0/1)				
Primigravidae	50	9	18.0	.4134 (.2729)
>1	106	16	15.1	

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Hepatitis C virus (HCV)

For analyzing the seroprevalence of HCV-specific antibodies, 174 plasma specimens were tested by MEIA (Abbott). Seroprevalence in the entire study population was 1.1 % (N=2). We found a slightly higher prevalence in the other districts compared to Ellembelle district (see table 22). However, due to low observation number, a statistical evaluation was not possible.

Table 22: Prevalence of HCV by subgroup

Anti-HCV	Total sample (N=174, N= 9 no origin)	Subgroup 1: Ellembelle (N=86)	Subgroup 2: Others (N=79)
Positive	1.1% (2)	1.2% (1)	1.3% (1)
Negative	98.9% (172)	98.8% (85)	98.7% (78)

Source: own depiction Note: Number of observations in parentheses.

Hepatitis E virus (HEV)

All blood samples (N=174) were screened for the presence of antibodies directed against HEV by commercial immunoassays (MEIA/Abbott). Serological parameters were anti-HEV-IgG and anti-HEV-IgM. 36 samples (20.7%) were anti-HEV-IgM reactive. Moreover, in 12 (6.9%) specimens anti-HEV-IgG could be detected. It follows, according to this result an unusual high IgM seroprevalence together with a low IgG seropositivity. Table 23 shows the serological status of 172 study participants (n=2 missing values; only IgG or IgM). Thus, nearly 21% (N=35) of study participants showed a serological profile (IgM+/IgG+ or IgM+) which may be consistent with an acute infection by HEV. In the context of this survey, no further laboratory data like levels of liver transaminases (AST/ALT) or liver function (bilirubin, coagulation parameters, and cholinesterase) were determined.

Table 23: Distribution of HEV-specific IgM/IgG antibodies (N=172)

IgG	IgM	Interpretation	Prevalence (N)
+	-	Subsided Infection	6.4 (11)
+	+	Acute infection	.6 (1)
-	+		20.3 (35)
-	-	No infection	72,7 (125)

Source: own depiction

A rather high proportion of 27.8 % of the pregnant women specifying their water source as unprotected well, lake or river, were tested positive for anti-HEV-IgM antibodies, compared to only 18.5% of women using tap water. However, this distribution was not statistically significant. All other potential variables did not significantly influence the HEV-IgM seroprevalence on the basis of a logit model (see table 24).

Table 24: Results of the logit model with Anti-HEV IgM as dependent variable

Independent variable	Observations	Number of IgM-HEV +	Prevalence (%)	Odds ratio (standard error)
Age (years)	/	/	/	.9343 (.04867)
Origin (0/1)				
Ellembelle	86	19	22.1	.8244 (.4490)
Other	79	16	20.3	
Education (0/1)				
No	46	9	19.6	1.416 (.8971)
Educational degree	99	21	21.2	
Water source (0/1)				
Tap water	124	23	18.5	1.342 (.8203)
Unprotected	36	10	27.8	
Gravidity (0/1)				
Primigravidae	48	10	20.8	1.520 (.9940)
>1	108	21	19.4	

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Cytomegalovirus (CMV)

We screened 172 (N=8 missing observations) blood samples for the presence of cytomegalovirus-specific IgM- and IgG antibodies. None of the tested sera contained IgM antibodies against CMV making it likely that acute CMV infections were not present in the study population. In contrast, all participants showed serological signs of subsided CMV infections because they presented with high IgG antibody levels.

Rubella virus

All participating pregnant women (N=172, N=8 missing observations) were screened for infections with rubella virus. None of them had received a vaccination in childhood, since rubella – as in many African countries - is not part of the Ghanaian immunization schedule. Serological markers were specific IgM and IgG antibodies in the maternal serum. Eight pregnant women (4.7%) were seropositive for IgM and IgG. This finding indicates an acute or more recently acquired infection with relevance for the pregnancy outcome. High percentages (84.3 %, N=145) of our study participants were tested positive for the presence of Rubella virus-specific IgG antibodies without IgM antibodies, confirming a subsided rubella infection in non-vaccinated individuals. No serological signs of a previous contact (no IgM/no IgG) could be identified in 15.7 % (N=27) of the

study participants. This group of study participants would be susceptible for a primary rubella virus infection with a consequently higher risk of vertical transmission.

Human immunodeficiency virus (HIV)

173 (N=7 missing observations) blood samples were tested for the presence of HIV specific antibodies or/and antigens. The obtained data nearly equaled the one that were given in a nationwide survey of the adult population in Ghana (UNAIDS 2012b). According to that, 1.8 % of Ghanaian adults were HIV positive in 2009. The 2012 sentinel survey in Ghana estimated a prevalence of 1.37 % (Service 2012). In our analysis, only one woman showed serological signs of an HIV infection, resulting in a prevalence of 0.6 %, which is slightly lower compared to the general population. Due to this low prevalence, a statistical evaluation was not possible.

Varicella-zoster virus (VZV)

A total of 169 (N=11 missing observations) blood samples were analyzed on the immunological status with regard to a primary or subsided infection with VZV. Specific IgA and IgG antibodies were found in 2.4 % (N=4) of the samples. This result may indicate an acute or active infection. In addition, specific IgG antibodies without IgA antibodies were detected in 54.4 % (N=92), making a previous infection likely. Altogether 56.8 % (N=96) were VZV seropositive. All 73 seronegative individuals (43.2%) could be judged to be potentially susceptible for a primary VZV infection.

Furthermore, the impact of socioeconomic and obstetric parameters was analyzed. According to regression results, maternal age was not significantly different between seropositive and seronegative women. The results of a multivariate data analysis (logit model) show a significant impact of the number of pregnancies (primigravidae/>1), included as a dummy variable as well as the origin (Ellembelle/1) on the likelihood of VZV seropositivity. This means, for example, that having had at least one pregnancy increased the risk of IgG seropositivity by 2.38. A further important factor on VZV seroprevalence is the number of children in the household. No significant effect of the education level on the IgG seroprevalence was detectable (see table 25).

Table 25: Results of the logit model with Anti-VZV-IgG as dependent variable

Independent variable	Observations	Number of IgG-VZV	Prevalence (%)	Odds ratio (standard error)
Age (years)	/	/	/	.9767 (.03591)
Origin (0/1)				
Ellembelle	85	42	49.4	2.572** (1.092)
Other	77	50	64.9	
Education (0/1)				
No	46	28	60.9	1.255 (.5576)
Educational degree	96	53	55.2	
Gravidity (0/1)				
Primigravidae	48	20	41.7	2.388*** (1.226)
>1	104	64	61.5	
Children in household (# of children)	/	/	/	1.282*** (.171)

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Herpes simplex virus (HSV)

All 174 (N=6 missing observations) blood samples were analyzed for serological markers of infection with Herpes simplex virus type 1 and type 2. In addition, all vaginal swabs were screened for the presence of DNA of the respective viruses by real-time PCR (see table 26). Our complete study sample (N=174) showed consistently high levels of IgG antibodies against Herpes simplex virus 1 and 2. This fact indicates subsided HSV infections among all study participants. Twenty pregnant women (11.5 %) were tested also positive for IgM antibodies, indicating an active or recently acquired infection. None of the study participants showed isolated elevated IgM antibody titers. Two (1.1%) of the analyzed vaginal swabs (N=180) were positively tested for HSV DNA. HSV-1 and HSV-2 DNA were detected only in one pregnant woman each. None of the women were IgM/IgG seropositive, indicating a very acute infection.

Table 26: Distribution of serological markers against HSV (N=174) and positive PCR (N=180)

IgG	IgM	+ PCR	Prevalence (N)
+	-	-	88.5 (154)
+	+	-	11.5 (20)
+	+	+	.0 (0)
-	-	+	1.1 (2)

Source: own depiction

Parvovirus B 19 (PB19)

Blood samples of 170 (N=10 missing observations) study participants were tested for the presence of PB19 IgM and IgG antibodies with two different commercial ELISA test systems. Since the obtained results were divergent, PB19 DNA was also determined in all serum samples by qPCR.

Ninety-three (54.7%) of the study sample were tested positive for PB19 IgG antibodies when using test system A (Serion ELISA classic). Different results were obtained by test system B (Mikrogen, recomWell); 80% (N=136) were IgG seropositive. Like with IgG, there were major differences in the results for seroprevalences of IgM antibodies when comparing both test systems: Test system A detected specific IgM antibodies in 30.6% (N=52) of the tested samples, while only 4.7% (N=8) of all samples were tested IgM-positive, when using test system B. In 1.2 % (N=2) of cases, the serum samples contained low concentrations (<400 copies/ml) of PB19 DNA. As very low levels of PB19 DNA were detectable in two anti-B19V-IgM-positive women, acute B19V-infection could be suspected in these two cases (see table 27).

Table 27 Results of PB19 screening with two different Elisa systems and by using qPCR

Ig class	Test systems		
	Serion ELISA classic	Mikorgen recomWell	qPCR
IgG	55%	80%	
IgM	31%	4.7%	1.2% (IgM+/IgG+)

Source: own depiction

Applying a logit model, we showed that neither origin nor maternal age influenced the seroprevalence significantly (see table 28). But like with VZV infection, a significant dependency could be shown between seropositivity and number of pregnancies (gravidity); women with more than one pregnancy had a 3.4-fold higher risk for being

seropositive for parvovirus B19. Furthermore, women with at least a primary educational degree had also a 3.4-fold higher probability for a subsided PB19 infection.

Table 28: Results of the logit model with Anti-PB19-IgG as dependent variable

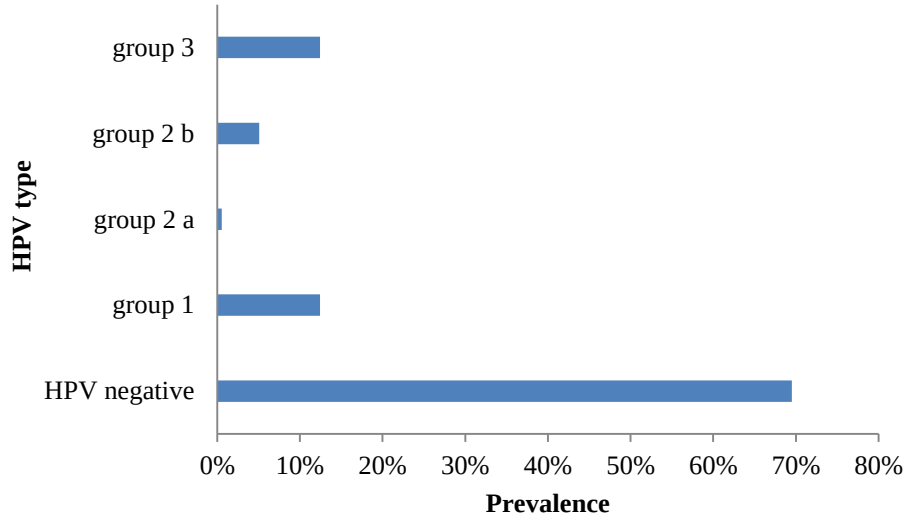
Independent variable	**Observations**	**Number of IgG-PB19 +**	**Prevalence (%)**	**Odds ratio (standard error)**
Age (years)	/	/	/	1.023 (.04882)
Origin (0/1)				
Ellembelle	84	68	81.0	1.435 (.7608)
Other	78	63	80.8	
Gravidity (0/1)				
Primigravidae	48	33	68.7	3.350** (2.012)
>1	106	91	85.8	
Education level (0/1)				
No	45	31	68.9	3.369** (1.792)
Educational degree	95	81	85.3	
Children in household (# of children)	/	/	/	.949 (.1433)

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Human papillomavirus (HPV)

A total of 177 study participants were tested for the presence of HPV-DNA. The prevalence of any of the 37 HPV types in our study population was 30.5% (N=54). In 18 cases (10.2%) more than one HPV genotype was found. To apply our results to the WHO classification of HPV virus, the following distribution, shown in figure 7 could be detected.



Source: own depiction

Figure 7: HPV prevalence by HPV type

Group 1 HPV types (definite carcinogens) could be detected in 12.4% (N=22) of the observations. The prevalence of group 3 HPV types (questionable carcinogens or low risk types) was also 12.4% (N=22). The most frequent HPV types were HPV6, 61, 67, 52, 53, 54, 45, 18 and 42. The prevalence of HPV 16 and 18 was low; HPV 16 was found in 1.1% (N=2) and HPV 18 was found in 2.3% (N=4) of the tested women.

Data indicate a clear peak in HPV at younger ages. Figure 8 shows the distribution of HPV positive women in relation to the age. The prevalence among study participants under 20 years is 41.7%. In all other age groups HPV infection rates decrease more and more. Due to very low prevalences of HIV (0.6%), no association between HIV and HPV could be found.

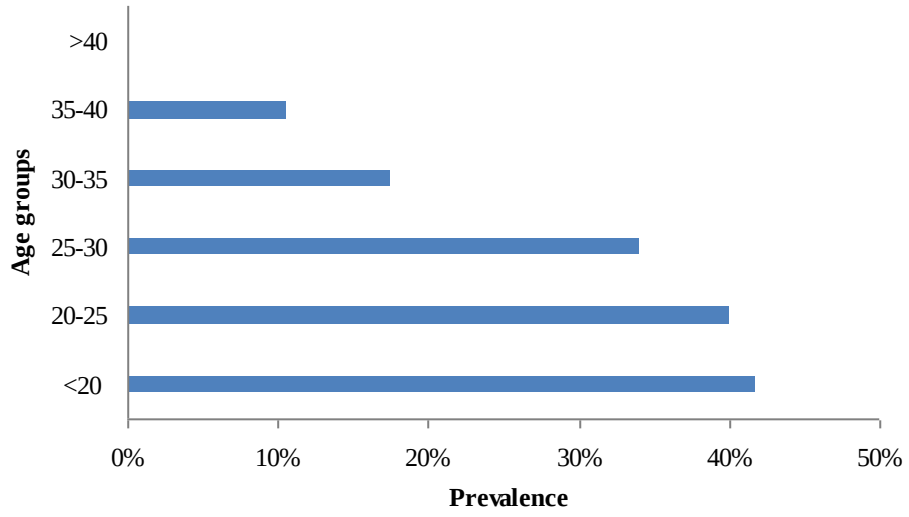


Figure 8: Prevalence of HPV infection by age group

Source: own depiction

5.2.3 Prevalence of bacterial infections

All 180 study participants were screened for bacterial infections with *Treponema pallidum* and *Brucella spp.* or the presence of vaginal colonization with group B streptococci (GBS), *Listeria monocytogenes, Neisseria gonorrhoeae,* and *Chlamydia trachomatis* by analyzing serum samples and vaginal swabs

Group B Streptococci (GBS)

A low vaginal swab was taken of 180 participating pregnant women in order to determine the maternal carriage rate of GBS. Nineteen (10.6%) of our study population were colonized by GBS. Table 29 shows the impact of several sociodemographic and obstetric parameters on the vaginal carriage of GBS. Our survey revealed a higher carriage rate in the mean age group of 25-29 years (see figure 9). In this age range, 14.9% of the pregnant women were colonized by GBS compared to lower percentages in younger and older age groups. However, the difference was not statistically significant applying either the chi square or logit regression. Although an accumulation of GBS cases was found among multigravid (≥1 pregnancy) women. This association was equally not significant. However, study participants from Ellembelle district had significantly higher GBS colonization rates compared to pregnant women from other districts, as tested using a chi

square test as well as logit regression. The distribution showed a GBS rate of subgroup 1 that is more than double as high as the rate of subgroup 2 (15.9% vs. 6.0%). According to our logit regression model, pregnant women from Ellembelle district had a 3-fold higher risk having a vaginal GBS colonization than women from other districts.

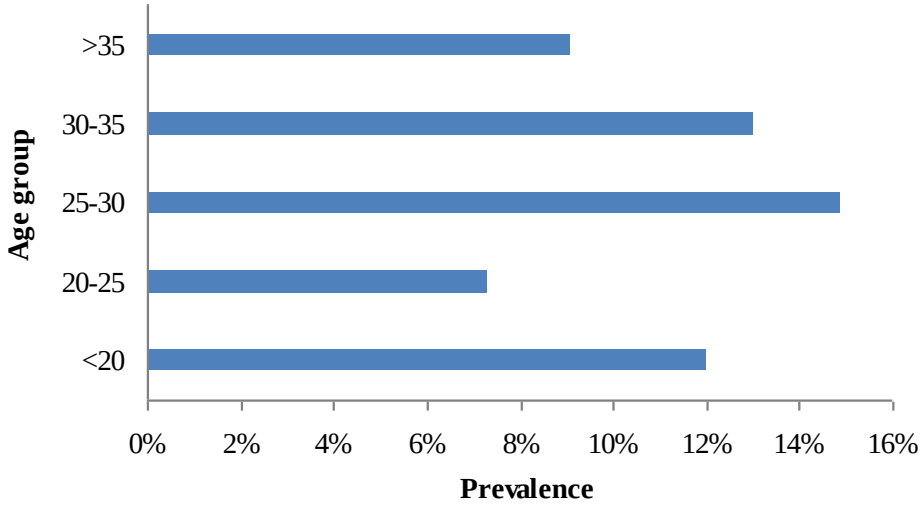


Figure 9: GBS prevalence by age group

Source: own depiction

Table 29: Results of the logit model with GBS carriage as dependent variable

Independent variable	Observations	Number of GBS +	Prevalence (%)	Odds ratio (standard error)
Age (years)	/	/	/	1.055 (0.05216)
Origin				
Ellembelle	88	14	15.9	0.3011*** (0.1943)
Other	83	5	6.0	
Gravidity				
Primigravidae	51	2	3.9	2.470 (2.203)
>1	111	12	10.8	

p<0.1* significance at 10% level, p<0.05** significance at 5% level, p<0.01*** significance at 1% level

Source: own depiction

Listeria monocytogenes and Neisseria gonorrhoeae

Both pathogens were not detectable in all 180 vaginal specimens. For the absence of *Listeria monocytogenes* it is noteworthy that all pregnant women specified canned milk as their only source of dairy products.

Chlamydia trachomatis

Altogether 177 (N=3 missing observations) vaginal specimens were tested for the presence of the gram-negative obligate intracellular organism *Chlamydia trachomatis*. Only three (1.7%) out of 177 samples were tested positive for *Chlamydia trachomatis* DNA. Due to low observation numbers, the determination of correlation to socioeconomic, demographic or obstetric impact factors was not possible.

Brucella spp.

Serum samples of all 180 study participants were screened for the presence of *Brucella spp*.-specific IgG antibodies. However, none of the study participants' sera contained specific IgG antibodies. Appropriately, 100% of the study participants specified canned milk as their only source of dairy products.

Treponema pallidum

All study participants were screened for previous or acute lues/syphilis using the Treponema palladium particle agglutination (TPPA) test, FTA-Abs and VDRL. Procedures and diagnostic conclusions of these serological tests are given in chapter 4. Table 30 shows the immunological status relating to syphilis in the 180 pregnant women. Nine of them (5%) showed a seroreactive TPPA result. A minor percentage (2.8%, N=5) was simultaneously tested positive by the TPPA and the FTA-ABS, a *treponema pallidum* -specific test. However, all patients were tested negative by using the VDRL. Hence, in five cases a treated and consequently subsided syphilis seemed to be likely. The individuals with an isolated positive TPPA (N=4) were probably serological relicts of infections with other *Treponema pallidum ssp.* or immunological cross-reactions. In this regard, it is important to note that yaws caused by *Treponema pallidum ssp. pertenue* is endemic in Ghana. Due to low observation numbers, a correlation to socioeconomic, demographic or obstetric impact factors could not be analyzed. Table 30 summarizes the results of our syphilis screening.

Table 30: Serological profile and their prevalences

Stage of Syphilis	TPPA	FTA-ABS	VDRL	Prevalence (N)
No infection	-	-	-	95 (171)
Primary	+	+	+	0 (0)
Secondary	+	+	+	0 (0)
Tertiary	+	+	+	0 (0)
Treated	+	+	-	2.8 (5)
Cross reaction/Treated	+	-	-	2.2 (4)

Source: own depiction

5.3 Prevalences of coinfections

A further research goal was to determine the frequency/prevalence of simultaneous co-infections and their potential interdependencies. As shown in 5.2, we were able to determine a multiplicity of different infections in our study sample. However, co-infections were rare. This fact was presumably linked to our limited sampling. Thus, co-infections only incidenced among pregnant women infected with HBV, the most prevalent infection in our study sample. 4.7% of the pregnant women were positively tested for HBV and HEV. Furthermore, several HBV-positive women were concurrently infected with GBS (1.1%) or showed serological signs of former contact with *Treponema pallidum* (0.6%). In our limited study sample, we were not able to show an expected high rate of co-infection among STIs (HIV, *T. pallidum*, HBV, HSV1/2, *Chlamydia trachomatis*).

6 Discussion

This chapter compares our data with the current scientific knowledge outlined in the literature review (see chapter 2). Furthermore, we discuss the concrete impact of our results on mother and child health in Western rural Ghana.

6.1 Parasitic pathogens

Plasmodium spp.

In our sample we determine a malaria prevalence of 10.6%. This result is roughly in accordance with other studies from Ghana, where prevalences decreased after the introduction of the intermittent preventive treatment during pregnancy (Hommerich et al.

2007). In our rural population, prevalence of malaria seems to be even slightly lower compared to Southern Ghana (see table 31).

Table 31: Comparison of our results on malaria to data from previous studies

HIC	LIC	Ghana	Our data
No incidence	10.4% (Newman et al. 2003) 15.2% (Ouedraogo et al. 2012)	34.9% (Hommerich et al. 2007) 15.0%* (Hommerich et al. 2007)	**10.6%**

Source: own depiction * after implementation of IPTp

However, it must be pointed out, that our data were collected during dry season from October until January. Hence, prevalence of malaria is most likely higher during raining season. Statistical analysis of our study sample showed that the probability of acquiring malaria during pregnancy decreases with age. This might be linked to a rising immunity against *Plasmodium spp.* in the course of life. Another risk factor was the origin; participants from more distant districts had a higher risk of having malaria. This could be due to better medical support and medical education in the immediate vicinity of St. Martin de Porres Hospital.

Despite this relatively low prevalence, further antimalarial actions, besides IPTp and other primary preventions, are needed to prevent malaria during pregnancy in this region. It would be possible to implement a screening for malaria at regular intervals during antenatal medical care services and treat only in case of proven infection (intermittent screening and treatment, IST). So far, no studies on the comparison of the effectiveness of IPTp and of IST are available.

Toxoplasma gondii

In our study population we are able to determine an IgG seroprevalence of 75% (N=126). Among these IgG seropositive women, 1.1% (N=3) were also positive for IgM antibodies. According to Fig. 4, IgG avidity measurement is performed in the case of this serological status (IgG+/IgM+). Since all IgM seropositive women presented with high IgG avidities an acute infection acquired within the last four months could be excluded (Hedman et al. 1989; Montoya et al. 2002; BioMérieux 2014). Indeed, an acute infection during pregnancy could not be completely excluded, since our serological screening was conducted in the last month of pregnancy. 25% of the tested women were seronegative and therefore considered to be at risk for primary infection. We determined also risk

factors for *T. gondii*-IgM seropositivity in our study sample. Accordingly, the probability of IgM seropositivity decreased with age. This fact is in accordance with a study from Burkina Faso that showed significant higher rates of IgG and IgM seropositivity among younger pregnant women (Linguissi et al. 2012).

Compared to the only other existing study from Ghana (Ayi et al. 2009), our results indicate a slightly lower seroprevalence of *T. gondii*-specific antibodies among the population of rural areas in Western Ghana. Similar seroprevalences were found in Nigeria with 75.4% (Onadeko et al. 1996). Astonishing low seropositivity rates were determined by Linguissi et al. (2012) in Burkina Faso. In this study, among 182 pregnant women only 20.3% were tested positive for IgG antibodies. Surprisingly, Kistiah et al. (2012) found an extremely low seroprevalence of 6.4% among the South African general population. Prevalences of our study population were very high compared to the ones published for Europe (see table 32). Altogether, seroprevalences seem to be very variable among different country.

Table 32: Comparison of our results on toxoplasmosis to data (IgG seroprevalence) from previous studies

HIC	**LIC**	**Ghana**	**Our data**
27.4% (Lebech et al. 1993)	75.4% (Onadeko et al. 1996)	88.7% (Ayi et al. 2009)	**75%**
43.8% (Berger et al. 2009)	20.3% (Linguissi et al. 2012)		
	6.4% (Kistiah et al. 2012) *		

Source: own depiction, *general population

The higher prevalences in our study sample might be explained by a more frequent exposure to transmission paths in rural areas. In general, *T. gondii* is transmitted by contaminated food or by cats and their feces (Jones et al. 2001). It is conceivable that the general population in Ghana is more frequently exposed to high-risk contacts. This may already lead to an increase of the IgG seroprevalence in the first decades of life and consequently to a higher seroprevalence among women of reproductive age.

Our data show that 75% have specific immunity. Hence, 25% are at risk to acquire a primary infection during pregnancy and might thus benefit from dietary and hygienic advices and serological surveillance during pregnancy.

6.2 Viral pathogens

Hepatitis B virus (HBV)

A high prevalence of HBsAg seropositive pregnant women (16.7%) were shown in our study population. This result is in accordance with two recently performed studies in urban areas of Ghana (Candotti et al. 2007; Cho et al. 2012).

Table 33: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data
1.2% (Stück et al. 2001)	8.0% (MacLean et al. 2012)	10.6% (Cho et al. 2012)	**16.7%**
0.60% - 5.79% (Euler et al. 2003)	8.2% (Olokoba et al. 2011)	16% (Candotti et al. 2007)	
0.1% (Salleras et al. 2009)			

Source: own depiction

Also in other African countries with similar socioeconomic and medical settings, such as Nigeria and Mali, HBs-carrier rate is considerably high but lower than in our sample population (Olokoba et al. 2011; MacLean et al. 2012) (see table 33).

We also determined possible risk factors for HBV infection. Increasing age was not significantly associated with higher prevalences of HBV infection. This is in correspondence to other studies confirming that the majority of infections occur in endemic areas below the age of five and mostly by vertical transmission. Horizontal transmission plays a minor role (Allain et al. 2003; El-Serag and Rudolph 2007; Cho et al. 2012). Furthermore, statistical analysis showed that HBV prevalence is independent of all other tested possible impact factors (origin, education, gravidity).

Our data indicate that a remarkable high percentage of pregnant women and children are exposed to the risks of acute and chronic HBV infection. 15% to 40% of infected patients later develop cirrhosis and/or hepatocellular carcinoma (HCC) (Lok and McMahon 2009). Incidence of HCC in West Africa is approximately 20 cases / 100.000 inhabitants (El-Serag and Rudolph 2007). At the moment, there is no data available on the incidence of HCC in the Western Region of Ghana.

Vertical (mother to child) transmission is common and responsible for this alarming high prevalence of HBV and its sequelae. The rate of maternofetal transmission is estimated to be between 3.3% and 55.5%, depending on the maternal virus load, as shown by Candotti

et al. (2007). Without vaccination or other medical intervention, up to 90% of perinatally infected newborns develop a chronic progression. In contrast, maternal morbidity and mortality rate is most likely not influenced by HBV infection. However, HBV may have adverse effects on the pregnancy-outcome (low birth weight, preterm birth), as shown by Jonas et al. (2009).

Although prenatal HBV screening is part of nationwide guidelines for gynaecology and obstetrics in Ghana, in practice many deliveries occur without the knowledge of the maternal HBV status: in our study population, only a minority of 17.2% was tested on HBV by a rapid serological test (ACON, San Diego, USA). Reasons for this are multifarious. Informal interviews show that rare visits of antenatal care services, no test possibilities in many smaller health centers and a low capacity of the laboratory in St. Martin de Porres Hospital are the main factors for this problem. Also passive and active immunization for newborns of seropositive pregnant women is currently not refunded by the public health insurance in Ghana. The importance of horizontal transmission is hard to estimate. Recent studies performed in Kumasi/Ghana characterized the risk of hepatitis B infection by blood transfusion as low due to consequent screening of blood donors (Allain et al. 2003). The role of sexual polygamy and application of contraception is unclear.

Hence, the implementation of an intensified nationwide antenatal screening and in St. Martin de Porres Hospital should be considered. We could show that a reliable rapid test system (HBsAg EIA test kit, ASCON) already exists. In addition, newborns of HBs-positive mothers need to be consequently vaccinated. Future research should investigate the exact impact of vertical HBV transmission on neonatal and child health in rural Western Ghana. In addition, the investigation of further risk factors for HBs-positivity, like promiscuity or traditional medicine, is of interest.

Hepatitis C virus (HCV)

We emphasized an HCV seroprevalence rate of 1.1%; only two women were tested positive on anti-HCV antibodies. Both of them were not aware of their infection. In general, our data confirm other studies from Ghana. Wansbrough-Jones et al. (1998) determined an HCV prevalence among females of 1.0% which underlines our findings. Only one study conducted in Central Ghana reports a considerably higher prevalence with 5.1% (Apea-Kubi et al. 2006). In most African studies, infection rates vary between 2-6%

(Zahran et al. 2010; Zeba et al. 2011). Compared to Europe, the prevalence of HCV in Western Ghana is similar (Martyn et al. 2011) or slightly higher (RKI 2013b). The very limited number of observations did not allow an evaluation of potential risk factors (HIV, history of blood transfusion). Therefore, a reported accumulation of HBV, HCV and HIV among pregnant women in Nigeria (Okeke et al. 2012) cannot be confirmed nor rejected in the case of rural Western Ghana.

Table 34: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data
0.4% (RKI 2013b) *	6.4% (Zahran et al. 2010)	5.1% (Apea-Kubi et al. 2006)	**1.1%**
1.4% ((Martyn et al. 2011))	3.6% (Ugbebor et al. 2011)	2.8% (Apea-Kubi et al. 2006)	
	2.14% (Zeba et al. 2011)		

Source: own depiction * general population

The medical impact of HCV during pregnancy should be less serious compared to adverse effects of considerably more frequent HBV infections. Nevertheless, maternofetal transmission occurs in 5-10% of the cases and is responsible for the majority of HCV infections during childhood (Le Campion et al. 2012). Further research projects should focus on the impact of HCV on neonatal and child health in rural Western Ghana.

Hepatitis E virus (HEV)

The seroprevalence of HEV was surprisingly high; among 174 pregnant women, we determined an anti-HEV IgM seropositivity rate of 20.7% (N=36). Specific IgG antibodies were detected in 6.9% (N=12) of the cases. However, the majority of pregnant women (71.8%, N=125) did not show serological signs of an acute or past HEV infection (see table 35). Only one serological profile clearly proved an acute HEV infection (IgM+IgG seropositive). Since isolated elevated IgM titers do not prove an acute infection, these patients should be retested. Fortunately, no apparent clinical infections with acute jaundice were recorded but can not be excluded, since no further tests (transaminases, liver function values) were conducted. The seroprevalence of HEV was not different between Ellembelle and the surrounding districts. Surprisingly, Caron and Kazanji (2008) showed a significant higher seroprevalence in urban areas compared to rural areas in Gabon.

Table 35: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data	
			IgM	IgG
16.8% ** (Faber et al. 2012)	14.1% IgG (Caron and Kazanji 2008)	18.5% IgM (Adjei et al. 2009) 10.2% IgG (Adjei et al. 2009)	**20.7%**	**6.9%**

Source: own depiction, ** general population

Our socioeconomic data did not indicate a significant relation between HEV serostatus and unsafe drinking water sources (unprotected surface water). However, other studies described a relation between unhygienic conditions (including contaminated surface water) and high anti-HEV antibody titers (Guthmann et al. 2006; Adjei et al. 2009). In addition, endemic outbreaks are described in the context of flooding and successive drinking water contamination in Africa and Southeast Asia (Sudan: Guthmann et al. 2006; Uganda: Howard et al. 2010). In our study area, comparable flooding occurs during raining season.

Furthermore, bush meat (mostly rats) is discussed as an origin of infection in the study area. Several studies give some evidence that close contact to animals and consumption of swine, wild boar or game (Clayson et al. 1995; Tamada et al. 2004; Adjei et al. 2009) is a major risk factor for HEV infection. In our study population, only 3% declare bush meat as part of their regular diet. Pork can largely be eliminated as a source of infection due to the fact that livestock farming is completely focused on poultry and goat.

Unlikely but not excludable is the possibility that false-positive IgM antibodies were measured due to polyclonal stimulation in the context of an immune response against malaria, other infections or against traditional herbs.

The burden on mother and child health during pregnancy is hard to estimate. At least in our study sample, clinical HEV infections seemed to be unlikely. Hence, further investigation could focus on the exact clinical process of HEV infections in rural areas of Western Ghana and determine the exact incidences of different HEV serotypes among pregnant women and potential risk factors. In addition, environmental specimen (water sources, meat, stool samples of farm animals, fish/seafood) could be tested on HEV-RNA to detect possible sources of an infection. If further investigations lead to an increased suspicion, additional medical measures, including screening should be implemented.

Cytomegalovirus (CMV)

To the best of our knowledge, our results substantiate the first data on CMV during pregnancy in Ghana. The seroprevalence of CMV was high with 100%. All the study participants were IgG seropositive and hence indicated past CMV infection. Our results are within the range reported for other African countries (see table 36).

Table 36: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data
56.3% (Alanen et al. 2005)	87.0% (Bello and Whittle 1991)	No data	**100%**
46.8% (Picone et al. 2009)	97.2% (Rodier et al. 1995)		
	72.2% ((Hamdan et al. 2011)		

Source: own depiction

In Europe, the seroprevalence rates of CMV among pregnant women are considerably lower. Reasons for that may be a differently intense exposition to transmission paths in industrialized and developing countries. CMV is usually acquired through sexual contact or through contact to young children (Alanen et al. 2005). Therefor, the seroprevalence of CMV in rural parts of Africa might be higher. The primary infection rate in our study population is unclear, since at least two subsequent tests need to be done to detect a seroconversion. Studies from Europe determine the prevalence of primary infection during pregnancy with 1-4% (Johnson et al. 2012). Higher IgG seroprevalences in our study group may indicate lower rates of CMV susceptibility among Ghanaian women in the childbearing age and thereby lower risk to acquire primary infection during pregnancy.

Of importance are findings that indicate a perinatal or postnatal CMV transmission from IgG seropositive mother to their child without maternal signs of an acute primary infection. Up to 90% of IgG seropositive women transmit CMV through breast milk feeding in the first four months postpartum. A symptomatic neonatal CMV infection may result in 50 per cent of all cases (Vochem et al. 1998; Johnson et al. 2012). In this context, a study performed in Gabun provides interesting data: 14% of newborns were infected by CMV (viral excretion in urine or saliva) although maternal primary infection was absent (Bello and Whittle 1991).

This stresses that without a serological and clinical screening of newborns for CMV, a negative impact of CMV on mother and child health in Western Ghana is not excludable. Further research should focus on the prevalence of congenital CMV infections.

Rubella virus

We could determine a seroprevalence of 84.6% for specific IgG antibodies resulting in a susceptibility rate of 15.4% of our study participants. Hence, this result is in the range of other studies (see table 37). Based on this maternal prevalence, the impact of rubella infections remains questionable.

Table 37: Comparison of our results on rubella virus IgG seroprevalence to data from previous studies

HIC	LIC	Ghana	Our data
85.8% (Calimeri et al. 2012) 95% (Hernandez Diaz et al. 2011)	95,3% (Barreto et al. 2006) 96.5% (Corcoran and Hardie 2005)	92.6% (Lawn et al. 2000)	**84.6%**

Source: own depiction

A study from Ghana investigated the incidence of congenital rubella syndrome (CRS) in the Kumasi region. This research project estimated a CRS rate of 0.8 per 1,000 live births but probably in connection with a rubella outbreak (Lawn et al. 2000). The further data situation is poor and a national reporting obligation does not exist. Consequently, more scientific data on the burden of this disease is needed to create a basis for policy implications like a nationwide rubella vaccination program. This may be established through the installation of a national register in Ghana with the aim of achieving exact data on incidences of maternal rubella infections and of CRS. Alternatively, major studies are needed to determine the frequency of maternal rubella by monitoring the serological profile of pregnant women during the course of pregnancy.

Human immunodeficiency virus (HIV)

In our study sample, only one pregnant woman was tested positive on HIV. Consequently, we determined a prevalence of 0.6%. In comparison to other studies, this HIV prevalence is surprisingly low (see table 38). Since the sample number of our pilot study was low subsequent studies should focus a broader HIV screening among pregnant women and additionally among neonates in order to further improve our database.

Table 38: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data
<0.19% (Nicoll et al. 1998)	2-8%* (Asamoah-Odei et al. 2004)	2.1% (UNAIDS 2012b) 3.1% (Duda et al. 2005) 6.0% (Apea-Kubi et al. 2006)	**0.6%**

Source: own depiction, * general population

Varicella-zoster virus (VZV)

Surprisingly, in our study sample the seroprevalence of anti-VZV IgG was very low (see table 39). The seropositivity rate as determined by us in Ghana was half as high as in industrialized countries, but in accordance with data from the U.S. that indicate a higher seronegativity rate among immigrants from (sub-) tropical countries (Leikin et al. 1997; van Rijckevorsel et al. 2012). In combination with a non-existing VZV vaccine policy in Ghana, a lower natural virus circulation within the population might be the reason for this low seroprevalence rate. Additionally, we investigated the effect of origin, number of pregnancies and the number of children in the household on the VZV-specific seroprevalence, indicating that seropositivity is linked to contact to young children (Daley et al. 2008).

Table 39: Comparison of our results to data from previous studies

HIC	LIC	Ghana	Our data
≥94% (Alanen et al. 2005)	80.9%* (Hannachi et al. 2011)	No data	**56.5 %**

Source: own depiction; * limited data

Currently, general VZV screening during pregnancy or selective testing of high-risk groups among pregnant women is not part of the clinical practice in Ghana. Furthermore, hospitals in rural areas do not have the means and resources to treat maternal or neonatal varicella. As a consequence, the incidence of maternal varicella and of its sequelae (congenital varicella syndrome, CVS) is not known in Sub-Saharan Africa. Hence, further research is needed to estimate the burden of this particular disease and to plan adequate medical and political interventions. Possibly reasonable measures would be a nationwide vaccine program and the supply of acyclovir treatment during pregnancy and of a post-exposure prophylaxis (varicella-zoster immunoglobulin).

Herpes simplex virus (HSV)

HSV-specific IgG seroprevalence was 100% in our study population. This is a remarkably high seropositivity, compared to other studies from Europe and Africa (see table 40). All 174 tested pregnant women were IgG positive. In 20 cases, the serological profile (IgM+/IgG+) indicated an acute HSV infection or a reactivation during pregnancy, since IgM antibodies have a high sensitivity and specificity for active infection (both 98%), and long persistency of IgM antibodies is rare in HSV infections.

Table 40: Comparison of our results on HSV-IgG seropositivity to data from previous studies

HIC	LIC	Ghana	Our data
2.0% ** (Anzivino et al. 2009)	51.1% *(Kurewa et al. 2010)	No data */**/***	**100%***
54.3%* (Alanen et al. 2005)	0.9%** (Latif et al. 1999)		
82.7%* (Kucera et al. 2012))	49.1%*** (Munjoma et al. 2010)		

Source: own depiction *IgG seroprevalence HSV1/2/ **prevalence of Herpes genitalis/ ***IgG seroprevalence HSV-2

Potentially, a high viral load in the general Ghanaian population and prevalent risk factors for HSV transmission in the study area, may explain the high IgG seroprevalence. Comparable studies show that HIV infection, low educational level, and polygamy significantly increase the risk for HSV-2 seropositivity (Munjoma et al. 2010). We were not able to search for risk factors and coinfections, because of two limitations: the serological test used by us does not differ between HSV-1 and HSV-2, and our study population was consistently seropositive. We detected genital herpes in two cases (1.1%) by real-time PCR. This prevalence is in accordance with another study from Africa (0.9%, Latif et al. 1999) and data from the US (2.0% Anzivino et al. 2009). Type-specific testing showed HSV-1 in the first positive vaginal sample and HSV-2 in the second one.

Since the majority of neonatal HSV infections occurs by perinatal transmission from pregnant women with asymptomatic genital HSV infections (≈ 70% Anzivino et al 2009), we assume that 1.1% of infants in our study population were at risk to acquire a neonatal HSV infection. However, data on the effective transmission rate and on the prevalence of neonatal HSV infections in Sub-Saharan countries are limited. Consequently, further research should focus on the prevalence of HSV infection among newborns and its impact on child health. Furthermore, the prevalence of HSV-1 versus HSV-2 among pregnant

women in Ghana, risk factors and prevention measures should be a future area of research.

Parvovirus B19 virus (PB19)

Our data show that prevalences of anti-PB19 IgM and IgG antibodies are highly dependent on the test system. The test system B (Mikrogen recomWell Parvovirus B19 IgG/IgM) is used by the German Consulting Laboratory for Parvovirus B19 Infection at the University of Regensburg, and is thus thought to be the more valid one. In addition, the presence of PB19-DNA should be determined in order to maximize sensitivity and specificity of the overall diagnosis (Bredl et al. 2011). However, and to date, most of the published studies just determine IgM and IgG antibodies for diagnosis. Consequently, those data must be regarded with care.

Table 41 Comparison of our results on PB19 infection parameters with data from previous studies

HIC*	**LIC***	**Ghana**	**Our data**			
			Test	IgM	IgG	DNA
66% (Jensen et al. 2000)	27.5% (Emiasegen et al. 2011)	No data	A	31%	55%	3.0%
58.6% (Alanen et al. 2005)	61% (Elnifro et al. 2009)		B	4.7%	80%	
69.2% (Enders et al. 2007)	24.9% (Schoub et al. 1993)					

Source: own depiction; * IgG seroprevalence

Our data, using test system B indicate that about 80% of our study population were seropositive for PB19-specific IgG antibodies. When considering both test systems 20-45% of our study population could be considered to be susceptible to a parvovirus infection. In only two cases (1.2%), we found evidence for a recently acquired or active parvovirus infection (IgM+/IgG+/DNA+). In contrast to most other studies from Africa, we found a higher seroprevalence of PB19-specific IgG antibodies. A possible explanation for high seroprevalences is close contact to children (Jensen et al. 2000). Accordingly, our data showed a significant impact of the educational level and the number of pregnancies on PB19-specific IgG seroprevalences. Thus, the higher seroprevalence could be explained by social impacts, such as families with many children and closer contacts in school.

Current data on PB19 infection in Ghana and other African countries is scarce and the incidence of adverse pregnancy outcomes due to acute PB19 infection in developing

countries is questionable. Therefore, further research should focus on concrete incidences of maternal PB19 infections during pregnancy and their impacts on pregnancy outcome and fetal/neonatal health in developing countries. Based on this data, the purpose of interventional measurements could be considered.

Human papillomavirus (HPV)

The prevalence of HPV infection in our study population was 30.5%. The prevalence was higher compared to two major studies that estimated a prevalence of 24% in Sub-Saharan Africa and considerably higher compared to the only existing Ghanaian study (see table 42).

Table 42 Comparison of our results on HPV prevalence to data from previous studies

HIC	LIC	Ghana	Our data
11-12% (Forman et al. 2012)	24% (Forman et al. 2012) 24% (Bruni et al. 2010) 37% (Akarolo-Anthony et al. 2014)	10.7% (Domfeh et al. 2008)	**30.5%**

Source: own depiction

Furthermore, we observed an inverse association between age and the the rate of HPV. This peak of HPV infection at younger ages (<30 years) is in accordance with other studies (Bruni et al. 2010; Akarolo-Anthony et al. 2014).

On the pathogenic level, we detected low prevalences of HPV 16 (1.1%, N=2) and HPV 18 (2.3%, N=4) in relation to the worldwide prevalence of HPV 16 of 3.2% and HPV 18 of 1.4%, shown by one major meta-analysis (Forman et al. 2012). Concurrently, HPV 67 another definite carcinogen seems to be more frequent in our study population with a prevalence of 4.0% (N=7).

6.3 Bacterial pathogens

Group B streptococci (GBS)

The prevalence of maternal colonization by GBS in our study population was 10.6% and thus in the range with previous studies (see table 43). The fact that a slight tendency towards a lower prevalence was found in our study may be explained by the mode of data collection. In our study, only vaginal specimens were analyzed for the presence of GBS. In all other studies, a combined recto-vaginal swab was taken for analysis.

Table 43: Comparison of our results on GBS carriage to data from previous studies

HIC	LIC	Ghana	Our data
25.6 % (Kieran et al. 1998) 6.6 % (Citernesi et al. 1996) 5-40% (Halle et al. 2000)	4-25% (Marai 2001) 23% (Joachim et al. 2009)	19% (Enweronu-Laryea et al. 2011)	**10.6%**

Source: own depiction

Furthermore, we did not use a specific culture medium to increase the sensitivity for detection of GBS. Another limitation of our approach might be the use of human donor blood for preparing blood and chocolate agar. Hence, a possible contamination by antibiotics may result in limited bacterial growth.

Surprisingly, our results showed a higher prevalence of GBS colonization in Ellembelle district than in the surrounding districts. On the basis of our data, this result could not be explained.

The impact of this result on mother and child health in Western rural Ghana is hard to estimate. Current data indicate a similar importance of GBS in neonatal sepsis with an even higher mortality rate in developing countries compared to industrialized countries (Watson et al. 2003; Gray et al. 2007). Insofar, the role of GBS in neonatal sepsis in rural Western Ghana needs to be investigated more precisely in the future. To deepen the scientific basis, it would be helpful to determine the effective prevalence of vertical GBS transmission and of subsequent neonatal infections. In the case of high neonatal GBS prevalences, a general or risk-adapted GBS screening should be carried out during the last trimester of pregnancy. Furthermore, an intrapartum antibiotic prophylaxis (IAP) in conformity with the 2010 American Guidelines for the Prevention of Perinatal Group B Streptococcal Disease (Prevention 2010) should be discussed.

Listeria and Neisseria gonorrhoeae

Both vaginal pathogens were absent in the 180 differentiated vaginal swabs of our study population. A very low prevalence of *Neisseria gonorrhoeae* was expected, since several studies from Africa and one study from Ghana show comparable results (see table 44). Same holds true for the prevalence of *Listeria spp*. No epidemiological data during pregnancy is available from other African countries.

Similarly, two studies from Europe show very low prevalences among women of reproductive age in Serbia (see table 45). The non-existence of *Listeria spp*. in our study

sample is in accordance with the fact that canned milk was the only consumed diary product. Consequently, the impact on mother and child health seemed to be rather small compared to other infections at least in our sample.

Table 44: Comparison of our results on Neisseria gonorrhoeae to data from previous studies

HIC	LIC	Ghana	Our data
2.3% (Farley,2003)	3.9 % (Latif et al. 1999) 0.5 - 14 % (Marai 2001) 1.7 % (Gray et al. 2001)	0.4 % (Apea-Kubi et al. 2004)	**0%**

Source: own depiction

Table 45: Comparison of our results for Listeria to data from previous studies

HIC	LIC	Ghana	Our data
2.0% (Lamont and Postlethwaite 1986) 0.1% (Stepanovic et al. 2007)	No data	No data	**0%**

Source: own depiction

Chlamydia trachomatis

The prevalence of *Chlamydia trachomatis* in our study population was lower than outlined in other epidemiological studies. Regarding Ghana, a study from the Korle-Bu Teaching Hospital in Accra (Apea-Kubi et al. 2004) describes a prevalence of 3.4 % among pregnant women (see table 46). Prevalence in our rural study area is considerably lower. Reasons for this difference are multifarious and possibly strongly related to a tendency towards an increased number of risk exposures in urban centres. Table 46 integrates our result into the current data situation.

Table 46: Comparison of our results for Chlamydia trachomatis to data from previous studies

HIC	LIC	Ghana	Our data
10.1 % (Farley,2003)	5.3% (Latif et al. 1999) 9.0% (Fonck et al. 2000) 7-31% (Marai 2001)	3.4 % (Apea-Kubi et al. 2004)	**1.7%**

Source: own depiction

Brucella

No seroprevalence of *Brucella* was detectable in our study population. To best of our knowledge, apart from one Egyptian study, that tries to determine the effect of maternal brucellosis, no further epidemiological study for African countries exists. Hence, in our study area a negative impact of Brucellosis on maternal and child health may be most likely excluded. Like with listeriosis, the absence of raw milk consumption might be the main factor explaining this result in rural Western Ghana.

Treponema pallidum

In developing countries, syphilis remains an important factor that impacts mother and child health in the 21th century. A plurity of African scientific projects determines the prevalence of syphilis over the past decade. These studies show that prevalences vary between 2 and 17% (see table 47). However, it must be considered that all these studies have methodological restrictions: namely the majority of studies use either only non-treponemal tests (VDRL/Rapid Plasma Reagin, RPR) or only one specific treponemal test (TPHA/TPPA/FTA-Abs). This may lead to false-positive results due to a cross-reaction with other species of *Treponema*, i.e. *T. pallidum ssp. pertenue*, the infectious agent of yaws. An alternative source for false-positive results is the lifelong persistence of TPHA- and TPPA-reactive antibodies after successful antibiotic treatment (Parija 2009; Groß 2013). Our results would be in accordance to recent studies, if only TPPA is considered. In this case, syphilis prevalence of our study population would be 5% (see table 47). However, after conducting a confirmatory test (FTS-ABS) and a further screening test (VDRL), all positive TPPA tests would have to be interpreted as subsided and/or treated *T. pallidum* infections or cross reactions. Consequently, we could not find an acute *T. pallidum* infection among our pregnant study participants. However, our study sample was too small for transferring our results to a larger study population. The fact, that only 53% of pregnant women were prenatally tested on syphilis is alarming. Consequently, in the sector of antenatal screening further efforts are needed. Additionally, further research would be needed to investigate the impact of *T. pallidum* on neonatal health.

*Table 47***:** *Comparison of our results to data from previous studies*

HIC	LIC	Ghana	Our data
4.5% (Genc and Ledger 2000) 0.04% (Meyer Sauteur et al. 2012)	3.8% (Latif et al. 1999) 7% (Fonck et al. 2000) 2-17% (Watson-Jones et al. 2002)	7.1% (Apea-Kubi et al. 2004)	**5%** **(TPPA)** **0%** **(TPPA+VDRL+ FTS-ABS)**

Source: own depiction

7 Conclusion

In developing countries of Sub-Saharan Africa, mother and child morbitiy and mortality rates are still among the highest in the world. In this context, infectious diseases play a major role. Current knowledge on the prevalence of bacterial, viral and parasitic infections during pregnancy in rural settings is very limited. We therefore conducted a cross-sectional pilot study in a rural area of Western Ghana with the aim to systematically develop an infectiological profile of pregnant women. For this, we screened 180 pregnant women for the presence of infectious diseases, which are known to have an influence on mother and child health.

The microbiological methods were based on either the direct detection of the pathogens by culture, MALDI-TOF, or PCR, or the indirect identification of an infection by serological markers. Additionally, all study participants were interviewed for the presence of potential risk factors, outcome of previous pregnancies, socioeconomic aspects and antenatal medical care services.

Our results show a high prevalence of acute or past infections with a broad spectrum of parasitic, viral and bacterial pathogens. Maternal malaria was shown to be frequent, despite increasing acceptance of the intermittent preventive treatment (IPT) and primary prevention. Seroprevalence of *Toxoplasma gondii*-specific antibodies was 75% and we determined at least three potential acute infections acquired during pregnancy. Concerning viral pathogens, we showed a high prevalence of infections caused by the hepatotropic viruses HBV, HCV, and HEV. Hence, in our sample 16.7% of the study participants were tested positive for HBs antigenemia, indicating active hepatitis B. The prevalence of HIV was very low and can be qualified as an encouraging step in the fight

against HIV/AIDS. Furthermore, we determined a high IgG seroprevalence for other pregnancy-related viral pathogens, such as CMV, HSV, VZV, rubella virus, and PB19 virus. Group B streptococci, the major risk factor for neonatal sepsis, were isolated from the birth canal of 10.6% of all pregnant women.

In developing countries, so in Ghana, many disease burdens compete for limited public health attention and funding. Therefore, and based on our results, we particularly recommend to focus at the moment on a more intense control of hepatitis B and infections caused by group B streptococci. With regard to hepatitis B, intensified antenatal screening and postnatal vaccination of the newborn in the case of maternal infection should be discussed. Furthermore, an intrapartum antibiotic prophylaxis (IAP) seems to be an appropriate instrument to minimize the risk of vertical GBS transmission and consequently of neonatal infections.

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Appendix

(1) Informed consent

Study: Prevalence of pregnancy-related infectious and non-communicable diseases in Ghanaian women

Dear lady,

I would like to ask you and your future child for participation in the above-mentioned study about risk factors during pregnancy. We can imagine that a couple of days before delivery there are more important things for an expectant mother than considering the participation in a medical study. But let me please shortly describe our work and its aim. If there are some words you do not understand, please ask me and we will take time to explain.

My name is Fabian Voelker. I am a 25-year-old medical student from Goettingen/Germany. In the context of my doctoral thesis I would like to determine infectious (bacteria/viruses) and non-infectious (pregnancy-associated diseases) risk factors during pregnancy. My work on this subject is guided by the experienced professor Prof. Dr Uwe Groß from the University Medical Centre of Goettingen.

Pregnancy, one of the big miracles of life, is a nine month-long journey. We are happy that in the majority of cases a healthy mother gives birth to a healthy little child. Unfortunately, there exist also some risks for mother and child during pregnancy. For example, bleedings or preterm births can occur. The mother or her child can get ill.

Particularly infections can cause complications because they are able to move from mother to child and in this way infect the child. In general, there are two possible ways. On the one hand, some infections can infect the child while it

grows in his mother (intrauterine). At the worst this may cause a miscarriage or disturb the normal development of the child. On the other hand, there exist some infections which are transmitted to the child during delivery. Certain bacteria may populate the birth canal and in so doing they have contact to the new-born child (perinatal). Unfortunately, some of these bacteria are able to provoke so-called neonatal infections like for example high fever or pneumonia.

Furthermore, there are diseases of the mother which typically occur during pregnancy. For example, the blood pressure or the level of sugar in the blood can be extraordinary high. These complications may affect the health of mother and child.

Therefore, we want to find out the frequency of potentially dangerous infections and diseases during pregnancy in order to develop suitable strategies in the future.

Participation in the study is entirely voluntary. It is your choice whether to participate or not. If you choose to participate or not in this study, all the services you receive in the clinic will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier. Medical risks are not elevated by participating in this study project.

If you like to participate we would like to take a blood sample of 5-10 ml, which means only some drops of blood and a little prick in your arm. Then we would like to perform a vaginal and anal smear test. According to your wish these medical examinations will be realized by me or by a nurse. Blood taking and the gynecological examination will take around 30 minutes. Following this, we will analyze the samplings in our laboratory. In addition to this we would ask for answering the questionnaire which should help us to understand the general medical situation of pregnant women in Ghana. If your child is born without any signs of infection like for example fever, the study is completed. Only in case of infections of your baby, we would like to perform further diagnostic methods what means an examination of its blood and its spinal fluid.

The information that we will collect by these examinations will be kept confidential. Information about you and your child will not be identified by your name but by a number.

You do not have to decide immediately whether you will or not participate in the research. Just take a couple of hours to reflect.

We would highly appreciate your support.

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Study:

Prevalence of pregnancy-related infectious and non-communicable diseases in Ghanaian women

Informed consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any question that I have asked has been answered to my satisfaction. I understand that it will involve a vaginal and anal smear test and a blood taking. I know that my child will be examined in case of any signs of infection. I have been informed that the risks are extremely minimal. I am aware that there will be no benefit for me personally and that I will not be compensated.

I consent voluntarily to participate together with my future child as a participant in this research and I understand that I have the right to withdraw from the research at any time without in any way affecting my medical care.

Print name of Participant _____

Signature of Participant _____

Date _____ (Day/Month/Year)

Informed consent if illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Thumb print of Participant 

Print name of witness _____

Signature of witness _____

Date _____ (Day/Month/Year)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of researcher _____

Signature of researcher _____

Date _____ (Day/Month/Year)

(2) Questionnaire

Pilot Questionnaire Eikwe/Ghana risk factors during pregnancy

Q1. Name of home region _____

Q2. Name of home district _____

Q3. Name of home village _____

IF THE ANSWER TO ANY QUESTION IS 'DON'T KNOW', WRITE 97 NEXT TO THE CODES. IF THE PERSON DOES NOT WANT TO ANSWER WRITE 98.

Demographic data

1. How many people live in your household? _____
2. How many adults (>17) live in your household? _____
3. How many children (<12) live in your household? _____
4. How many children younger than 5 years old live in your household? _____
5. Does your husband live in your household the whole year?
   1- Yes ☐ 2- No ☐
6. What was the highest education level you achieved? _____
7. Which vaccinations did the youngest children receive? **Use the card** CODING
   - ☐ BCG
   - ☐ OpV0
   - ☐ OpV1
   - ☐ OpV2
   - ☐ DPT1
   - ☐ DPT2
   - ☐ Others (please specify_____)

Nutrition

8. Do you use the water from the lake for drinking? 1- Yes ☐ 2-No ☐

9. How often did you eat meat during the last 7 days? Number ☐

10. What kind of meat?

☐ Chicken/Duck
☐ Goat
☐ Pork
☐ Beef
☐ Fish
☐ Bushmeat (please specify _____)
☐ Others (please specify_____)

11.How often did you drink milk during the last 7 days? Number ☐

12. How often did <u>your children (< 5 years)</u> drink milk during the last week?

Number☐

13. Where is the milk coming from?

☐ 1- Directly from my own animals
☐ 2-Directly from animals of friends/neighbours
☐ 3- Unpacked milk from Shop
☐ 4- Packed milk from shop

14. If it is own milk, from neighbours/friends or unpacked is it mainly from?

☐ 1- a cow
☐ 2- a goat/sheep
☐ 3- not applicable

15. If it is own milk, from neighbours/friends or unpacked, how do you treat the milk before drinking?

☐ 1- Raw/Untreated
☐ 2- Heated/boiled
☐ 3- Fermented/Yoghurt

Health data ONLY Children < 12 years in your household

16	17	18	19	20	21	22	23	24	25	26	27	28
Child No.	Age	Place of birth	Is the child immunized against								Growth Rate	Age of mother birth
			BCG	OPV			DPT			HeB		
				1	2	3	1	2	3			
1												
2												
3												
4												
5												
6												

1- at home without TBA

2- at home assisted by TBA

3- Hospital Eikwe

4- other Hospital

1-Upper Red

2-Green

3-Lower Red

4-Grey

29. How often did you contact your health facility with your youngest child in the first year of life?

1. every month ☐
2. regularly ☐
3. only in case of illness ☐
4. never ☐
5. Don't know ☐

30. How often did you contact your health facility with your youngest child afterwards until it reached the age of five?

1. every month ☐
2. regularly ☐
3. only in case of illness ☐

4. never ☐
5. Don't know ☐

31. When your children fall ill, what do you do?

1.Self Treatment traditional medicine ☐
2.Traditional healer ☐
3.Drug store ☐
4.Health facility ☐
5.Other, specify ☐ _____

32. Have any of the children in your household died before she/he reached the age of five?

1- Yes ☐ 2- No ☐ **if no Q39**

33. How many children have died? Number ☐

34. What symptoms did the child have before deaths?

1- Fever ☐
2- Diarrheal ☐
3- Cough ☐
4- Convulsion ☐
5. Fast and frequent breathing ☐
5- Other, specify ☐ _____

36. How old was the child at deaths? _____ months OR _____ years

37. Did this child receive treatment before deaths of

1- No treatment ☐
2- Self treatment ☐
3-Health facility ☐
4-Traditional healer ☐
5- other specify _____

38. How many days was the interval between onset of the disease and death? ____days

39. From your point of view what is the main problem in taking care of the health of your children?

_____

40. How many of your children below the age of 5 slept under an impregnated bed net the last night? Number _____ (101 if no children below five)

Obstetric History Gravida __ / Para __

Fill in the Table with the respective codes given below!

41	42	43	44	45	46	47
Child No.	Year of birth	Mode of delivery	Place of delivery	Complications (multiple answers)	Pregnancy outcome	Birth weight
1						
2						
3						
4						
5						
6						

Mode of delivery:

1. spontaneous vaginal delivery
2. vaginal operative delivery (Vacuum extraction)
3. primary caesarean section
4. secondary cesarean section
3. APH (Ante Partum Hemorrhage)
4. PPH (Post Partum Hemorrhage)
5. Puerperal sepsis
6. Anemia
7. Malaria
8. Premature labour
9. Breech
10. Twins
11. Multiples

Place of delivery:

1- at home without TBA
2- at home assisted by TBA
3- Eikwe Hospital
4- other Hospital

Pregnancy outcome:

1.live birth
2. still birth
3. neonatal death (within 28 days)
4. abortion (weeks of gestation)

12. Asphyxia

13. other (please specify)

48. In your last pregnancy, did you attend ANC? 1- Yes ☐ 2- No ☐

49. Are you pregnant at the moment of interview? 1- Yes ☐ 2- No ☐ if no Q53

50. Have you started attending ANC clinic (Ante Natal Care clinic)? Yes ☐ No ☐

51. If yes, how many months are you pregnant? _____ months

52. If not started ANC, when do you intend to start? _____ months

Have you been vaccinated against rubella?

Malaria treatment?

53. What are the things you like most in the ANC (Ante Natal Care clinic) clinic?

☐ Not attended
☐ Professionalism
☐ Safety
☐ Good for health
☐ Friendliness
☐ Others (please specify_____)

54. What are the things you dislike in the ANC clinic?

☐ Costs
☐ Waiting
☐ Long distance to get there
☐ Others (please specify_____)

55. What do you think are the advantages of delivery at home?

☐ No Costs
☐ No Waiting
☐ Traditional
☐ Others (please specify_____)

56. What do you think are the advantages of delivery at the health facility?

- ☐ Safety
- ☐ Professionalism
- ☐ Easier
- ☐ Others (please specify_____)

58. Do you know any women, who <u>died from complications of pregnancy within the last two years in your neighborhood?</u> 1- Yes ☐ 2- No ☐ if No Q 62

59. How long ago did she die? Years ☐ Months ☐ Days ☐

60. If yes, where did she deliver?

at home without TBA	☐
at home assisted by TBA	☐
Eikwe Hospital	☐
Other Hospital	☐
Other, specify	☐

61. If yes, what was the cause of death?

Complications:

1. Preeclampsia ☐
2. Eclampsia ☐
3. APH (Ante Partum Hemorrhage) ☐
4. PPH (Post Partum Hemorrhage) ☐
5. Puerperal sepsis ☐
6. Anemia in Pregnancy ☐
7. Malaria in Pregnancy ☐
8. Abortion complications ☐
9. Don't Know ☐
9. other (please specify) ☐

Transfusion Medicine

62. Do you suffer from anemia? 1- Yes ☐ 2-No ☐

63. If yes, are you aware of the diagnosis

☐ 1- Malaria
☐ 2- Parasitic worm infection
☐ 3- Sickle cell disease
☐ 4- Thalassemia
☐ 5- iron deficiency
☐ 6- other (specify) _____

64. Have you ever received blood transfusion or is blood transfusion planned during the next days? 1- Yes ☐ 2-No ☐

65. If no, is this because of unavailability of blood donations? 1- Yes ☐ 2-No ☐

66. If yes, have you been asked for payment? 1 - Yes ☐ 2-No ☐

67. If yes, have you been asked for a replacement donation by a relative or friend?

1 - Yes ☐ 2-No ☐

Agricultural data

62. Which quality problems <u>at harvest of crops (maize, beans, rice, cassava) do you know</u>?

1- Insect damage ☐
2- Spoilage ☐
3- Fungi growth ☐
4- Discoloration ☐
3- Others (specify:_____) ☐

63. What are the main quality problems <u>during storage of crops above</u>?

1- Insect damage ☐
2- Spoilage ☐
3- Fungi growth ☐
4- Discoloration ☐
5- Odour ☐
4- Others (specify:_____) ☐

Water and Sanitation

64. What is your main water source for drinking? ☐ 1- Tap water (public)

Only one option

☐ 2- Tap water on compound/in house

☐ 3- Public Pump
☐ 4- Well (protected at surface)
☐ 5- Well (unprotected at surface)
☐ 6- Lake
☐ 7- Rain water

65. Which type of latrine do the majority of members of your household use? Only one option

☐ 1- Pit latrine on site with flush
☐ 2- Pit latrine on site NO flush
☐ 3- Public Latrine
☐ 4- No latrine/Bush

66. Where do you dispose the stool of your children? Only one option

☐ 1- Near the house (forest/bush)
☐ 2- Far away from the house (forest/bush)
☐ 3- In latrine
☐ 4- Trash
☐ 5- Buried

67. Where do you wash yourself?

☐ 1-Lake
☐ 2- River
☐ 3- Pond
☐ 4- Home
☐ 5- Others (specify: _____)

68. Where do you wash your children below the age of 5?

☐ 1- Lake
☐ 2- River
☐ 3- Pond
☐ 4- Home
☐ 5- Others (specify: _____)

69. How many of your children <5 years had red urine now or during the last 4 weeks?

Number ☐

70. Do you know about the disease schistosomiasis (bilharzia)?

1- Yes ☐ 2- No ☐

Household characteristics

71. Does your household have electricity supply? 1- Yes ☐ 2- No ☐

72. Is the house you live in ☐ 1- Rented
☐ 2- Your own

73.Which of the following assets does your household posses

READ OUT THE ANSWERS AND WRITE DOWN THE NUMBERS!

1- Car		15- Jewelry	
2- Motorbike		16- Sewing Machine	
3- Bicycle		18- Chicken	
4- Boat		19- Ducks	
5- Radio		20- Pigs	
6- TV		21- Donkey	
7- Refrigerator		22- Goat	
8- Gas cooker		23- Sheep	
9- Fan		24- Cattle	
10- Mattress		25- Cats	
11- Beds		26- Dogs	
12- Table		27- Other Animals	
13- Chairs		28- Hand tools for working on the field	
14- Cell phone		29- Plough	
		30- Small Tractor	

74. Do your animals suffer from any visible disease?

- ☐ 1- Diarrhoea
- ☐ 2- Respiratory
- ☐ 3- Skin
- ☐ 4- Others (specify: _____)

75. Does <u>you</u> or your husband have a bank account? 1- Yes ☐ 2-No ☐

Questionnaire Health Seeking Behavior:

ONLY FOR WOMEN WHO COME FROM OUTSIDE OF EIKWE!

Q1. Distance from the village to Eikwe (if only hours, write 0 days, number of hours, number of minutes):

	Days		Hours		Minutes

Q2. Mode of transport:

1	Car
2	Motorbike
3	Bike
4	By foot

Q3. Distance <u>from home village</u> to next delivery facility:

	Days		Hours		Minutes

Q4. Mode of transport:

1	Car/Bus
2	Motorbike
3	Bike
4	By foot

Q5. Why was this facility close to the home town <u>not chosen</u> for this birth?

Bad quality of facility (dirty, ….)	1
Staff is not qualified	2
Staff is rude	3
Staff is corrupt	4
Price for delivery too high	5

Too expensive	6	
Coincidental (woman was close to Eikwe when labor pain started)	7	
Expected problems during birth	8	
Other	9	Specify here:_____

Q6. Why was Eikwe hospital chosen?

Good reputation/image	1	
Trust in nuns	2	
Religious reasons (trust in god)	3	
Modern techniques (e.g. ultrasound)	4	
Fear, because of problems during pregnancy	5	
Friends recommend Eikwe	6	
Relatives recommend Eikwe	7	
Other	8	specify here:_____

Q.7 Who made the decision to go to Eikwe?

Woman who gives births herself	1	
Husband	2	
Mother of women	3	
Mother of husband	4	
Other family member	5	specify here: _____
Other	6	specify here: _____

Q8. How much did the transport cost from home town to Eikwe hospital?

	Cedi

Q9. Why do other women from your village not go to Eikwe?

Too far	1	
Too expensive	2	
Husband does not allow	3	
They don't know Eikwe	4	
Other	5	specify here:_____

For women from Eikwe village

Q1.Why was Eikwe hospital chosen?

Good reputation/image	1	
Trust in nuns	2	
Religious reasons (trust in god)	3	
Modern techniques (e.g. ultrasound)	4	
Fear, because of problems during pregnancy	5	
Friends recommend hospital	6	
Relatives recommend hospital	7	
All women give birth there	8	
Other	9	specify here:_____

Q.2 Who made the decision to go to Eikwe?

Woman who gives births herself	1	
Husband	2	
Mother of women	3	
Mother of husband	4	
Other family member	5	specify here: _____
Other	6	specify here: _____

Do you know women from Eikwe who give birth at home and not at the hospital?

Yes	1
No	2
Don't know	3
Don't want to answer	4

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Göttingen, Frühjahr 2016

Fabian Völker 

Lebenslauf

Ich wurde am 04. April 1986 in der oberfränkischen Kleinstadt Coburg geboren und verbrachte dort sowohl meine Kindheit als auch meine Schulzeit. Nach der Grundschulzeit besuchte ich von 1996 bis zum Abitur im Jahr 2005 das Gymnasium Ernestinum. Anschließend leistete ich meinen Zivildienst in Freiburg im Breisgau.

Im März 2007 erhielt ich die Zusage für einen Studienplatz im Fach Humanmedizin an der Georg-August-Universität Göttingen und nahm das Studium zum Sommersemester 2007 auf. Im April 2009 schloss ich den Ersten Abschnitt der Ärztlichen Prüfung an der Universitätsmedizin Göttingen erfolgreich ab. Ab September 2010 absolvierte ich ein Auslandsjahr an der Université Claude Bernard in Lyon, Frankreich. Im Herbst 2011 begann ich in der Abteilung für Medizinische Mikrobiologie meine Promotionsarbeit zum Thema „Mutter und Kind Gesundheit in ländlichen Regionen Ghanas“.

Mit dem erfolgreich abgeschlossenen zweiten Abschnitt der Ärztlichen Prüfung im Juni 2014 beendete ich mein Studium der Humanmedizin an der Georg-August-Universität Göttingen und absolviere seit September 2014 die Facharztausbildung im Bereich Kinder- und Jugendmedizin an der Universitätsklinik Göttingen.