Université de Montréal

Association between Human Papillomavirus 16 (HPV-16) viral load in pregnant women and preterm birth

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Mémoire présenté en vue de l'obtention du grade de
Maîtrise en épidémiologie

Juin 2023

Université de Montréal
Département de médecine sociale et préventive, École de santé publique

Ce mémoire intitulé

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Résumé

L'accouchement prématuré a été récemment associé à la persistance du virus du papillome humain de type 16 (VPH-16) en grossesse. Il demeure toutefois difficile de savoir si cette association est causale et d'en expliquer les mécanismes biologiques potentiels. Afin de mieux caractériser cette association, nous avons étudié l'association entre la charge virale du VPH-16 en grossesse et l'accouchement prématuré. Les données de 48 femmes enceintes qui étaient positives pour le VPH-16 dans la cohorte HERITAGE ont été analysées avec un modèle de régression logistique, où la confusion a été ajustée avec scores de propension et pondération par l'inverse de probabilité de traitement. La charge virale du VPH, mesurée avec test PCR en nombre de copies/cellule au 1er et 3ième trimestre de grossesse, a été analysée en continue et dichotomisée à l'aide de différents seuils (0,5, 1,0 et 2,0). La charge virale (en continue) au 1er trimestre de grossesse a été associée à l'accouchement prématuré avec un OR ajusté (aOR) de 1,13 [IC 95% 1,03-1,25]. Le aOR pour la charge virale catégorisée avec seuil de 1,0 copie/cellule au 1er trimestre était de 15,03 [IC 95 % 1,75- 129,26]. Les analyses avec des seuils différents et au 3ième trimestre de grossesse ont données des résultats similaires quoique les ORs n'étaient pas toujours statistiquement significatifs. Nos résultats suggèrent une forte association entre la charge virale du VPH-16 et l'accouchement prématuré. Cette étude contribue à une meilleure compréhension des mécanismes tout en supportant la causalité.

Mots-clés : Virus du papillome humain (VPH), Virus du papillome humain de type 16 (VPH-16), charge virale, accouchement prématuré, grossesse.

Abstract

Preterm birth has recently been associated with the persistence of human papillomavirus 16 (HPV-16) during pregnancy. However, it remains difficult to determine whether this association is causal and to explain its potential biological mechanisms. To better characterize this association, we investigated the association between HPV-16 viral load during pregnancy and preterm birth. Data from 48 pregnant women positive with HPV-16 infection from the HERITAGE cohort were analyzed using a logistic regression model, where confounders were adjusted with propensity scores and inverse probability treatment weighting. HPV viral load, measured with a PCR test as copy numbers/cell during the 1st and 3rd trimester of pregnancy was analyzed continuously, and categorized using different cutoffs (0.5, 1.0 and 2.0). Continuous viral load at 1st trimester of pregnancy was associated with preterm birth with an adjusted OR (aOR) of 1.13 [95% CI: 1.03-1.25]. The aOR viral load categorized with cutoff 1 copy/cell at 1st trimester was 15.03 [1.75-129.26]. Analyses with different cutoffs in 3rd trimester of pregnancy gave similar results although the ORs were not always statistically significant. Our results suggest a strong association between HPV-16 viral load and preterm birth. This study contributes to a better understanding of the mechanisms and provides an additional argument on causality.

Keywords: Human papillomavirus (HPV), Human papillomavirus 16 (HPV-16), viral loads, preterm birth, pregnancy.

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List of acronyms and abbreviations

β-globin	beta-globin
BMI	body mass index
ºC	Degrees Celsius
CHUM	Centre Hospitalier de l'Université de Montréal
CI	Confidence Interval
CIHR	Canadian Institutes of Health Research
CIN	Cervical intraepithelial neoplasia
CIN1	Cervical intraepithelial neoplasia grade 1
CIN2	Cervical intraepithelial neoplasia grade 2
CIN2+	Cervical intraepithelial neoplasia grade 2 or grade 3
CRH	corticotropin-releasing hormone
DNA	deoxyribonucleic acid
e.g.	*Exempli gratia* (For example)
GA	gestational age
HERITAGE	Human papillomavirus perinatal transmission and risk of HPV persistence among children
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR	High-risk
HR-HPV	High-risk human papillomavirus
i.e.	*Id est* (That is)
IRNPQEO	Integrated Research Network in Perinatology of Québec and Eastern Ontario
LMP	Last menstrual period
LR	Low-risk
LR-HPV	Low-risk human papillomavirus
LSIL	Low-grade squamous intraepithelial lesion
mm	millimeter
ng	nanogram
OR	Odds ratio
ORF	Open reading frame
PPROM	preterm premature rupture of membranes
pH	potential of Hydrogen
Pap test	Papanicolaou test
PCR	Polymerase chain reaction
PV	Papillomaviridae
RR	relative risk

SD	Standard deviation
STI	Sexually transmitted infection
µL	microliter
US$	USD currency
USA	United States of America
vs	versus
WHO	World Health Organization

Dedicated to my family,
To my beloved parents Praduman Khayargoli and Pratibha Malla, my greatest source of inspiration and strength, for their endless love and support,
To my beloved siblings Primika Khayargoli and Prajin Khayargoli for their encouragement,
And to my baby Polo Khayargoli for his unconditional love and tail wags!

Acknowledgments

I would like to express my deepest appreciation to my supervisor Dr. Helen Trottier, for her unwavering guidance and support in completing this work. What a tremendous experience it has been to learn from her expertise in epidemiology and infectious diseases. I will always remember and be grateful for her warm welcome into her lab, and the genuine care she has for the success of her students. I would also like to extend my sincere thanks to my co-supervisor Dr. Marie-Hélène Mayrand, whose valuable comments and insights have been extremely helpful in putting together this work. Her input, rooted in her expertise in epidemiology and clinical background, have added tremendous significance to this work. Thank you both for your positive energies, encouragement, and unparalleled support!

I am also grateful to all the members of the HERITAGE research team. Many thanks to Louise Laporte for all the support and to Dr. Joseph Niyibizi for helping me with any questions I had on the data. Special thanks to Dr. François Coutlée and team for viral load data, and input on analysis. I also wish to thank all the women who took part in the HERITAGE study.

Many thanks also to the jury members of this thesis, Dr. Marie-Pierre Sylvestre and Dr. Mark Keezer for their insightful suggestions.

Finally, I am thankful to my family and friends for their unconditional love and support! My parents (Praduman Khayargoli and Pratibha Malla), my siblings (Primika Khayargoli and Prajin Khayargoli), my baby (Polo Khayargoli), extended family in Nepal and USA, and best friend Emma-Irene Jimenez, I am extremely grateful for all of you!

Chapter 1. Introduction

Preterm birth, a birth before 37 weeks of gestation, remains one of the leading causes of infant mortality and morbidity worldwide. Over 13 million babies were born prematurely in 2020, and complications related to preterm birth accounted for 900,000 deaths in 2019 [1]. The global preterm birth proportion was estimated at 10.6% of live births in 2014, with different proportions across industrialized countries [2]. In Canada, preterm birth accounts for nearly two thirds of infant deaths and almost 8% of Canadian babies are born prematurely [3]. Despite advances in obstetrics and neonatal research, preterm birth pathogenesis is not well understood. Important preterm birth risk factors have been identified and several theories of mechanisms leading to preterm birth have been suggested. Findings have highlighted the role of neuroendocrine and hormonal processes (e.g., stress, or infection/inflammation) during pregnancy in preterm birth etiology. The main consensus in the literature involves the role of (early activation of) inflammatory processes that lead to preterm birth. Bacterial infection is an important risk factor for preterm birth, and the role of bacterial genital infections through inflammatory processes in preterm birth has been confirmed [4-9]. However, despite advances in research, it is important to note that many preterm births are idiopathic; a meta-analysis of 4.1 million births in five high-income countries reported that 65% of preterm births do not exhibit any identifiable risk factors [10].

Recently, a possible role for HPV infection in preterm births has been raised. A meta-analysis including 19 studies on preterm births reported a pooled age-adjusted OR of 1.50 [95% CI: 1.19-1.88] for the relationship between HPV and preterm birth including 8 studies that showed significant positive associations and 11 that did not show significant associations [11]. Indeed, clinical studies have yielded inconsistent results due to important limitations including lack of control for confounders, detection of HPV at inappropriate time points (before/after but not during pregnancy), misclassification of both exposure and outcome, or more importantly lack of consideration of specific HPV genotypes [11]. The latter seems especially important given results from a recent study (HERITAGE: Human papillomavirus perinatal transmission and risk of HPV persistence among children, conducted by our team) that reported that this association between HPV and preterm birth could be essentially driven by HPV-16, more particularly, by the persistence of HPV-16 during pregnancy. We reported almost 4 times increased preterm birth risk

(3.72 [95% CI: 1.47-9.39]) among women with persistent HPV-16/18 infection during pregnancy compared to uninfected women [12]. A case-control study has also just confirmed an association for HPV-16 [13] and population data from different countries (Australia, Finland and Denmark) showed a reduction in preterm births following the implementation of mass HPV vaccination programs [14-16]. While it is becoming increasingly clear that there is a relationship between HPV-16 and preterm birth, it remains difficult to explain the biological mechanisms behind this association. An interesting factor to study would be viral load. Finding an association with viral load would not only strengthen the plausibility of a causal link between HPV-16 and preterm birth, but also further our understanding of the mechanisms underlying this association. The objective of this study was therefore to measure the association between HPV-16 viral load and preterm birth in the HERITAGE cohort study.

The following thesis (by article) is divided in five chapters. Following this introduction (chapter 1), the second chapter provides background information on the current state of the literature on preterm birth, HPV infections, and on the association between HPV (and HPV-16) and preterm birth as well as details on the rationale and aim of the study. Chapter 3 presents the methodology of the study, including the description of the study population in HERITAGE cohort study and sample (inclusion and exclusion criteria), data and specimen collection, relevant variables, statistical analyses, and the ethical considerations. Chapter 4 presents the results of the study in the form of a manuscript. The fifth and final chapter details the discussion and conclusions of the study, situating our findings with current literature and suggesting potential avenues for future research.

Chapter 2. Background and Literature Review

This chapter will provide background information and a full literature review on the relevant topics of this work, notably preterm birth, and HPV infection. The first section will summarize what is currently known about preterm birth, from its definition, to epidemiology, risk factors and suspected etiology. The second section will provide information on HPV and present its classification, epidemiology, related diseases, and prevention. The third section will summarize the current state of the literature (including gaps) on the relationship between HPV and preterm birth which will provide the rationale for this study. The last section will then state the objective of this study.

2.1 Preterm birth

2.1.1 Definition

Preterm birth, defined as birth before 37 completed weeks of gestation by the WHO, is an important public health issue as it remains one of the leading causes of death globally among children younger than 5 years [1]. Premature infants are those that are born with immature organs and therefore require additional support for survival for extrauterine life [7]. There are differences in defining preterm birth given that prematurity is not a disease identifiable by specific clinical manifestations and because fetal growth and development occurs as a continuum [10]. Thus, the lower limit of viability or maturity can be challenging to determine [17]. Preterm births in early epidemiological studies were defined using neonatal birth weights lower than 2300g (low birth weight), or even lower than 1500g (very low birth weight), irrespective of number of weeks of gestation as it was seen as an objective, accurate and easy measure [7, 17]. Given that conventional birth weights may vary due to growth restrictions, preterm birth is now measured using weeks of gestation (i.e., completed weeks of pregnancy, without rounding [7]), which is used as a proxy measure for maturity [7]. The International Statistical Classification of Diseases and Related Health Problems defines a live birth as one that shows any “signs of life” [10, 18]. The American College of Obstetricians and Gynecologists define the earliest preterm birth being one where delivery occurs at or after 20 weeks of gestation [19], which is the lower limit that will be chosen for this work.

2.1.2 Gestational age measures

Several methods of measuring gestational age (GA) exist [7, 20]. One method is the use of the first day of last menstrual period (LMP) to measure start of pregnancy [7]. This method may pose some difficulties to estimate pregnancy duration due to variations in menstrual cycles (as a result of factors such as age, ethnicity, BMI, physical activity, smoking [21]), i.e., differences in ovulation and implantation timing among women, as this method implies that conception occurs on ovulation day, and that both conception and ovulation occur 14 days after the start of the last of period [22]. The onset of LMP and conception may vary in women from 7 to 25 days [7]. Irregular menstrual cycles, oral contraceptive use and recall errors (which generally tend to overestimate gestational age [23, 24]) may provide inaccurate estimates of gestation duration based on LMP [7] relative to ultrasound-based estimates [24], which is another method for estimating GA. Early ultrasound (especially during first trimester) provides a more accurate pregnancy dating [7, 24, 25] and is considered the gold standard for GA assessment [10]. Ultrasounds, which are based on fetal measurements, if taken later in the pregnancy (third trimester), may be less accurate due to other factors that may influence fetal growth at that stage [7]. First trimester ultrasound, although more accurate, may be limited by health care access [7]; LMP is thus most frequently used in low-resource settings [26]. Studies have found differences in GA depending on the method used [27, 28]. Both LMP and ultrasound can also be combined using different algorithms to obtain the best estimate of GA [29].

2.1.3 Classification by gestational age

The severity and negative outcomes of prematurity is determined by pregnancy duration, which is indicative of maturity level [10]. GA is therefore the greatest indicator of preterm birth outcome [30], as infants born with shorter gestations have greater morbidity and mortality rates [10, 31]. Preterm birth can be further divided into subcategories according to GA. The WHO defines three categories: extremely preterm (< 28 weeks), very preterm (28 to 32 weeks) and moderate to late preterm (32 to 37 weeks) [1]. Other subdivisions involve early (< 32 weeks of gestation) or late preterm (34-36 weeks) [22]. Most preterm infants are born between 33 and 36 weeks of gestation [7].

2.1.4 Other classifications

In addition to GA, preterm birth can as also be subdivided according to clinical presentation: spontaneous or induced [20, 22]. Preterm birth can be spontaneous either due to spontaneous preterm labor with intact membranes and/or preterm premature rupture of membranes (PPROM) [22], which is defined as spontaneous rupture of membranes occurring < 37 weeks of gestation at least 1 hour before contractions [4]. Spontaneous births are those that follow both spontaneous labour and PPROM [4]. Preterm birth can be induced when either mother's or fetus's life is at risk due to medical conditions such as pre-eclampsia, placental abruption, fetal distress, or intrauterine growth restriction [10, 32]. Spontaneous preterm labour is the most common obstetric precursor leading to preterm delivery, followed by induced labor and PPROM [4, 20]. The etiology of these clinical presentations varies across populations and regions [32].

2.1.5 Prevalence

Global and regional estimates of preterm birth show varying prevalence between countries (from 5% of livebirths in European countries, and over 18% in African countries [20]), with highest proportions occurring in low- and middle-income countries [9, 33, 34]. Several systematic reviews have estimated global preterm birth prevalence: Beck et al. estimated a global prevalence at 9.6% [95% CI: 9.1-10.1%] in 2010 with data from 99 countries [34]; Blencowe et al. reported a global average at 11.1% [95 CI: 9.1-13.4%] with data from 184 countries in 2010 [33]; Chawanpaiboon et al. found 10% [95% CI: 9.0-12.0%] with data points from 107 countries in 2014. All three studies report higher proportions of preterm birth occurring in sub-Saharan Africa and South Asia [2, 33-35]. In 2021, 8% of babies were born preterm in Canada [36] and this proportion was at 10% in USA [31].

Global modelling of preterm birth data shows a declining trend [37]; the global number of preterm birth estimates decreased by 5.3% from 16.06 million in 1990 to 15.22 million in 2019 [37]. Most cases of preterm birth occurred in India and Pakistan in 2019 [37]. In Canada, preterm birth estimates initially increased from 7.4% in 1990 to 8.2% in 2004 but has remained stable since [38]. In Quebec, preterm birth proportion increased from 5.6 to 8.1 per 100 livebirths between 1981 and 2004, and decreased to 7.0 per 100 livebirths by 2017 [38].

2.1.6 Burden

Preterm birth causes enormous societal economic burden due to initial neonatal treatment [20] and continued increased healthcare utilization. In USA, preterm birth costs reached at least US $26.2 billion or US $51,600 per premature infant in 2005 [7]. In Canada, the cost per premature infant over the first ten years, based on administrative population-based databases from Quebec, was estimated at $67,467 for early preterm infants (<28 weeks), $54,554 for moderate infants (28-32 weeks) and $10,010 for late preterm infants (33-36 weeks) [39]. At the population level, this cost goes to $587.1 million for all preterm infants in Canada [39]. Earlier preterm births are most costly, but thankfully over the last decade, the survival probability of infants born before 28 weeks of gestation has increased due to advanced technological advances such as assisted ventilation, surfactants and earlier use of antenatal corticosteroids [30].

Preterm birth costs are also higher due to the need for long-term assistance as infants born prematurely are at risk for long-term morbidities such as asthma, hypertension, diabetes, attention deficit disorder, learning disabilities and social-emotional problems [40, 41]. Prematurity is also associated with cerebral palsy and neurodegenerative disorders [10]. Those with shorter gestations have greater complications and levels of dysfunction [30]. Short-term complications include increased risk of respiratory conditions and sepsis. Longer term complications include cerebral palsy, visual and hearing problems [10]. Other costs include caregiver as well as out-of-pocket costs such as transportation, childcare for outpatients or hospitalization visits [40]. Late preterm infants are still more likely to suffer from respiratory complications, hypoglycemia, cold stress, jaundice when compared to full term infants [7, 42].

2.1.7 Pathophysiology and causal pathways

The pathophysiology of preterm birth is not well understood. Different mechanisms and processes have been suggested, and preterm birth is currently thought to be a result of multiple biological pathways and processes. Before exploring the potential causes of preterm birth, it is important to understand the immunological and biological changes that occurs during labor. Labor involves a continuum of processes that undergo 5 distinct phases: implantation (fertilized egg attaches the uterus), uterine quiescence (uterus grows without contractions), activation (uterus prepares for contractions), stimulation (labor, uterine contractions) and involution (uterus returns of pre-pregnancy state) [9]. The majority of the pregnancy is spent in quiescence and activation phases

[9]. Preterm birth is induced by labor occurring prematurely, which may be attributed to disruptions in the common pathway of parturition (i.e., childbirth) [5]. Labor starts when a switch from a quiescent to contractile state of the myometrium is observed [5]. This occurs through activation of signaling between anti-inflammatory and pro-inflammatory pathways with chemokines (interleukin-8), cytokines (interleukin-1 and -6) and proteins that promote uterine contractility (oxytocin, prostaglandins) [5]. Progesterone maintains quiescence by blocking the gene expression of these inflammatory factors and by keeping the formation of uterine gap junctions [5, 7]. Changes in extracellular matrix proteins (affected by increases in prostaglandins, estrogen, and inflammatory cytokines production, and progesterone withdrawal [7]) prepare the cervix for dilation, leading to the next step of parturition, known as cervical ripening [5]. Finally, there is activation of the decidual/membrane, which prepares for ruptures of membranes, and separation of placenta from uterus [5]. The common pathway is thought to be activated physiologically during labor at term. Preterm labor may therefore be a result of disruptions in the normal timing of the common pathway of parturition [5]. Proposed pathways of preterm birth pathophysiology involve those induced by stress, uterine overdistension/cervical insufficiency, thrombosis/decidual hemorrhage, as well as infection/inflammation [4, 5, 7-9]. The following subsections will describe these potential causal pathways of preterm delivery, i.e., the components and biological mechanisms of how preterm birth may be induced. This information may help in understanding how and why (specifically, through which mechanisms) some preterm birth risk factors (presented in the next section 2.1.8) may increase premature delivery risk.

2.1.7.1 Stress

Stress (acute or chronic) is thought to lead to preterm birth through neuroendocrine processes which involve increased placental corticotropin-releasing hormone (CRH) [7, 9] levels and early activation of biomolecular parturition events [43]. Women with higher CRH levels are 3 times more likely [RR: 3.3 (95% CI: 1.2-9.4)] to have a spontaneous preterm delivery after 33 weeks of gestation compared to women with normal CRH levels [43]. Stress may also increase susceptibility of other known risk factors such as infection and other health behaviors [7].

2.1.7.2 Uterine overdistension/cervical insufficiency

Uterine overdistension increases preterm birth risk, especially during multiple gestations [4, 7] through premature myometrial contractions, PPROM and inflammation [7, 9]. Cervical

insufficiency, i.e., cervical length less than 25mm [44] increases risk of preterm delivery [9, 45]. Women with cervical length less than 25 mm are 6 times more at risk [RR: 6.19 (95% CI: 3.84-9.97)] to deliver before 35 weeks compared to women with cervical length above 40 mm [45].

2.1.7.3 Thrombosis/Decidual hemorrhage

Women with higher thrombin levels (mostly generated to maintain decidual homeostasis upon vaginal bleeding or decidual hemorrhage) are at increased risk for spontaneous preterm birth [5]. Thrombin can trigger myometrial contractions, leading to rupture of membranes and preterm labor [5].

2.1.7.4 Inflammation/Infection

Out of all the causes of preterm birth, infection/inflammation from bacteria (e.g., intrauterine infection, lower genital tract infections and maternal systemic infections [7]) is the most strongly established one. Women who experience earlier preterm births have higher frequency of bacterial intrauterine infections [4] as they cause 50% of preterm births before 28 weeks [7]. Bacterial infections lead to preterm birth through early activation of biological cascades that increase inflammation as bacteria stimulate the production of pro-inflammatory cytokines and prostaglandins in the amnion and decidua [7]. Lower genital tract bacterial infections also increase inflammation through early stimulation of chemokines, cytokines, and other inflammatory mediators that induce premature contractility and membrane ruptures [5]. Bacterial vaginosis, a change in the microbiome characterized by anaerobic bacteria, increases risk of spontaneous preterm birth (1.5-to-3-fold increase [4]) by changing the vaginal microbial ecosystem (to a pH greater than 4.5 [4]) and increasing risk of intra-amniotic infection [5, 7]. Genital infections from chlamydia [46], syphilis [9], gonorrhoea [4] have also been linked to spontaneous preterm birth. Other maternal infections including periodontal disease (anaerobic bacterial infection of the mouth) also increase spontaneous preterm birth [7] through inflammatory responses, but its exact mechanism remains unknown [4].

Unlike bacterial infections, the role of viral infections in preterm birth has not yet been established. Some studies report increased preterm birth risk due to infections with viruses such as hepatitis B [47], chronic hepatitis C [48] and HIV [49]. These viruses are, however, not frequent and would not explain many preterm births. Recently, a strong association was found by our team between persistence of human papillomavirus 16 (HPV-16) during pregnancy and preterm birth [12]. This

finding was recently supported by populational data in several countries that showed decreasing trends in preterm estimates with HPV mass vaccination [14-16]. Despite these recent data suggesting an important role of HPV-16 in preterm birth, it remains difficult to explain the biological mechanisms behind this relationship, since HPV infection is not typically associated with inflammation. It is therefore very important to continue research on this topic to better understand the nature of the relationship. The last two sections of this chapter will summarize the epidemiology of HPV and the current state of the literature on the association between HPV (and HPV-16) and preterm birth.

2.1.8 Risk factors

Given that an exact causal mechanism of preterm birth has not been established, identifying its risk factors is important in order to explain and further understand its etiology [4]. Studying preterm birth risk factors may also allow identification of at-risk populations in order to target interventions and conduct further studies [4]. It is important to note that there are many preterm births that are idiopathic, i.e., that occur without presence of known preterm birth risk factors: in a meta-analysis of 4.1 million births in five high-income countries, 65% of preterm births did not have any risk factors [10]. Therefore, it is important to continue research on the identification of risk factors to prevent preterm birth.

Relevant known risk factors may be divided into maternal risk factors, pregnancy history, history of cervical treatment and pregnancy characteristics.

2.1.8.1 Maternal risk factors

2.1.8.1.1 Age

Maternal age (low and high) is a risk factor for preterm birth [50]. Women in adolescence as well as women aged 35 and over (especially if it is their first pregnancy) are at higher risk of preterm birth [7, 10]. In Quebec, preterm birth is highest among women less than 20 and over 45 [38].

2.1.8.1.2 Race and ethnicity

There are racial and ethnic disparities in preterm birth. A meta-analysis of 13 systematic reviews reported an increased risk of preterm birth, with an OR of 2.0 [95% CI: 1.8-2.22] in Blacks compared to Whites across the world [51]. Other studies have also reported preterm birth differences between Black and White women [52]. Differences in preterm birth etiology between different races and ethnic groups have also been reported, with White women often having

spontaneous preterm births due to preterm labour whereas Black women had preterm birth due to PPROM [19]. In USA, African American (14.8%) women had 50% higher rates of preterm birth compared to White (9.5%) or Hispanic (10.2%) women in 2021[31]. Explanations for racial disparities include systemic racism, differential access to medical care before and during pregnancy, differences in socioeconomic conditions as well as prevalence of other risk factors [7].

2.1.8.2 Pregnancy history

Women that have a history of spontaneous preterm delivery have a 2.5-fold increased risk in their next delivery [53]. This may be due to maternal risk factors that may recur from one pregnancy to another, such as intrauterine infections, diabetes, hypertension or obesity [4].

2.1.8.3 History of cervical treatment

Several studies have shown the impact of treatment for cervical preinvasive and early invasive disease on pregnancy outcomes. Results from four meta-analyses reported increased preterm birth risk following excisional techniques [54-57]. For example, in a meta-analysis including 19 240 participants across 71 studies, risk of preterm birth increased for excisional techniques: Cold Knife Cone: 2.27 [95%CI: 1.70-3.02]; laser conisation: 1.77 [95% CI: 1.29-2.43]; and LLETZ (large loop excision of the transformation zone): 1.37 [95% CI: 1.16-1.62], compared to the untreated colposcopy group, whereas no differences were found for ablative methods (laser ablation: 1.05 [95% CI: 0.78-1.41]; cryotherapy: 1.01 [95% CI: 0.35-2.92]; and cold coagulation: 0.67 [95% CI: 0.02-29.15] [54]. It is important to note that women with untreated cervical intraepithelial neoplasia (CIN) may also have an increased risk of preterm birth compared to healthy women [54].

2.1.8.4 Pregnancy characteristics

2.1.8.4.1 Mother's health behavior and status

Cigarette smoking in later part of pregnancy increases risk of preterm birth by two-fold [4]. Reported RR have varied between 1.2-1.5 for smoking 10-20 cigarettes per day and 1.5-2.0 for 21 cigarettes or more per day [7]. Smoking patterns may also affect gestational age at birth [7]. Proposed causal mechanisms include restricted maternal blood flow among smokers [50] as nicotine and carbon monoxide are known vasoconstrictors that may lead to disruptions in the placenta and uterine blood flow [4]. Alcohol consumption during pregnancy may also increase preterm birth risk [58, 59]. It is important to note that because this variable is generally self-reported, it is difficult to quantify and be certain of its measure in epidemiological studies, and

thus this association remains uncertain [7, 50]. Women who consume drugs including marijuana and cocaine have increased risk of delivering prematurely [7]. Theories proposed for the mechanisms behind this association include drug relation to vasoconstriction (for cocaine), but the causality of this relationship has not been established either [7].

Some observational studies have hinted at the positive relationship between physically demanding work and preterm birth, but results have been inconsistent [4]. Mother's nutritional status has also been linked to preterm birth [50]. Studies have reported than women with low serum concentrations of iron, folate or zinc have more preterm births than those with normal measurements [4]. It is thought that women with lower BMI levels may have increased risk of spontaneous preterm birth due to decreased blood volume and reduced blood flow to the uterus [4]. Obesity is a risk factor for preterm birth [7]. Overweight and obese women are more at risk for spontaneous preterm birth than their non-overweight counterparts [50]. This is because they are more likely to suffer from complications such as hypertension, preeclampsia, gestational diabetes [4, 50]. Obese women are also more likely to have congenital anomalies that may have to be delivered prematurely [4]. Genital and urinary tract infections, specifically intrauterine infections as well as bacterial vaginosis also increase preterm birth risk [4, 7]. Other medical disorders such as vaginal bleeding, thyroid disease, periodontal disease, asthma, diabetes, and hypertension also increase complications during delivery, and thus may increase risk of preterm birth [4, 7].

2.1.8.4.3 Multiple gestations

Women with multiple gestations are at higher risk for preterm delivery; almost 60% of twins are born prematurely [4]. This is thought to occur through the causal pathway of uterine overdistension [4].

2.1.8.4.4 Psychosocial factors

Women that experience high levels of stress are almost 2 times more likely to deliver prematurely, even after adjusting for medical, behavioral and other sociodemographic risk factors [4]. The exact mechanism underlying this association has not been established, but some propose that it may involve a pathway with CRH as described in section 2.1.7.1 [4, 7]. Depression and anxiety have also been linked to preterm birth [4]. This may be due to other health behaviors that increase risk of preterm birth that associated with depression and anxiety, such as smoking, alcohol, and drug

use [4]. The biological mechanisms are thought to be due to depression reducing natural killer cell activity, and therefore increasing amount of proinflammatory cytokines and their receptors [4].

2.2 Human papillomavirus (HPV)

2.2.1 Classification and Taxonomy

HPV is part of a virus family called *Papillomaviridae* (PV) which includes over 50 genera and over 130 species [60]. PV has been identified in fish, reptiles, birds and mammals, though it has mostly been studied in humans [61]. It is composed of circular double-stranded DNA genomes with 8 genes [62], among which E1, E2, L1 and L2 open reading frames (ORFs) are thought to be present among all members of the family [61]. PV is further divided in genus according to the composition of the DNA sequence of the L1 gene. L1 has been used for virus classification for over 15 years because it is the most conserved gene within the genome [61]. Members of different genus within the PV family have 60% or more differences in nucleotide sequence identity of the L1 gene [61]. Species within a genus share 71-89% of nucleotide identity of the complete L1 ORF sequence [63]. PV are classified as different types if they have more 10% difference in DNA sequence of the L1 gene [63]. Those with 2-10% differences are known as subtypes, and those with less than 2% differences in L1 sequence are variants [63]. Over 170 HPV types have been sequenced, and they are divided in five genera according to DNA sequences: Alpha-PV, Beta-PV, Gamma-PV, Mu-PV and Nu-PV [64].

2.2.2 Tropism, oncogenic potential and lesions associated with HPV

HPV can also be classified according to its tropism to human epithelial cells: mucosal or cutaneous [65]. The Beta-PV, Gamma-PV, Mu-PV and Nu-PV are composed of HPV genotypes that are all cutaneous and that generally cause warts, and the Alpha-PV is composed of HPV genotypes that are both mucosal and cutaneous that not only cause warts, but also HPV-related cancers [66]. Alpha-PV are the most studied HPV types [67]. There are about 40 Alpha-PV that can infect the mucosal epithelia, and these can be further classified as low-risk (LR) or high-risk (HR) based on their oncogenic potential (LR = low oncogenic potential and HR= high oncogenic potential). The International Agency of Research on Cancer Evaluation classifies twelve HPV genotypes as HR oncogenic (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) and two genotypes as LR (6,11) [65]. Infections from LR-HPVs such as HPV-6 and HPV-11 causes benign lesions in anogenital regions also known as genital warts or condylomata acuminata [68]. These LR, non-cancerous HPV

genotypes have also been linked to low-grade squamous intraepithelial lesion (LSIL), which are characterized by abnormal cell formation in areas such as the cervix, vagina, vulva, and esophagus. LR-HPVs have also been linked to recurrent respiratory papillomatosis, i.e., laryngeal papillomatosis, which is a rare condition resulting in recurrent growth of papilloma in the larynx of the respiratory tract [68].

HR-HPVs genotype are responsible for 5.2% of all human cancers worldwide; they cause virtually all cervical cancers and more than 80% of anal cancers, 70% of vaginal and vulvar cancers, 60% of penile cancers and 30% of oral cancers [69, 70]. Two HR-HPVs, HPV-16 and HPV-18 are responsible for 70% of cervical cancers [69], and HPV-16 alone is responsible for 60% of all cancer cases [65]. Associations between HPV infection and other cancers such as cancer of the skin, nose, colon and rectum, breast, ovary, prostate, urinary bladder, urethra, have also been reported [71].

2.2.3 Transmission of HPV

The most common route of HPV infection is through sexual intercourse, which includes both vaginal and anal intercourse [72]. Other routes involve oral, skin-to-skin and perinatal transmission, [72]. A new HPV infection can be detected after a sexual contact with an infected partner, most often within 1 year of exposure [65].

2.2.4 Prevalence of HPV infection among women

Genital HPV is the most common STI in the world (2 in 3 sexually active women may have HPV infection once in their lifetime) [73]. According to a meta-analysis that included data from more than 1 million women with normal cytology in 59 countries, the prevalence of cervical HPV infection ranges from 1.6% to 41.9%, with a global prevalence estimated at 11.7% [74]. Authors reported highest prevalence in Sub-Saharan African regions (24.0%), Latin America and the Caribbean (16.1%), Eastern Europe (14.2%) and Southeastern Asia (14.0%) [74]. HR-HPV are most often seen in incident cases of HPV infections, especially HPV-16 [72].

2.2.5 Risk factors

Age is an established risk factor for HPV infection. Globally, HPV infection is highest in women younger than 35 years, and it decreases with older age [75]. A peak in HPV prevalence has been observed to occur around 20-25 years of age and to decline over the years due to frequent clearance

and lower number of new sexual partners (which also declines with age) [75]. Incidence of genital HPV also peak for sexually active young population, and lowers as they grow older [73].

HPV acquisition is more frequent in women who have more sex partners [71], do not use protection, and are younger at first intercourse [72]. A study reported almost two times (RR: 1.99, 95% CI:1.46-2.72] higher risk of HPV infection among women with multiple sex partners in the previous 12 months of the study [76]. Results from a prospective study in Colombia reported that determinants of new HPV infections also involved new sexual partners and having more than one sexual partner [77]. A shorter interval between sexual partners may increase the risk of HPV acquisition [72]. The age of first intercourse has been linked to prevalent HPV infection. Earlier sexual debut may be associated with riskier sexual behaviors (e.g., no condom use) [72]. Other factors such as smoking, co-infection with other STIs and infections (e.g., chlamydia, bacterial vaginosis [76]), immune suppression (through HIV infection, transplantation), genetics (presence of certain human leukocyte antigen), hormonal birth control (among users for more than 5 or 10 years) and diet may increase susceptibility of HPV infection although these associations have not been strongly established [71, 72, 78]. Condom use and link with HPV infection is still debated [72].

2.2.6 Prevalence of HPV infection among pregnant women

The highest HPV prevalence is observed in the age group at which fertility is maximum [36, 74, 77, 79]. A meta-analysis including 112 studies in 39 countries reported a pooled worldwide prevalence of HPV in cervico-vaginal samples among pregnant women at 30.38% [95% CI: 26.88%-33.99%] varying by region with 45.8% in Americas, 46.5% in Africa, 19.6% in Europe and 21.4% in Western Pacific regions. They reported also a pooled HPV detection in placental samples at 17.81% [95% CI: 9.81%-27.46%] [80]. Ambuhl et al., in their systematic review, reported a prevalence of cervical HPV at 17.5%, with higher prevalence in Latin America (35.5%) and the USA (24.6%), and lower ones in Europe (11.0%) and Asia (16.4%) [81]. Usually, higher prevalence is found in vaginal samples (versus cervical samples) [82, 83]. Three recent large studies among pregnant women with vaginal sample testing in first trimester of pregnancy have estimated the prevalence of HPV at 40%, 39% and 45% in Canada [84], India [85] and Italy [86] respectively.

2.2.7 Persistence of HPV infection

Generally, HPV infection does not persist and resolves on its own (80-90% of the time) within 6-24 months of infection through the body's natural immune responses (through viral clearance or viral latency) [65]. Yet, a small portion of HPV infections persist. Persistence of HPV infection has been defined as individuals having two consecutive positive HPV DNA tests (for either same HPV genotype or group of genotypes [71]) over a certain time interval [65], e.g., interval of 6-12 months [78] . The mechanisms behind the persistence of HR-HPVs are not well understood; HPV type and viral load have been suggested as major determinants of persistence [65]. HPV-16 has been found to persist the longest [65, 72], which may explain its significant role in cancer risk.

2.2.8 HPV viral load

HPV viral load has been studied in HPV-related neoplasia, precancerous lesions and invasive cancers. Studies have reported positive correlations between higher HPV viral loads and severity of cervical lesions [87-90], invasive cervical cancer [91-93], squamous intrepithelial lesions [94] and high-grade cervical neoplasia [95]. A study reported higher HPV-16 viral loads among patients diagnosed with low or high grade squamous intrepithelial lesions compared to those with normal cytology [94]. Another study reported that women with higher HPV-16 viral loads were 5.5 times more at risk for high-grade cervical neoplasia compared to those with lower viral loads [95]. It is important to note that studies reporting associations between HPV viral loads and HPV-associated diseases were mostly done for HPV-16 genotype [96]. A study of 17 235 women in China compared the viral loads of eight HR HPVs between $\leq$CIN1 and CIN2 patients and reported significant differences only for those with HR HPV-16 genotype (which was also the most prevalent) [97]. Another large longitudinal hospital-based cohort study in France investigated if HPV-16 and HPV-18 viral loads could predict severity of high-grade cervical lesions and reported that only HPV-16 viral loads predicted CIN2+ development although both HPV-16 and HPV-18 genotypes are most frequently linked to cervical cancer [98]. Similarly, Wang et al. also reported a positive correlation between HPV-16 viral load and cervical lesions, but not one with HPV-18 viral load [99]. There is therefore a particular interest around the viral load of HPV-16 when studying the issues that are associated with HPV. However, the impact of HPV viral load have never been studied on pregnancy outcomes.

2.2.9 Prevention of HPV

The most effective way to prevent HPV infection is through vaccination. To date, three vaccines (Cervarix, Gardasil, Gardasil9), highly effective at preventing HPV infection, are available [100]. Cervarix is a bivalent vaccine that provides protection against these two highly oncogenic HR-HPVs, namely HPV-16 and HPV-18 [100]. Gardasil is quadrivalent vaccine that provides protection against two LR-HPV, HPV-6 and HPV-11, in addition to HPV-16 and HPV-18 [100]. Gardasil9 is a 9-valent vaccine that protects against infection from additional HR-HPV genotypes than the first two (31,33,45,52,58) [100].

2.3 HPV and preterm birth, and rationale for study

HPV cause a range of adverse outcomes, which is concerning given the high prevalence of HPV in pregnancy. Several studies have analysed the association between HPV and preterm birth but with inconsistent results. Some studies showed no significant association between HPV and preterm birth [101-112] while others reported significant positive associations [11-13, 81, 102, 112-121], including three meta-analyses that reported significant associations. Huang et al. reported a pooled OR of 2.12 [95% CI: 1.51-2.98] from six cohort and two case-control studies [117]. Xiong et al. included 18 studies and reported an almost 3 times higher risk of spontaneous preterm birth among those with a HR-HPV infection (2.84 [95% CI: 1.95-4.14]) [122]. Another recent meta-analysis of 36 studies by Niyibizi et al. reported a pooled age-adjusted OR of 1.50 [95% CI: 1.19-1.88], and even higher significant associations when restricting to studies that used HPV testing (2.01 [95% CI: 1.06-2.73]), and that measured HPV during pregnancy (1.70 [95% CI: 1.06-2.73]) [11]. Most studies available in the literature however have limitations and do not consider the potential role of individual HPV genotypes- instead, they provide a measure for either the presence/absence of HPV, or for a cluster of genotypes. A recent study with analysis of HERITAGE data indicated that this association is essentially driven by the specific genotype HPV-16 and not by other genotypes; we found almost 4 times increased preterm birth risk (3.72 [95% CI: 1.47-9.39) among women with persistent HPV-16/18 infection during pregnancy (first and third trimester) [12]. The link between HPV-16 and preterm birth was also recently observed (p=0.04) in a recent case-control study in India [13]. From these recent data, it seems clear that there is a relationship between HPV-16 and preterm birth. However, the biological mechanisms underlying the association between HPV-16 and preterm birth remains difficult to explain. Two

specific mechanisms for HPV infection's role in preterm birth has been suggested [123]. Findings from *in vitro* studies have highlighted HPV infection's potential role in altering trophoblast physiology in the placenta, which could lead to early activation of biological and immunological processes leading to labor and thus compromise gestation [81, 106, 108, 120]. Genital HPV infection may also increase heterogeneity of the vaginal microbiome [124], and disrupt its homeostasis and cause early production of proinflammatory cytokines and consequently, early labor. Even with these hypotheses, we are left with important questions on the potential mechanisms underlying the association between persistent HPV-16 in pregnancy and preterm birth. An answer may lie in the viral load of HPV-16 infection given that higher viral loads may confer higher risk of persistence of HPV infection, as reported in several studies on viral loads and cervical lesions [65, 125-127].

Exploring if HPV-16 viral load in pregnancy is associated with preterm birth would therefore not only help to strengthen the plausibility of the causal relationship between HPV-16 and preterm birth but it will also be an important steppingstone into better understanding the biological mechanism behind this association. Given that this has never been studied before, findings will provide new evidence on the role of HPV-16 viral loads beyond (already known) cancerous lesions, in important pregnancy outcomes such as preterm birth. This may open new lines of research in understanding the role of viral genital infections (especially one as frequent in women as HPV) in preterm birth pathophysiology, which is of high importance. Hence, a study looking at the association between HPV-16 viral load in pregnancy and preterm birth, with robust measures of all relevant variables, is needed. With the prospective design of our HERITAGE study, we have measures of HPV-16 viral load during both first and third trimesters, allowing us to study its association with preterm birth.

2.4 Objective

The objective of this study was to measure the association between HPV-16 viral load in pregnant women during first and third trimesters of pregnancy and preterm birth.

Chapter 3. Methodology

This chapter describes the study design, participants, data and specimen collection, the statistical analysis, power calculation and ethical considerations.

3.1 The HERITAGE cohort: design and aims

Data used for this study comes from the HERITAGE cohort study, a CIHR-funded study led by Dr. Helen Trottier. Its design, methods, and preliminary results have previously been published [12, 84, 128-130]. HERITAGE is a prospective cohort study that aimed to better understand the perinatal route of HPV transmission as well as the impact of HPV during pregnancy. The HERITAGE study had five aims, specifically to: 1) estimate HPV perinatal transmission (in conjunctival, pharyngeal, buccal, and genital mucosa of newborns), 2) estimate the risk factors that are associated with perinatal transmission, 3) assess HPV persistence risk (in conjunctival, pharyngeal, buccal and genital mucosa) in children as well as the risk factors associated with infection persistence, 4) determine and correlate HPV antibody presence in mothers and children, and 5) measure the HPV prevalence in the placenta, as well as the impact of HPV infection (during pregnancy and in the placenta) on pregnancy outcomes such as birth weight, gestational age at delivery, and other adverse outcomes (e.g. preterm birth). This thesis builds upon the fifth aim of HERITAGE.

3.2 Study participants

Recruitment for HERITAGE was done in two phases from three academic hospitals in Montreal, Canada. Pregnant women aged 18-30 years were first recruited in the pilot phase (n=167) from November 2010 to June 2012 from Sainte-Justine hospital, the CHUM and from Saint-Mary's Hospital if they were between 8-14 weeks of gestation. Recruitment was limited to women aged 18-30 in the pilot phase to ensure sufficient prevalence of HPV infection in the sample. Recruitment was opened to participants over 18 years old in the second phase. Specifically, participants aged 18 and over were recruited for the second phase (n=883) from February 2015 to July 2016, from Sainte-Justine hospital centre and from the CHUM and their affiliated clinics if they were between 6-14 weeks of gestation and had a birth anticipated to take place at a participating hospital. Women who were HIV positive or unable to provide written consent were excluded. A total of 1303 eligible women were approached during the two study phases. Among

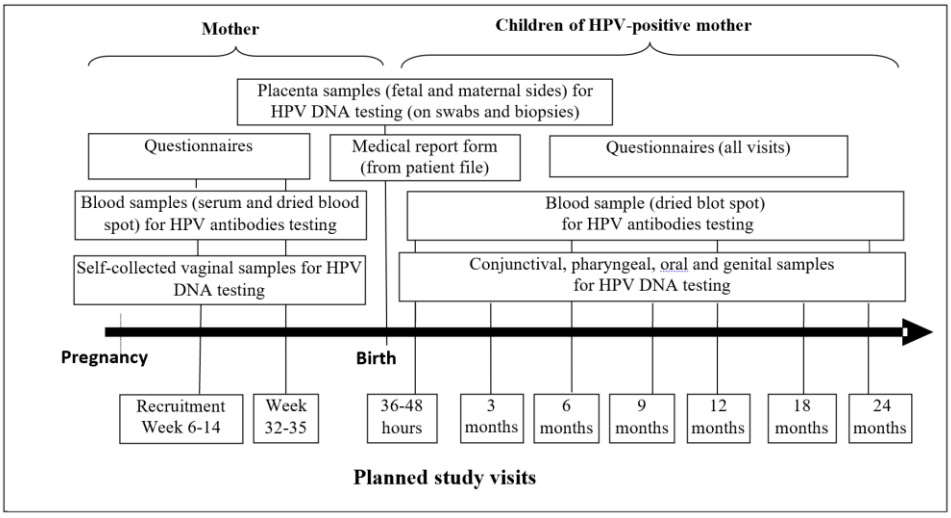
them, 251 (19.3%) declined and 1052 (80.7%) enrolled in HERITAGE. Women were followed up until they gave birth, and overall retention rate was high (95.3%).

3.3 Data and specimen collection

3.3.1 Specimen collection

The HERITAGE cohort involved specimen collections from women at the first trimester (week 8-14), third trimester (week 32-35) and at birth, as well as from newborns after birth. See Figure 3.1 for study design and sample collection. Women self-collected vaginal samples for HPV DNA testing and genotyping during the first trimester of pregnancy at the recruitment visit. Blood samples from mothers were also collected at recruitment for HPV antibody testing. Women who were HPV positive in the first trimester collected a second vaginal sample during the third trimester for HPV DNA testing and genotyping. After birth, 2 placental swabs (from the maternal and foetal side) and four placenta biopsies (two from the maternal side and two from the foetal side) were collected for HPV DNA testing. Specimens were collected from newborns for HPV DNA testing and genotyping at 36-48 hours of life and at 3, 6, 9, 12, 18 and 24 months in the eye, pharynx, mouth, and genital surface. Blood samples from newborns were also collected during some of these visits on dried blood spot. All swabs were placed in plastic vials with liquid preservatives and kept at room temperature while being transferred. The DNA in swabs was then purified with the Master pure procedure and stored at -80^{o}C. The biopsies were stored in cryogenic tubes at −80°C until HPV-DNA testing. Dried blood spots were stored at -80^{o}C until serology testing.

Figure 3.1. HERITAGE study design and follow-up visits



3.3.2 Data collected from questionnaires

Participants provided sociodemographic information at recruitment during the first trimester (see Annex I) including information on their age, ethnic group, marital status, work, education level, annual household income, gestational age at recruitment, medical (HPV vaccination, Pap test abnormalities) and sexual (number of lifetime sexual partners, number of sexual partners during the last year) history, as well as drug, alcohol, and tobacco consumption. Women who tested positive for HPV at the first trimester visit filled out another questionnaire after birth (see Annex II), which included information on medical history during pregnancy, as well as updates on other medical and sexual activity, drug, alcohol and/or tobacco consumption since start of pregnancy.

3.3.3 Data collected from medical files

Additional information was extracted from participants' medical charts for all participants, including history of preterm birth (for those who had previously given birth), history of cervical intraepithelial neoplasia treatment, gestational hypertension, and diabetes, as well as urinary tract or genital infections during pregnancy and details on birth history (delivery methods, timing of labour start and length, timing of membrane rupture, time of birth, etc.). A standardized case report form (available in Annex III) was used for all participants to extract information from their medical files. Data from participants' medical charts was also used to verify history of cervical lesion that was reported in the questionnaires filled out by the participants at recruitment. If there were disagreements between medical charts and self-reported measures, data from charts were used.

3.4 HPV genotyping and viral load testing

Linear array assay (Roche Diagnostics®) was used for HPV DNA detection and genotyping. It uses the enhanced PGMY09/11 primer system and involves a PCR with co-amplifications of HPV and β-globin DNA sequences. It is a consensus PCR-based assay that is both sensitive and specific for detecting individual HPV genotypes, which limits false positive and false negative results [131, 132]. Samples that had both negative results for HPV and β-globin DNA sequences were considered inadequate for analysis; only those that tested positive at least for β-globin were further tested for viral load. This PCR permits the detection of 36 HPV genotypes including 6, 11, 16, 18, 26, 31, 33, 34 (formerly known as type 4), 35, 39, 40, 42, 44 (formerly known as type 55), 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89 were identified. High-risk (HR-) HPV genotypes (categorized according to oncogenic potential) included: 16, 18,

31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 whereas low-risk (LR-) HPV included: 6, 11, 26, 34, 40, 42, 44, 53, 54, 61, 62, 67, 69, 70, 71, 72, 81, 83, 84, and 89.

HPV viral load testing was done for HPV genotypes 6, 11, 16, 18, 33, 45 and 52 using real-time PCR assays. This method enables the exact quantitation of HPV DNA over a wide range of concentrations. The cellular content of the samples was also measured using quantification of β-globin DNA. Unknown amounts of HPV DNA were determined through plots of HPV threshold cycle (Ct) in each sample vs the logarithm of the concentration of a standard curve of an HPV DNA plasmid in a background of 100 ng of human fibroblasts. Viral load was recorded as the crude copy number as well as copy numbers per cell. All HPV testing was done blindly to the researchers in the laboratory.

3.5 HERITAGE participants and data included in this study

In this study, we included women from the HERITAGE cohort who tested positive for HPV-16 in the first trimester. Women with pregnancies that ended before 20 weeks were excluded (miscarriages and induced abortion). We also excluded women with multiple pregnancies (twins or more), or those with a history of cervico-isthmic incompetency with a prophylactic cerclage placed in the 1st trimester. A sample of 48 women were included in the analysis, as summarized in Figure 2 in Chapter 4.

3.6 Statistical analysis

Descriptive statistics (mean, SD, median, quartiles (25th and 75th) and proportions (%)) were used to describe participants' characteristics. The viral loads of participants in the first and third trimesters were plotted in a line graph, with each line representing the viral loads of one participant. The x-axis of the line graph shows the visit (first or third trimester) and the y-axis shows the HPV-16 viral load in copies/cell. The association between HPV-16 viral load during pregnancy and preterm birth was estimated using logistic regression, given that our outcome (preterm birth) was binary. Crude odds ratios (OR) and 95% confidence intervals (CIs) as well as adjusted ORs (aORs) were computed. A log-binomial regression model was not used for this analysis because the frequency of our outcome was below 10%, and thus with this rare outcome assumption, similar results would be obtained using a logistic regression model. Inverse probability treatment weighting (IPTW) with propensity scores of HPV-16 viral loads in first trimester were used to

adjust for confounders. Propensity scores were used rather than multivariable logistic regression to adjust for confounders because weighting with propensity scores produce estimates that are less biased (aOR non collapsible), more robust and more precise, especially in studies in which the outcome is rare and where there are multiple confounders to adjust for [133, 134]. Further information on the choice of confounders is detailed below in the directed acyclic graph (DAG). All variables relevant for the analysis are also detailed in Table 3.1. Missing values were replaced by the mode. Three variables had missing values: smoking (1 out of 48 [2.1%]), history of cervical intraepithelial neoplasia treatment (6 out of 48 [12.5%]), gestational diabetes (2 out of 48 [4.2%]). All tests were two-sided, and p values were considered statistical at $p< 0.05$. Stata/SE version 14.0 was used for all analysis.

Table 3.1. Descriptions of variables

Variable name	Type of variable	Coding	Missing values*	Collection visit	Source
HPV-16 viral load	Continuous	Viral load in copies of HPV-16/cell		Recruitment & follow-up	Self-collected vaginal samples for HPV DNA testing
	Categorical	Referent ≤ 0.5 or 1.0 or 2.0 copies/cell			
Age	Continuous	Years		Recruitment	Sociodemographic questionnaire
Race[a]	Categorical	0: White 1: Other		Recruitment	Sociodemographic questionnaire
Education	Continuous	Number of completed years of education		Recruitment	Sociodemographic questionnaire
Smoking	Categorical	0: Non-smoker[b] 1: Smoker	1	Recruitment & follow-up	Sociodemographic questionnaire
Days of alcohol consumption[c]	Categorical	0: None 1: 1-4 days 2: ≥ 5 days		Recruitment & follow-up	Sociodemographic questionnaire
History of preterm birth	Categorical	0: Multiparous women without history of preterm birth 1: Multiparous with history of preterm birth 2: Nulliparous		Recruitment	Medical records
History of cervical intraepithelial neoplasia treatment	Categorical	0: No 1: Yes	6	Recruitment	Medical records
Gestational diabetes	Categorical	0: No 1: Yes	2	Follow-up	Medical records
Pregnancy-induced hypertension	Categorical	0: No 1: Yes[d]		Follow-up	Medical records
Genital or urinary tract infections	Categorical	0: No 1: Yes		Follow-up	Medical records

Preterm birth	Categorical	0: No 1: Yes		Follow-up	Medical records

*Missing values were imputed by the mode.
[a]Participants could select at least one ethnic group from a list of eight ethnic groups (White, Latin-American, African, African-American, Autochthone, East-Asian, South-Asian and Arab/Occidental Asian). They also had an option to specify an ethnic group not part of the list if relevant as "Other". Given the distribution of HPV infections in 1st trimester that showed low frequency in certain ethnic groups, this variable was dichotomized as White or Other.
[b]Participants were considered non-smoker if they never smoked, stopped smoking before or at the start of pregnancy.
[c]Number of days where at least one alcoholic beverage was consumed since start of pregnancy.
[d]Participants were put in the yes category if they had a hypertension diagnosis since the 20th week of gestation or if they had preeclampsia (which is indicative of gestational hypertension associated with one of these following signs: proteinuria or dysfunction of vital organs such as kidneys, liver, central nervous system, or other coagulation disorders).

3.6.1 Dependent variable

Preterm birth was the dependent variable in the analysis. Preterm birth was defined as a birth occurring between 20 weeks and 0 days and 36 weeks and 6 days of gestation. The gestational age was estimated based on date of last menstrual period and confirmed by ultrasound in the first trimester (this information was extracted from clinical notes in participants' medical records as aforementioned and as detailed in Table 3.1). In case of discrepancy, dating based on first trimester ultrasound was used.

3.6.2 Independent variable

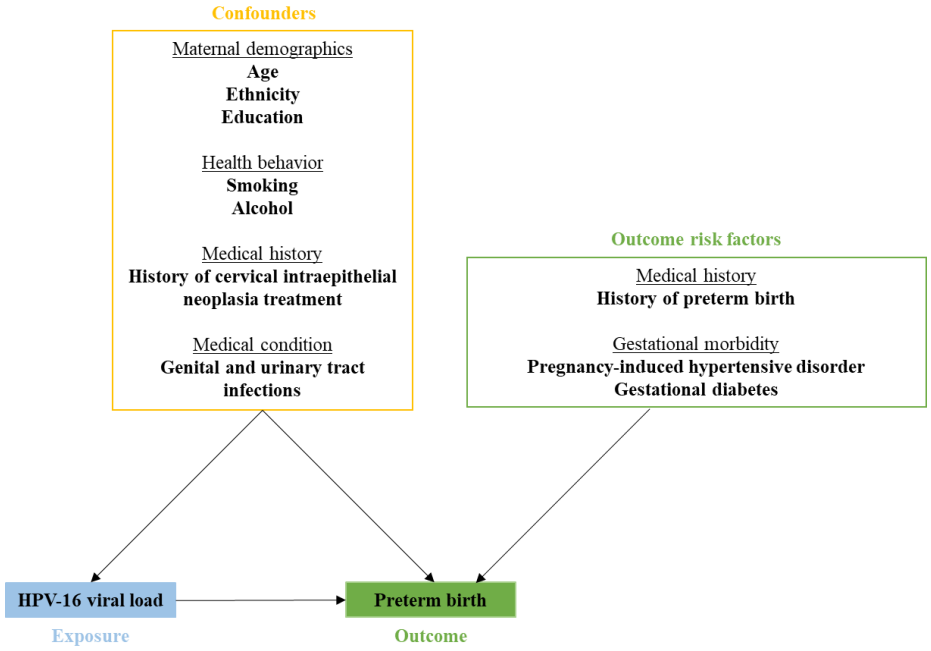
HPV-16 viral load was the independent variable in the analysis. Viral loads in first and third trimester were analyzed separately, and this variable was analyzed both continuously and categorized using exploratory cutoffs of 0.5 copy/cell, 1.0 copy/cell and 2.0 copy/cell. Viral load below or equal to the cutoff (either 0.5, 1.0 or 2.0) was the referent (and considered to be a low viral load).

3.6.3 Confounders and other relevant covariates

Potential confounders were identified *a priori* through a full and critical literature review. Data for relevant confounders in the study were extracted both from participants' sociodemographic questionnaires and medical files. We included all covariates as confounders if they were associated to both HPV infection and preterm birth, without being a mediating variable or a collider in this relationship. The confounders that were included in our analysis were the following: maternal age, race, completed years of education, smoking at enrollment, total days of use of alcohol during pregnancy genital and urinary tract infections and history of cervical intraepithelial neoplasia treatment. Variables that were only associated with preterm birth were also included when

calculating propensity scores for each participant. This was done because including both confounders as well as covariates only associated with the outcome (and excluding those associated with the exposure only) provides the most efficient estimates: i.e., reduced variance with no increase in bias [135-137]. The covariates only associated with preterm birth included in our analysis were the following: history of preterm birth, pregnancy-induced hypertensive disorders (which were either hypertension diagnosed 20 weeks after start of pregnancy or preeclampsia) and gestational diabetes. Figure 3.2 illustrates the DAG with the covariates that were included in our propensity score; confounders as well as those associated with preterm birth only.

Figure 3.2. Directed acyclic graph (DAG) of covariates included in the analysis



3.6.4 IPTW using propensity scores

The inverse probability of treatment weighting (IPTW) technique is a method based on estimation of propensity scores to control for confounding in observational studies [135]. It involves two steps: 1) estimation of propensity scores, which is the probability of being exposed given participants' baseline characteristics, 2) calculation of weights for each participant, which is done by taking the inverse of the probability of being exposed [135, 138]. This method allows for equal distribution of important baseline covariates across both exposed and non-exposed groups [135, 138], and thus adjusting for the confounding that these variables could have caused as long as all

the relevant cofounders are measured. In our study, propensity scores of HPV-16 viral loads were calculated using logistic regression. In these models, HPV-16 viral load during the first or third trimester were considered the dependent variable and the independent variables were maternal age, ethnicity, education, smoking and/or alcohol consumption, genital and urinary tract infections, history of preterm birth and/or cervical intraepithelial neoplasia treatment, pregnancy induced hypertensive disorder or gestational diabetes (detailed in Table 3.1). In our sample, we did not have any participants with urinary or genital infections nor pregnancy-induced hypertensive disorder, therefore these variables were not included in our final propensity scores. Once the propensity scores were estimated, we checked the weights of the propensity scores. We then checked the balance of the baseline covariates by comparing the standardized difference before and after weighting with IPTW for every model [138]. We considered covariates as improperly balanced if their standardised differences exceeded 10% [138]. As shown by the red lines (which limit 10% differences) in Annex IV, the standardised differences remained usually on/between the lines, thereby confirming adequate weighting of baseline covariates between exposed and non-exposed participants. With sensitivity analyses, we also compared the adjustment with multivariate models which produced similar adjusted ORs, although generally somewhat higher given the non-collapsibility of the ORs. For the adjustment of the models with continuous viral load, the propensity score used to weigh the model was estimated by dichotomizing the sample based on the viral load cutoff with 0.5 copy/cell (although using different cutoffs provide similar adjusted estimates).

3.6.5 Power estimation

Given 48 HPV-16 positive women in first trimester (18 exposed as having high viral load and 30 non-exposed/low viral load), the minimum detectable OR was estimated at 5.9 with a 5% alpha level and power of 80%. We had a power of 30% and 40% to detect ORs of 2.5 and 3, respectively. We acknowledge that the power of the study was limited. Since this association was never studied, we thought that this secondary analysis from HERITAGE data would be a steppingstone in understanding the overall effect of HPV-16 viral load in preterm birth, and thus, that it was worthwhile despite its low power.

3.7 Ethical considerations

Ethical approval was obtained from all participating institutions for HERITAGE. All participants signed a consent form and participated in the study in a voluntary manner. Participants were informed that all data and specimens were collected for research purposes only, and that all their personal information would remain confidential. Ethical approval from CERES (Ethics Committee for Health Research at the Université de Montréal) for access to data (containing no identifying information) from HERITAGE for this thesis was also obtained. (Initial REB approbation certificate and renewal are provided in Annex V and VI respectively).

Chapter 4. Results (Manuscript)

Association between Human Papillomavirus 16 viral load in pregnancy and preterm birth

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Running Title: Human Papillomavirus-16 (HPV-16) viral load and preterm birth

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ABSTRACT

Background: Human papillomavirus 16 (HPV-16) infection during pregnancy has been associated with preterm birth. To better characterize this association, we analyzed the association between HPV-16 viral load during pregnancy and preterm birth.

Methods: We used data from HPV-16 positive women at first trimester of pregnancy recruited in HERITAGE study. HPV DNA testing and genotyping on self-collected vaginal samples at 1st and 3rd trimesters were performed using the Linear Array assay. HPV viral load (copy numbers/cell) were measured with real-time PCR and analyzed continuously as well as categorized using 0.5, 1 and 2 copies/cell cutoffs. Preterm birth was defined as birth before 37 weeks of gestation using gestational age based on first day of last menstrual period and confirmed by ultrasound in 1st trimester. Logistic regression was used to measure the association between HPV-16 viral load during pregnancy and preterm birth. Odd ratios (OR) were adjusted with inverse probability treatment weighting (IPTW) of propensity score.

Results: The study included 48 HPV-16 positive women at 1st trimester of pregnancy. Mean viral load for HPV-16 (± Standard deviation (SD)) was 1.6 ±5.6 copies/cell (median [25%-75%], $8.0*10^{-3}$[$1.2*10^{-3}$ – 0.1]). The adjusted OR (aOR) and 95% confidence intervals [95% CI] for the association between HPV-16 viral load measured (continuously) at 1st trimester was significantly associated with preterm birth (aOR= 1.13 [95% CI: 1.03-1.25]). When viral load at 1st trimester was categorised with a cutoff of 0.5 copy/cell, higher viral load category was significantly associated with preterm birth (aOR= 13.04 [95% CI:1.58-107.57]. Similar associations were found using different cutoffs for categorisation for viral load at 1st and 3rd trimesters.

Conclusions: High HPV-16 viral loads during pregnancy was strongly associated with preterm birth risk.

Keywords: Human papillomavirus (HPV), HPV-16, viral load, pregnancy, preterm birth

Background

Preterm birth, usually defined as birth before 37 weeks of gestation, remains one of the leading causes of infant mortality and morbidity worldwide [1]. The global preterm birth proportion was estimated at 10.6% in 2014 [2], varying across industrialized countries with: 8.6% in Australia, 7.2% in Denmark, 8.4% in France, 8.9% in Germany, 9.6% in USA and 8.2% in Canada [3]. Although preterm birth survival proportions in high-income countries have risen in the last years [2], preterm births still account for nearly two thirds of infant deaths in Canada [4]. Despite advances in obstetrics and neonatal research, risk factors for preterm birth are not well understood. Bacterial infections and inflammation are known risk factors for preterm delivery [5-10]. More recently, human papillomavirus (HPV) infection has been associated with preterm birth. Systematic reviews and meta-analyses have found an association between HPV and preterm birth [11, 12]. We recently published results from a large cohort study that detected specific HPV genotypes at different time-points during pregnancy and reported a strong relationship between persistence of HPV-16 during pregnancy and preterm birth [13]. Decreasing trends in preterm rates in several countries have also been associated with mass HPV vaccination [14-16]. Although several studies suggest a potential role of HPV infection in preterm birth [11-13, 17-28], and more specifically for HPV-16 persistence [13], the biological mechanisms underlying this relationship remains unresolved. Showing a biological gradient between HPV-16 infection and outcome would enhance biological plausibility and provide support for a causal relationship. Higher HPV-16 loads may confer higher risk of persistence of HPV infection and thus translate into greater viral impact on the genital tract [29-32]. Therefore, this study aimed to investigate the association between HPV-16 viral load during pregnancy and preterm birth.

Methods

Design and Participants

We used data from HERITAGE (Human Papillomavirus perinatal transmission and risk of HPV persistence among children) cohort study, whose design, methods, and preliminary results have previously been published [13, 33-36]. The cohort included 1052 pregnant women, recruited between 2009-2016 from three academic hospitals in Montreal, Quebec, Canada. Participants were

eligible for HERITAGE if they were at least 18 years of age, pregnant at 14 weeks or earlier of gestation, able to provide written consent, and negative for HIV.

In this analysis, we included participants if they had HPV-16 infection detected at baseline. Participants were excluded if they had multiple pregnancies (twins or more), spontaneous or induced abortions or a history of cervico-isthmic insufficiency with a prophylactic cerclage in the first trimester. Study flow diagram (Figure 1) presents the details of the 48 participants included in this analysis.

Sample Collection

The participants self-collected vaginal samples for genotype-specific HPV DNA testing at the first recruitment visit (1st trimester of pregnancy) and at third trimester visit (32 to 35 weeks). Samples were processed as described elsewhere [33].

HPV testing

Extracted DNA from vaginal samples was tested for HPV DNA detection and genotyping with the Linear array HPV genotyping assay (Roche Molecular Systems) [37].

HPV-16 viral loads were measured in HPV-16 positive samples from the first and third trimester visits using real-time PCR assays in a Light Cycler PCR and detection system (Roche Molecular Systems) by measuring HPV-16 and β-globin copy numbers in 2 µL of processed sample. Results were recorded as crude number of copies as well as copy numbers per cell. Briefly, HPV-16 positive samples were screened for the presence of PCR inhibitors by amplification of an internal control, as described previously [38]. The presence of PCR inhibitors was suspected when 1000 copies of the internal control generated a signal corresponding to <700 copies. All samples tested were shown to be free of inhibitor activity. HPV-16 E6 DNA was quantified using a standard protocol [39]. Cycle thresholds obtained for each sample were compared to those of a titration curve obtained by serial 10-fold dilutions of HPV-16 DNA plasmid in 75 ng of human genomic DNA (Roche Diagnostics) in 10 mM Tris-HCl (pH 8.2). Processed samples were then tested for quantification of β-globin DNA to estimate the cell content of samples [39]. Viral loads were calculated by dividing the number of HPV DNA copies by the total number of cells, which was estimated by the number of β-globin copies.

Exposure, outcome, and covariates

HPV-16 viral load measured as copy numbers per cell was the exposure of interest. The outcome of interest was preterm birth, which was defined as a birth between 20 weeks and 0 days to 36 weeks and 6 days of gestation. First trimester ultrasound, which is part of routine prenatal care in the recruiting centers, was used to confirm gestational age based on menstrual period. One participant who underwent an emergency cerclage in the second trimester was considered as having experienced a spontaneous preterm birth, although she ultimately delivered at 36 weeks of gestation.

Sociodemographic information, medical and sexual history, as well as alcohol and tobacco consumption were collected at both recruitment, follow-up visits and at birth using self-reported questionnaires. Information on pregnancy outcomes and delivery information (labor onset, duration, date and time of membrane rupture and delivery, type of delivery) as well as medical history (history of preterm birth and cervical intraepithelial neoplasia treatment, gestational diabetes, hypertension, urinary tract, or genital infections) was extracted from participants' electronic medical records.

Statistical analysis

Characteristics of participants were described using means and standard deviations (SD) or medians with quartiles (25^{th} and 75^{th}) for continuous variables and proportions (%) for categorical variables. HPV-16 viral loads measured in 1^{st} and 3^{rd} trimesters were plotted in a line graph.

The association between HPV-16 viral load and preterm birth was measured using logistic regression. Viral loads measured at first and third trimesters were analyzed as a continuous variable, as well as considered as binary variable using cutoffs of 0.5, 1 and 2 copies/cell (at above or below the cutoff), with the lowest viral load category being the referent in regression models. Crude odds ratios (OR) and 95% confidence intervals (CIs) were computed. Adjusted ORs (aORs) (and 95% CI) were estimated using propensity scores with inverse probability treatment weighting (IPTW). Propensity scores were estimated including potential confounders such as maternal age, ethnic origin (White or other), completed years education, smoking at enrollment (yes or no), total days of use of alcohol since pregnancy (none, 1-4 days, or ≥ 5 days), history of preterm birth among parous women (yes or no), history of cervical intraepithelial neoplasia treatment (yes or

no), gestational diabetes (yes or no). Three variables had some missing values: smoking (1 out of 48 [2.1%]), history of cervical intraepithelial neoplasia treatment (6 out of 48 [12.5%]), gestational diabetes (2 out of 48 [4.2%]) that were imputed by the mode. Tests were two-sided, and p-values were considered statistically significant at $p< 0.05$. Analysis was done using Stata/SE version 14.0.

Results

Table 1 summarizes the characteristics of the 48 participants. Overall, the mean (± SD) age was 31.2 years (± 4.7). Most of the participants were White (83.3%), had a university education (median of 17 completed years of education), did not smoke (89.6%), did not have a history of preterm birth (94.7%), nor of cervical intraepithelial neoplasia treatment (75%). At the first trimester visit, we found a mean HPV-16 viral load of 1.63 copies/cell (±5.64) (median= $8.0*10^{-3}$ copies/cell) with a maximum value of 31.46 copies/cell. Among these, 35 women remained positive in the third trimester with a mean HPV-16 viral load of 0.32 copies/cell (±0.97) (median=$5.29*10^{-3}$ copies/cell) with a maximum value of 5.07 copies/cell. Ten women cleared their infection and three had missing data of HPV status in the third trimester. Figure 2 shows the values of HPV-16 viral load for each woman in the first and third trimester. Interestingly, we observe an overall decrease in the average viral load between the first and third trimester (paired t-test p-value=0.0562). Five women (10.4%) had preterm birth (four delivered at 36 weeks and one at 35 weeks) and among them, one woman was multiparous without history of preterm birth. The five women with preterm birth had a mean HPV-16 viral load of 8.93 copies/cell (±13.51) (median=1.41 copies/cell and maximum value of 31.46 copies/cell) in the first trimester and remained positive for HPV-16 in the third trimester with a mean viral load of 1.12 copies/cell (±1.72) (median=$7.74*10^{-3}$ copies/cell and maximum value of 3.91 copies/cell). Only one woman with preterm birth (who gave birth at 36 weeks) had an increase in viral load between the two trimesters while the others had a decrease in viral load. Table 2 provides a description of individual data on viral load and other characteristics for each woman.

Table 3 shows the associations between HPV-16 viral load and preterm birth. The association between viral load (as a continuous variable) at 1st trimester was significantly associated with preterm birth; each unit increase in viral load at 1st trimester was associated with an increased risk of preterm by 13% (aOR [95% CI], 1.13 [1.03-1.25]). When viral load measures

were dichotomized using cutoff of 1 copy/cell, highest viral load values measured at both 1st and 3rd trimester were associated with preterm birth with aORs of 15.03 [95% CI: 1.75-129.26] and 14.02 [95% CI: 1.28-153.48], respectively. Similar results were obtained for the other categorisation although not always statistically significant.

Discussion

We found strong significant associations between HPV-16 viral load and preterm birth. When viral load was analyzed as a continuous variable among 48 HPV-16 positive women, we found that each unit increase of viral load in the first trimester was associated with an increased preterm risk by 13% [95% CI: 3-25%]. When viral loads were analyzed dichotomously, women with more than 1.0 copy/cell of HPV-16 at first trimester were 15.03 [95% CI: 1.75-129.26] more likely to experience preterm birth compared to women who had less than 1.0 copy/cell. Similar results were found for viral loads mesured at third trimester.

Several studies have shown positive associations between HPV and preterm birth, but most studies have not considered the potential role of individual HPV genotypes. Instead, they either provide a measure for either the presence/absence of HPV, or for a cluster of genotypes [11]. A meta-analysis including 36 studies reported a pooled age-adjusted OR of 1.50 [95% CI: 1.19-1.88] for the relationship between HPV and preterm birth [11]. The sensitivity analyses in this meta-analysis showed that this association was even stronger when restricting to studies of higher quality, such as those using either HPV testing or those measuring HPV during pregnancy. Other meta-analyses on the association between HPV and preterm birth reported pooled ORs of 2.84 [95% CI: 1.95-4.14] [40] and of 2.12 [95% CI: 1.51-2.98] [12]. Moreover, recent studies seem to indicate an important role of HPV-16 specifically in this association. A strong association between persistent HPV-16 infection and preterm birth was recently found in our large cohort study [13]. A recent case-control study has also reported a significant association (p=0.04) between HPV-16 and preterm birth and not for other genotypes [28]. Population data from Australia, Finland and Denmark show a reduction in preterm births following the implementation of mass HPV vaccination programs. A population-level study in Australia [14] found that among maternal cohorts with 60-80% vaccination coverage, there was a relative reduction in preterm birth proportion of 3.2% [95% CI: 1.1-5.3%]. After adjusting for infant birth year and maternal age,

they predicted a relative 1% reduction in preterm birth proportion with every 20% increase in vaccination coverage [14]. Similarly in Finland, results from a registry-based follow-up study reported lower preterm birth risk among women previously vaccinated for HPV compared to unvaccinated women of similar age [16]. The authors reported that reductions were more noticeable and statistically significant in early preterm (less than 32 weeks), which is when mortality and morbidity related to preterm is highest [16]. This was not observed in our sample, as all five women experienced late preterm. A nationwide study of over 240,000 singleton births in Denmark also reported reductions in spontaneous preterm birth among women vaccinated against HPV before 17 years of age [15].

To our knowledge, our study is the first exploring the impact of HPV-16 viral load during pregnancy on preterm birth risk. Current published literature on HPV viral load and persistence have mainly focused on clinical outcomes involving HPV-related cancers or precancerous lesions. Higher HPV viral load is correlated with severity of cervical lesions [41-46] and invasive cervical cancer [47-49]. Given the important clinical implication of HPV-16 viral load on cervical lesions, it appears plausible that viral load may play a role in other outcomes, such as preterm birth. We found a strong association between HPV-16 viral load and preterm birth. As the amount of extracellular virus can affect the inflammatory environment of the cervix, we also looked at the association between crude HPV-16 copy number (per μL) and preterm birth, and results were also similar although the ORs were somewhat attenuated (data not shown).

Our finding reinforces the plausibility of the link between HPV-16 and preterm birth. However, while HPV-16 viral load on vaginal specimens during pregnancy could serve as a biomarker for the risk of preterm birth, the mechanism at the basis for the relationship is still unresolved. Specifically, two mechanisms for HPV infection's role in preterm birth has been suggested [50]. Findings from *in vitro* studies suggest that HPV can alter trophoblast physiology and morphology with an increasing rate of apoptosis in the placenta, possibly causing abnormal placentation and compromised gestation [20, 26, 51, 52]. HPV infection has also been suspected to disturb vaginal microbiome and increase its heterogeneity [53], which could in turn increase production of pro-inflammatory cytokines and lead to early delivery. Yet, it seems that vaginal HPV viral load during pregnancy is an important parameter to consider. Higher viral load may cause greater cellular reactions in the cervix, and disrupt regular cellular pathways of parturition,

which may either increase risk of HPV transmission to the placenta or disrupt the vaginal microbiome during pregnancy and lead to preterm birth.

Our study has several strengths but also a few limitations. Given its prospective design, HPV DNA was tested during pregnancy with repeated testing allowing documentation of HPV persistence. A sensitive, type specific HPV detection technique was used. Viral load was also measured with a specific and sensitive technique, considering the number of copies per cell, attenuating possible errors that may be caused by fluctuations in cell content. Preterm birth estimates were also reliable given that first trimester ultrasound was routinely available. It is noteworthy that important confounders were measured and were adjusted for in our analysis using inverse probability treatment weighting (IPTW) with propensity scores, but we cannot excluded the possibility that there remains residual confounding because of unknown confounders or mesurement errors.

Conclusion

Our findings suggest that higher viral loads of HPV-16 infection during pregnancy are associated with increased risk of preterm birth. The presence of a biological gradient reinforce the biological plausibility of the link between HPV-16 and preterm birth, although it remains difficult to explain the exact mechanisms behind this relationship. Given that preterm birth remains a major health concern, it is important to better understand its etiology. It goes without saying, however, that if a causal relationship exists between HPV-16 and preterm birth, mass HPV vaccination with the currently available vaccines will have a significant impact in reducing the number of preterm births globally.

Role of coauthors:

All authors have directly contributed to the conception and design (HT, MHM, FC, FA, AMC, JL, FC, JN, LL) or acquisition of data (JN, LL, HT, MHM, FC, JL, EC) or analysis and interpretation (PK, HT, M-HM, FC) of the study. PK, HT, MHM and FC wrote the first draft of the manuscript. All authors have subsequently read, revised and approved the version that is being submitted. HT is responsible for the overall content as the guarantor and had the full responsibility for the work and/ or the conduct of the study, had access to the data, and controlled the decision to publish.

Competing interests :

FC has received grants to evaluate HPV detection tests through his institution from Becton-Dickson and Roche Molecular systems. HT has received occasional lecture fees from Merck and unrestricted grants form ViiV Healthcare. All other coauthors have no conflict of interests.

Funding

This work was supported by a grant from the Canadian Institutes of Health Research (CIHR) (Grant MOP-93564 and MOP-136833) to HT. HT holds a salary award (chercheur-boursier) for the duration of this study from the Fonds de la recherche du Québec en santé (FRQ-S), and from CIHR (new investigator salary award). M-HM held a salary award (chercheur-boursier clinicien) from the FRQ-S for the duration of this study. Funding for quality control of HPV testing was provided in part by the Réseau FRQS SIDA-MI to FC.

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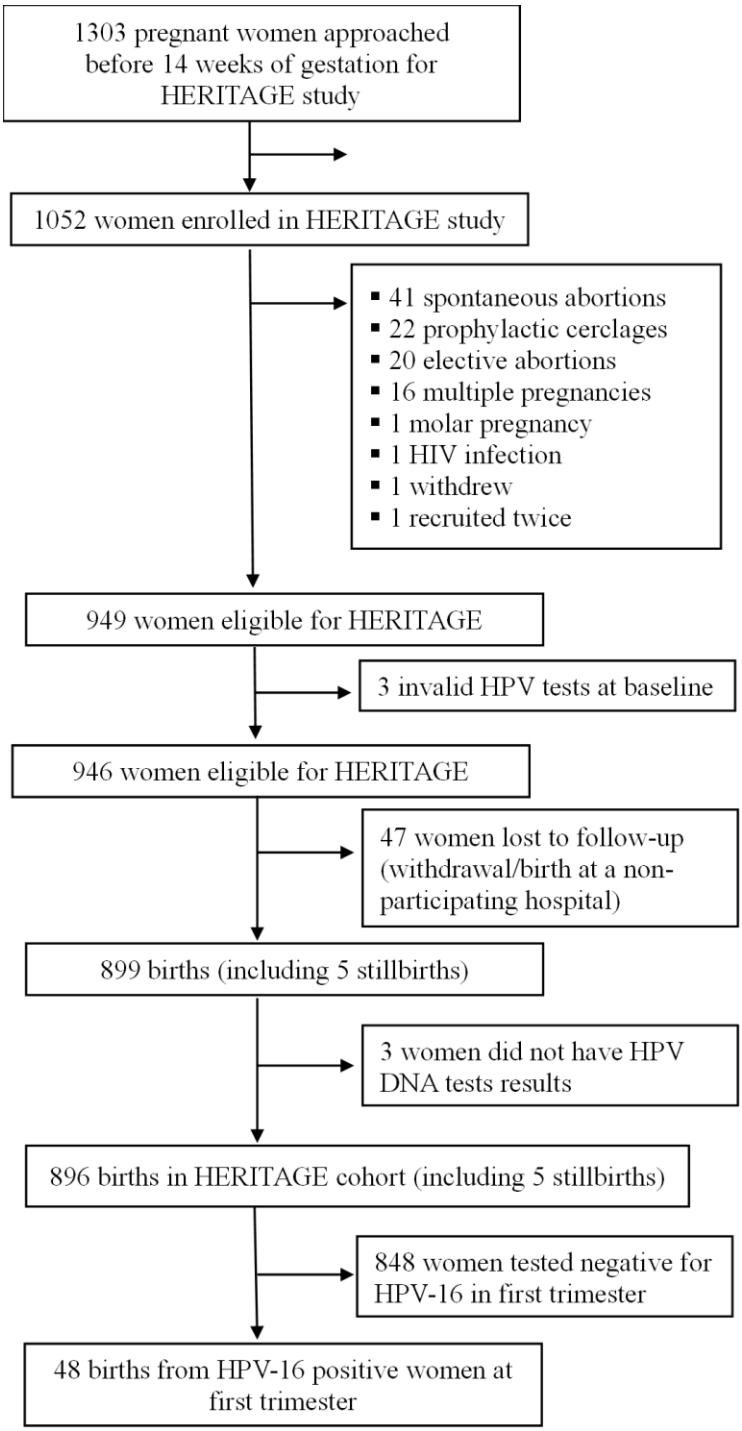
Tables and Figures

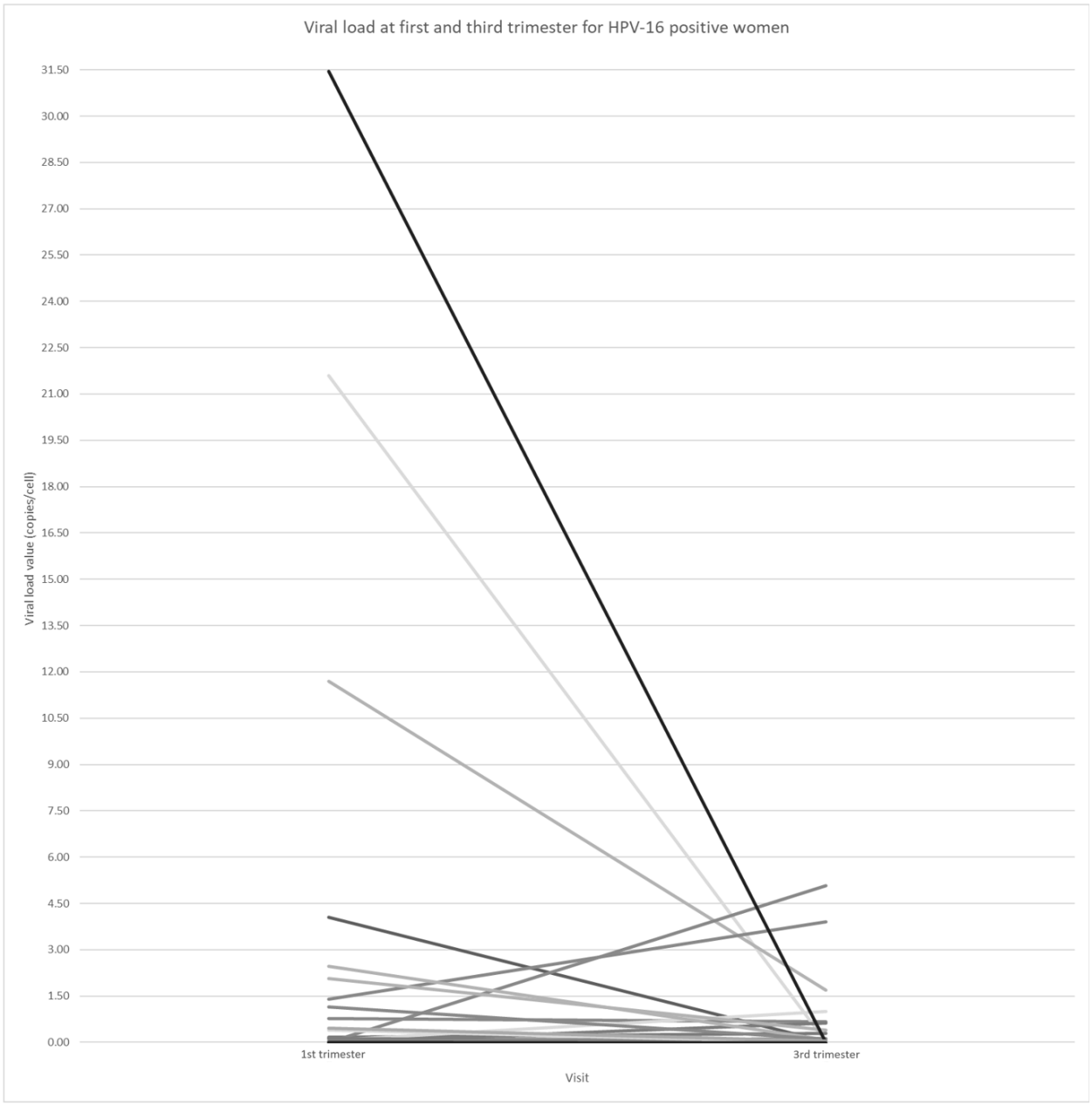
Figure 1. Study recruitment flowchart*



HPV: human papillomavirus, HIV: human immunodeficiency virus

*Figure was adapted from Niyibizi et al. [13]

Figure 2. Viral load measured at first and third trimester among HPV-16 positive woman (n=45)[a]



HPV: human papillomavirus

[a]Among the 48 HPV-16 positive pregnant women in first trimester, 35 remains positive at the third trimester, 10 cleared their infection and 3 participants had missing HPV DNA testing.

Table 1. Characteristics of HPV-16 positive women in first trimester of pregnancy

	Low HPV-16 viral load (≤1 copy/cell) n=40	High HPV-16 viral load (>1 copy/cell) n=8	Total sample n=48
Characteristics at baseline			
Mean age (SD); median [25%-75%]	31.4 (4.6); 31 [28-34.5]	30.1 (5.5); 29 [26-33.5]	31.2 (4.7); 31 [28-34.5]
Completed years of education, median [25%-75%]	17 [16-19]	17 [15.5-17]	17 [16-18.5]
Ethnicity, n (%)			
White	34 (85.0)	6 (75.0)	40 (83.3)
Arabic-West Asian	3 (7.5)	0	3 (6.3)
Native African	0	2 (25.0)	2 (4.2)
East Asian	1 (2.5)	0	1 (2.1)
Others[a]	2 (5.0)	0	2 (4.2)
Smoker, n (%)			
Yes	3 (7.5)	1 (12.5)	4 (8.3)
No	36 (90.0)	7 (87.5)	43 (89.6)
Missing	1 (2.5)	0	1 (2.1)
Alcohol consumption (number of days since the beginning of pregnancy)[b],n (%)			
None	21 (52.5)	6 (75.0)	27 (56.3)
1-4	13 (32.5)	2 (25.0)	15 (31.3)
≥ 5	6 (15.0)	0	6 (12.5)
Nulliparous, n (%)			
Yes	25 (62.5)	4 (50)	29 (60.4)
No	15 (37.5)	4 (50)	19 (39.6)
History of pre-term birth among parous women (n=19), n (%)			
Yes	1 (6.7)	0	1 (5.3)
No	14 (93.3)	4 (100)	18 (94.7)
History of cervical intraepithelial neoplasia treatment, n (%)[d]			
Yes	5 (12.5)	1 (12.5)	6 (12.5)
No	31 (77.5)	5 (62.5)	36 (75)
Missing	4 (10.0)	2 (25.0)	6 (12.5)

HPV-16 viral load (copies/cell)			
Mean (SD)	$5.9x10^{-2}$ (0.2)	9.5 (11.4)	1.6 (5.6)
Min-Max	$4.0x10^{-5}$ – 0.77	1.15 - 31.46	$4.0x10^{-5}$ - 31.46
Median [25%-75%]	$3.2x10^{-3}$ [$6.9*10^{-4}$ – $3.8x10^{-2}$]	3.3 [1.7-16.6]	$8.0x10^{-3}$ [$1.2x10^{-3}$ – 0.1]
Characteristics during pregnancy			
Gestational diabetes, n (%)			
Yes	3 (7.5)	2 (25.0)	5 (10.4)
No	35 (87.5)	6 (75.0)	41 (85.4)
Missing	2 (5.0)	0	2 (4.2)
Pregnancy-induced hypertensive Disorders, n (%)			
Yes	1 (2.5)	0	1 (2.1)
No	38 (95.0)	8 (100)	46 (95.8)
Missing	1 (2.5)	0	1 (2.1)
Urinary tract or genital infections[c], n (%)			
Yes	0	1 (12.5)	1 (2.1)
No	40 (100)	7 (87.5)	47 (97.9)
Pregnancy outcome, n (%)			
Preterm birth	2 (5.0)	3 (37.5)	5 (10.4)
Term birth	38 (95.0)	5 (62.5)	43 (89.6)

Note: Percentages may not sum to 100% due to rounding.
HPV: Human papillomavirus; SD: standard deviation; n: number.
[a]Participants were categorized in the group "others" if they self-identified as being in two different ethnic groups.
[b]Number of days where there was at least one drink of alcoholic consumption since the start of pregnancy.
[c]Urinary tract or genital infections include cystitis, bacterial vaginosis, active herpetic lesion and non-specified urinary tract or genital infection.
[d]Cervical intraepithelial neoplasia treatment includes 1 ablative treatment and 5 excisional treatments.

Table 2. Viral load, preterm birth, and other characteristics of HPV-16 positive women at first trimester (n=48)

Number	Age	1st trimester HPV-16 loads (copies/cell)	3rd trimester HPV-16 loads (copies/cell)	Difference viral load between 1st and 3rd trimester	Preterm birth	Gestational age (weeks)	History of cervical treatment
1	28	11.69219	1.69494	-9.99725	Yes	36	No
2	40	1.40437	3.90897	2.5046	Yes	36	No
3	30	0.03511	0.00529	-0.02982	Yes	36	No
4	31	0.03989	0.00774	-0.03215	Yes	35	-
5	36	31.4574	0.00114	-31.45626	Yes	36	Yes
6	26	0.00284	0.00009	-0.00275	No	38	No
7	26	0.01402	0.00166	-0.01236	No	40	No
8	36	0.7735	0.65959	-0.11391	No	37	No
9	34	0.00049	0	-0.00049	No	39	No
10	22	0.05343	-	-	No	38	No
11	26	21.59379	0.01448	-21.57932	No	39	No
12	31	0.09955	0.08503	-0.01451	No	38	No
13	36	0.00165	0.00589	0.00424	No	38	Yes
14	30	4.05012	0.02146	-4.02866	No	41	No
15	28	0.00579	-	-	No	38	No
16	27	0.01189	0.00415	-0.00774	No	40	No
17	29	0.00051	-	-	No	41	-
18	26	0.00279	0	-0.00279	No	38	No
19	26	0.00312	0	-0.00312	No	39	No
20	28	0.00025	0	-0.00025	No	39	Yes
21	33	0.00011	0.0011	-0.00099	No	40	-
22	30	0.16459	0.29646	0.13187	No	39	No
23	35	0.00036	0.00036	0	No	41	No
24	33	0.04063	0.00375	-0.03688	No	39	No
25	32	0.00134	5.06819	5.06686	No	39	No
26	28	0.00004	0.0004	0.00036	No	39	No
27	33	0.05176	1.00443	0.95267	No	40	No
28	38	0.00015	0	-0.00015	No	38	-
29	33	0.00486	0.62714	0.62228	No	40	No
30	26	0.02287	0.01027	-0.0126	No	40	No
31	26	2.46677	0.08264	-2.38413	No	39	-

32	24	1.14868	0.10666	-1.04202	No	40	No
33	47	0.00065	0	-0.00065	No	39	No
34	31	0.00327	0.00133	-0.00194	No	40	No
35	31	0.00108	0.01091	0.00983	No	41	Yes
36	32	0.12291	0.04096	-0.08195	No	39	No
37	30	0.00473	0.01793	0.0132	No	37	No
38	31	2.07335	0.38704	-1.6863	No	40	-
39	33	0.00063	0	-0.00063	No	40	No
40	29	0.01605	0.12466	0.10861	No	40	No
41	29	0.00227	0	-0.00227	No	39	No
42	32	0.39554	0.00759	-0.38795	No	39	No
43	38	0.45647	0.03041	-0.42606	No	41	Yes
44	35	0.00308	0.00004	-0.00304	No	39	No
45	36	0.0001	0	-0.0001	No	40	No
46	36	0.01013	0	-0.01013	No	41	No
47	35	0.00072	0.00524	0.00451	No	41	Yes
48	26	0.00307	0.00062	-0.00244	No	39	No

HPV: Human papillomavirus; -: missing data

Table 3. Odds ratio (OR) for associations between HPV-16 viral load and preterm birth

HPV-16 viral load (number of copies/cell)		Odds ratio (95% CI)	
	Number of preterm birth/total women	Crude	Adjusted[c]
Viral load at first trimester (continuous)	5/48 [a]	**1.15 (1.01-1.31)**	**1.13 (1.03-1.25)**
Viral load at third trimester (continuous)	5/45 [b]	1.75 (0.90-3.41)	1.84 (0.80-4.23)
First trimester			
Low-viral load (≤0.5 copies/cell)	2/39	Referent	Referent
High viral load (>0.5 copies/cell)	3/9	**9.25 (1.27-67.42)**	**13.04 (1.58-107.57)**
Third trimester			
Low-viral load (≤0.5 copies/cell)	3/39	Referent	Referent
High viral load (>0.5 copies/cell)	2/6	6.00 (0.76-47.36)	6.75 (0.76-59.67)
First trimester			
Low-viral load (≤1 copy/cell)	2/40	Referent	Referent
High viral load (>1 copy/cell)	3/8	**11.40 (1.52-85.73)**	**15.03 (1.75-129.26)**
Third trimester			
Low-viral load (≤1 copy/cell)	3/41	Referent	Referent
High viral load (>1 copy/cell)	2/4	**12.67 (1.29-124.51)**	**14.02 (1.28-153.48)**
First trimester			
Low-viral load (≤2 copies/cell)	3/42	Referent	Referent
High viral load (> 2 copies/cell)	2/6	6.50 (0.83-51.20)	6.24 (0.66-59.06)
Third trimester			
Low-viral load (≤2 copies/cell)	4/43	Referent	Referent
High viral load (>2 copies/cell)	1/2	9.75 (0.51-187.53)	14.67 (0.72-300.70)

HPV=human papillomavirus.

[a]Total number of women with HPV-16 DNA infection at first trimester of pregnancy

[b]Total number of HPV-16 DNA infection at third trimester of pregnancy (n=45, excluding 3 participants with HPV-16 infection at first trimester of pregnancy who had missing HPV DNA testing at third trimester).

[c]Adjusted estimates obtained for each model using propensity score-based inverse probability treatment weights including the following variables: maternal age (years; continuous), ethnic origin (White or other), completed education (years; continuous) smoking at enrollment (yes or no), total use of alcohol days since pregnancy (none, 1-4 days, or ≥ 5 days), history of preterm birth (yes or no), history of cervical intraepithelial neoplasia treatment (yes or no) and gestational diabetes (yes or no). For the adjustment of the model with continuous viral load, the propensity score used to weight the model was estimated by dichotomizing the sample based on the viral load cutoff with 0.5 copy/cell (although using different cutoffs provided similar adjusted estimates).

Chapter 5. Discussion

This chapter will summarize the findings as well as interpret them in light of the current state of the literature. The strengths and limitations of the study will also be described, with details on methodological considerations of internal and external validity. The impact of study's findings in public health will be discussed and future avenues of research will also be outlined.

5.1 Summary, interpretation, and contextualization of study results

The objective of this study was to estimate the association between HPV-16 viral load during pregnancy and preterm birth. We found strong and significant positive associations between HPV-16 viral load during pregnancy and preterm birth; higher HPV-16 viral loads in pregnant women during first and third trimesters were associated with increased preterm birth risk. These results were consistent both when viral load was analyzed continuously, as well as when it was dichotomized. Each unit increase of viral load in the first trimester was associated with an increased preterm birth risk by 13% [95% CI: 3-25%]. When viral load was dichotomized, those with more than 1.0 copy/cell of HPV-16 viral load in the first trimester were 15.03 [95% CI: 1.75-129.26] more likely to experience preterm birth compared to women who had less than 1.0 copy/cell. Similar results were found for viral load mesured at third trimester, and when categorized with a cutoff of 0.5 copy/cell, although results were not statistically significant. To our knowledge, this is the first study to explore the impact of HPV-16 viral load during pregnancy on preterm birth risk. Current published literature on HPV viral load has mainly focused on the impact of HR HPV viral load on HPV-related cancers [91-93] and precancerous lesions [87-90, 95]. Most studies report significant associations with HPV-16 viral loads with cancer and lesions. However, the impact of HPV-16 viral load has never been studied in relation to pregnancy outcomes.

A strong association between persistent HPV-16 infection during pregnancy and preterm birth was recently reported by our group in a large cohort study [12]. This followed a 19 study (included meta-analysis by Niyibizi et al. [11] that showed a pooled age-adjusted OR of 1.50 [95% CI: 1.19-1.88] for the association between HPV and preterm birth, with inconsistent results due to biases such as lack of control for confounders, detection of HPV at inappropriate times, misclassification of exposure and outcome, but most importantly lack of consideration of specific HPV genotypes. The asssociation between HPV-16 and preterm birth was also found in a recent case-control study

from India [13]. Authors evaluated the role of placental HPV in preterm neonatal intensive care admissions among 100 women with singleton live pregnancies admitted in labor ward of a tertiary care teaching hospital. Specifically, they compared placental HPV DNA of spontaneous preterm births between 24 and 36 and 6 weeks (n=50) to full term deliveries ≥ 37 weeks (n=50). What was most interesting is that a statistically significant association (p=0.04) was found only between HPV-16 genotype and preterm neonatal intensive care admissions, and no associations were found when other HR HPVs genotypes were considered together, which speaks to the importance of this HPV-16 genotype. It is important to note that this study had a small sample size and no control for confounders.

However, other teams have found a population-level decrease in preterm risk with HPV mass vaccination [14-16]. Reduction in preterm birth proportion of 3.2% [95% CI: 1.1-5.3%] was reported in a population-level study after 8 years of national HPV vaccination program among Australian maternal cohorts with 60-80% vaccination coverage [14]. In this study, data from the National Perinatal Data Collection between 2000-2015 on HPV vaccination and preterm births were compared. Authors suggested a 1% reduction in preterm birth proportion with every 20% increase in vaccination coverage after adjusting for infant birth year and maternal age [14]. There were some limitations in this study, including effect of unmeasured confounders such as smoking but more importantly there is also a possibility that women in the catch-up age groups may have been infected with HR HPVs before vaccination (as they may already have been sexually active) which would have underestimated the effect of HPV vaccination on preterm birth risk.

In Finland, lower preterm birth risk was also reported from a registry-based study among women previously vaccinated for HPV (n=6226 females vaccinated for HPV16/18) compared to unvaccinated women of similar age (n=1770 females who did not receive HPV vaccine at age of 18) [16]. For the first pregnancy, preterm birth rate was 3.2% among HPV-vaccinated women and 5.1% among non-vaccinated women although findings were not significant (OR: 0.61 [95% CI: 0.34-1.09]). However, for early preterm (less than 32 weeks; early preterm birth rates were 0% in HPV-vaccinated women and 1.0% in non-HPV-vaccinated women), reductions were more noticeable and statistically significant (p=0.04), which is a very important finding given that earlier preterm births have highest mortality and morbidity rates [16]. Limitations of this study include small sample size and lack of adjustment for confounders.

A nationwide study of over 240,000 singleton births in Denmark looking at the association between HPV vaccination and spontaneous preterm birth among primiparous women born between 1961-2004 with a singleton delivery at >22 weeks of gestation from 2006-2018 [15], also suggested a reduction of preterm birth with HPV vaccination. Reductions in spontaneous preterm birth among women vaccinated against HPV before 17 years of age (adjusted OR with maternal age at childbirth: 0.86 [95% CI: 0.71-0.98]) were reported. Although these findings should be interpreted with caution because of potential unmeasured confounders, this study also suggested a potential role of HPV-16 on preterm risk.

It is becoming increasingly clear that there is a link between HPV-16 and preterm birth, but it is difficult to establish with certainty whether this association is causal and to explain the biological mecanisms. It is important to note, though, that a few of Hill's criteria for causation, including strength, temporality, and biological gradient are met in our study. First, we found very strong odds ratios in our study, speaking to the strength of this association. Temporality criterion was also respected as the exposition of HPV-16 infection preceded the outcome of preterm birth. A biological gradient was also observed as there was a dose-response relationship between higher viral loads and preterm birth risk. Our results may therefore reinforce the biological plausibility of a causal relationship between HPV-16 and preterm birth. Yet, how HPV viral loads may increase risk of preterm birth remains challenging to explain. Could it be that higher viral loads may lead to greater cellular reactions in the cervix? It is still unclear if higher viral loads may increase preterm birth risks through previously suggested mechanisms such as trophoblast physiology changes in the placenta [81, 106, 108, 120] or increased disturbance of vaginal microbiome [124], or even through other biological pathways yet to be known. Nevertheless, our findings suggest that HPV-16 seems to be an important element in the chain of events leading to the preterm births.

5.2 Methodological considerations

This study has several strengths but also a few limitations. The prospective design of the study was a great strength as it allowed for measurement of exposure during the pertinent exposure time window, i.e., HPV-16 viral load during pregnancy. Our prospective design with repeated measurements also permitted documentation of HPV persistence throughout pregnancy, as well as collection of important confounders (related to maternal behaviors) bound to change throughout

pregnancy. Bias such as residual confounding may have remained due to unknown confounders or measurement errors in known confounders. The validity of the results are discussed below.

5.2.1 Internal validity

5.2.1.1 Precision

It is important to note that we did not have a large sample size (n=48). Confounders were adjusted for using a robust method (IPTW) which generally provides more precision. Nevertheless, important variations between crude and adjusted ORs were observed in some cases which may be due to the low frequencies of some categories of confounders, that may have impacted the weighting with propensity scores. Our estimates might also lack precision due to imbalance between our exposed and non-exposed groups [139]. When we categorized HPV-16 viral load, we always had less women in the exposed (higher viral load) compared to the non-exposed group (lower viral load, the referent), which could have affected the precision of the results. For example, when we dichotomized viral loads with cutoff of 1 copy/cell in first trimester, 83.3% of our sample were in the non-exposed group, and 16.7% were in the exposed group. Similar percentages were seen for other cutoffs in first trimester (81.25% in non-exposed group for 0.5 copy/cell and 87.50% for 2.0 copy/cell). Our small proportion of participants that experienced the outcome of preterm birth also affected the precision of the study, which may explain large width of confidence intervals. However, the power of the study was adequate as many odds ratios in our study were above the minimum detectable ORs of 5.9 that we calculated with a 5% alpha level.

5.2.1.2 Selection bias

The probability of a selection bias is very low in this study given the prospective design of the HERITAGE cohort study, the low numbers of missing data and the fact that there were no losses to follow-up. Generally, the main source of selection bias in prospective cohort studies is losses to follow up, which did not occur in our study (no attrition was present in our study). It seems unlikely that exposure and outcome could have influenced participation in the study given that participants' exposure to HPV was not known at recruitment (not an inclusion criterion, and asymptomatic), and the outcome had not occurred yet (participants were enrolled at the start of their pregnancy before they did (or not) experience preterm birth). A question of self-selection (or volunteer) bias may arise in this study given that participation was voluntary. For example, although the HPV infection status of participants was not known prior to enrollment in HERITAGE, there may be a

question of selection bias if we consider that some women, e.g., those who had abnormal Pap tests in the past, may have been more inclined to take part in this study. But, for this to threaten the study's internal validity, this would have to be related to not only the exposure, but also to the outcome. There is no reason to believe that this might be the case, and thus self-selection bias is unlikely to affect the results.

5.2.1.3 Information bias

Error of classification can occur when measuring exposure and outcome. HPV-16 was identified with a sensitive and specific HPV detection and genotyping technique, linear array (LA) assay (Roche Diagnostics®) [131, 132] used in Dr. François Coutlée's laboratory, which is the most recognized and experienced research lab with the LA assay around the world. The HPV-16 viral loads were measured as copies of HPV-16 DNA/cell, reducing possible errors that could have resulted due to differences in cell content in different samples. HPV-16 viral loads in currently published studies have been quantified either through semi-quantitative methods (such as the Hybrid capture II assay) [87-90, 95] which are not standardized thus hard to compare across studies, or by copies/human genome equivalents [91, 92] or absolute viral copies/μL [93], in which reported viral loads would vary depending on quantity of samples. There is thus great strength in quantifying viral loads by copies/cell as done in our study, as it allows for consistent results across different definitions and categorization of viral loads. Yet, given the lack of literature quantifying viral loads like in our study, it is challenging to define and interpret the clinical importance of our results on viral load data. Although quite accurate, no tool is perfect- there may therefore still be a possibility of misclassification of exposure, but this would be non-differential (and would be more likely to attenuate odds ratio towards null).

Error of classification when measuring outcome is also unlikely but possible. The outcome of preterm birth was measured in a reliable manner as gestational age information was measured from the first day of menstrual period validated by the first trimester ultrasound (the gold standard gestational age assessment [10]). However, preterm birth might still be misclassified, but this would again be non-differential and more likely to attenuate odds ratio towards null.

5.2.1.4 Confounding bias and effect modification

Important known confounders (as illustrated by the DAG Figure 3.2 in Chapter 3) were measured and adjusted for in the analysis. Confounding bias is thus minimized. Nevertheless, we cannot rule

out the possibility of unmeasured and unknown variables that may confound the relationship between HPV-16 viral load during pregnancy and preterm birth. There may thus be possibility of residual confounding bias. It is important to note that the propensity score used for IPTW included confounders (maternal age, ethnicity, education, smoking and alcohol consumption and history of cervical intraepithelial neoplasia treatment) but also variables associated to the outcome only (and excluding those only associated with exposure). This was done in order to increase precision of estimates (with lower variance) without increasing the risk of amplification bias [135-137, 140]. Therefore, variables only associated to the outcome (history of preterm birth, and gestational diabetes) were also included in our calculation of propensity scores. Moreover, our sample included women that already had the HPV infection, and that did not have many known risk factors for the outcome. Although the control for cofounding was done rigorously in our study, there is always a possibility of residual confounding.

There might also be error of classification when measuring confounders. Some confounders were self-reported, and therefore may be prone to recall and social desirability bias (especially when reporting variables related to health behavior). The question of recall bias may arise when reporting tobacco and alcohol consumption, as participants are asked to report their consumption not only during, but also prior to their pregnancy. However, this bias seems unlikely as participants are asked to recall behavior that happened in the recent past, and because there is no reason to think that either (exposed, or non-exposed) group would have a greater memory of past health behavior than another. If recall bias did occur, then tobacco and alcohol consumption may be both under- or overestimated. In case of underestimation of these confounders, the adjusted ORs would be overestimated, and vice versa. Social desirability bias is also possible when reporting these two variables. Indeed, participants may report lower levels of tobacco and alcohol consumption than actuality. The greatest impact may be with tobacco consumption. Underestimation of either confounder would overestimate the adjusted OR. Residual confounding bias is therefore possible because of measurement error.

The presence of effect modification is difficult to determine as little is known about the relationship between HPV-16 viral loads and preterm birth. One variable that may perhaps act as a modifier in this relationship is tobacco consumption. Given that increased smoking have been associated with higher viral loads (although not of HPV infection) [141], perhaps HPV-16 viral loads may be

amplified with smoking. However, we did not have the power to analyse the impact of smoking or other variable as an effect modifier in our study.

5.2.2 External validity

Findings may not be generalizable to all women infected with HPV-16 in different settings as preterm births can have several phenotypes (depending on maternal, fetal or placental conditions) [142] and the impact of HPV-16 viral loads may differ from one phenotype to another. HPV-16 viral loads may be more likely to be associated to spontaneous preterm births rather than induced ones as induced preterm births generally occur when there may be a danger to maternal or fetus' lives, and this may happen independently of HPV status. It is also difficult to generalize results to women from low-resource countries with limited health care access. Finally, findings may not be generalized to women with higher risk of preterm birth that were excluded in our analysis, including those with history of cervico-isthmic incompetency, as well as those with multiple pregnancies (twins or more).

5.3 Public health impact and future research

This study is the first to date to explore the effect of HPV-16 viral load during pregnancy and preterm birth. Findings suggest that high HPV-16 viral loads during pregnancy is strongly associated with preterm birth. Our findings reinforce the biological plausibility of the link between HPV-16 and preterm birth.

Of course, other large studies are needed to confirm this association. It would be interesting to also look at the association between HPV-16 viral load during pregnancy and the types of preterm birth based on weeks of gestation (early, late, etc.). Gaining a better understanding of the association between HPV-16 according to the type of preterm would be important because the impact of HPV-16 on early preterm birth is associated with higher morbidity and mortality. We were not able to conduct stratified analysis by weeks of gestation due to our small sample size, and because all women in our sample experienced late preterm births. Yet, our findings have great impact as late preterm births represent a substantial part of all preterm birth costs [143] as they are much more frequent than births before 34 weeks of gestation. Late term infants are also still more at risk for neonatal morbidity and mortality compared to full term infants [143]. It would be interesting also to look at the impact of smoking as a potential effect modification in this relationship in future studies.

Preterm birth is one of the leading causes of death among infants and establishing HPV-16 as another risk factor is very important especially because it can be prevented through adequate vaccination. In the light of the results of this study and the current literature, it is quite plausible to think that mass vaccination against HPV-16 deployed throughout the world will have a very significant impact on the prevention of preterm birth. Indeed, our results provide another argument in favor of vaccination against HPV to significantly reduce burdens brought on by preterm births globally.

5.4 Conclusion

This thesis aimed to better understand the role of HPV-16 during pregnancy in preterm birth. Our study found a significant association between HPV-16 viral load during pregnancy and preterm birth. Given that HPV is the most common genital infection among unvaccinated women, and that HPV-16 is the most frequent and persistent genotype, understanding its role in adverse pregnancy outcomes, especially in one as common as preterm birth, is crucial. Findings of this thesis suggest a potential role of HPV viral load in preterm birth, consequently opening new avenues of research for understanding idiopathic preterm birth. Finally, given the potential role of HPV-16 in the burden of preterm births, findings suggest that HPV mass vaccination may have an important impact at reducing burdens brought on preterm birth globally, one of the leading causes of infant mortality and morbidity in the world.

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Annex I. Sociodemographic questionnaire at first trimester

1- Critères d'éligibilité au projet HERITAGE: vous devez répondre OUI à toutes les questions:

		Oui	Non
1.1	Participante doit avoir 18 ans et plus au recrutement	☐	☐
1.2	Participante doit être enceinte et entre 6 et 14 de semaine de gestation	☐	☐
1.3	Participante doit accoucher dans un site participant	☐	☐
1.4	Participante doit pouvoir comprendre et signer un formulaire de consentement	☐	☐
1.5	Participante doit parler couramment le français ou l'anglais	☐	☐

2- CARACTÉRISTIQUES SOCIODÉMOGRAPHIQUES:

2.1 Date de recrutement: |_ _|_ _| / |_ _|_ _|_ _| / |_ _|_ _|_ _|_ _|
J J M M M A A A A

2.2 Date de naissance: |_ _|_ _| / |_ _|_ _|_ _| / |_ _|_ _|_ _|_ _|
J J M M M A A A A

2.3 Âge gestationnel au recrutement: ☐☐ nes jours/7 ☐

2.3.1 Date de vos dernières menstruations : |_ _|_ _| / |_ _|_ _|_ _| / |_ _|_ _|_ _|_ _| (premier jour de vos menstruations)
J J M M M A A A A

2.4 Origine ethnique: Les gens au Canada proviennent de divers groupes raciaux ou culturels. Vous appartenez peut-être à plusieurs des groupes suivants. Êtes-vous… *(SVP encerclez toutes les réponses possibles)*

1 = Blanc 2 = Latino-américain 3 = Africain 4 = Afro-américain
5 = Amérindien / people autochtone
6 = Asiatique de l'est (ex. Chinois, Japonais, Vietnamien, Cambodgien, Malaysien, Laotien, Indonésien, etc.)
7 = Sud-asiatique (ex. Indien de l'est, Pakistanais, Punjabi, Sri-Lankais, etc.)
8 = Arabe / asiatique occidental (ex.. Arménien, Égyptien, Iranien, Libanais, Marocain)
9 = Autre, spécifiez:_____
10 = Ne sait pas 11 = Refuse de répondre

2.5 État civil: SVP encerclez la bonne réponse

1 = Mariée 2 = Veuve 3 = Divorcée 4 = Séparée 5= Célibataire (jamais mariée)
6= Conjointe de fait ou vivant avec un partenaire
7 = Autre, spécifiez:_____
8 = Ne sait pas 9 = Refuse de répondre

2.6 Nombre d'années de scolarité complétées: ans ☐☐

SVP encerclez la bonne réponse

1 = Université 2 = Études Post-secondaire (CEGEP) 3 = Secondaire
4 = Professionnel

5 = Élémentaire 6 = Autre, spécifiez:_____
7 = Ne sait pas 8 = Refuse de répondre

2.7 Revenu annuel approximatif de votre ménage avant imposition, en dollars canadiens *(incluant le revenu de votre partenaire, et d'autres sources de revenu, ex. aide financière de la famille ou des amis).* SVP encerclez la bonne réponse

1 = Moins de 5,000$ 2 = 5,001$ - 10,000$ 3 = 10,001$ - 15,000$
4 = 15,001$ - 20,000$ 5 = 20,001$ - 30,000$ 6 = 30,001$ - 40,000$
7 = 40,001$ - 50,000$ 8 = 50,001$ - 60,000$ 9 = 60,001$ - 80,000$
10 = 80,001$ - 100,000$ 11 = ≥ 100,000$
12 = Ne sait pas 13 = Refuse de répondre

2.7.1 Combien de personnes vivent de ce revenu (incluant les enfants)? ☐☐

2.8 Travaillez-vous présentement? 1 = Oui 2 = Non

2.8.1 Si oui, spécifiez votre emploi:_____

2.8.1.1 Temps plein: 1 = Oui 2 = Non

2.8.1.2 Temps partiel: 1 = Oui 2 = Non

2.8.1.3 Combien d'heures/semaine: ☐☐

2.8.2 Si non, spécifiez:

2.8.2.1 Sans emploi: 1 = Oui 2 = Non

2.8.2.2 Étudiante: 1 = Oui 2 = Non

2.8.2.3 Femme au foyer : 1 = Oui 2 = Non

2.8.3 Autre, spécifiez:_____

2.9 Combien de grossesses avez-vous eues, quelle que soit son issue, en incluant la grossesse actuelle?

2.10 Combien d'enfants avez-vous eu? ☐☐

2.11 Âge à la première grossesse ☐☐

3. ANTÉCÉDENTS MÉDICAUX

3.1 Avez-vous déjà été vaccinée pour le virus du papillome humain (VPH)?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.1.1 Si oui: date approximative: mmm aaaa ☐☐☐ ☐☐☐☐

3.1.2 Veuillez indiquer le nom du vaccin que vous avez reçu:

1 = Gardasil (Quadrivalent) (4 types)

2 = Cervarix (Bivalent) (2 types)

3.2 Avez-vous déjà eu un test de VPH? 1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.2.1 Si oui: date approximative: mmm aaaa

3.2.2 Connaissez-vous le résultat? 1 = Oui 2 = Non 3 = Ne sai[illegible]se ☐☐☐ ☐☐☐☐

3.2.2.1 Si oui: 1 = Positif 2 = Négatif

3.3 Quelle est la date (réelle ou approximative) de votre dernier test Pap?

1 = jj mmm aaaa ☐☐ ☐☐☐ ☐☐☐☐

2 = Ne sait pas

3 = Refuse de répondre

3.3.1 Lieu où ce test Pap a eu lieu :_____

_____

_____

_____

_____

2 = Ne sait pas

3 = Refuse de répondre

3.4 Avant votre grossesse, avez-vous eu un test Pap anormal?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.4.1 Si oui: date approximative ☐☐☐ ☐☐☐☐

3.4.2 Connaissez-vous le résultat du test Pap?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.3.2.1 Si oui, spécifiez :_____

3.4.3 Avez-vous déjà subi une colposcopie?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.3.3.1 Si oui, avez-vous eu une biopsie? 1 = Oui 2 = Non

3.3.3.2 Si oui, Connaissez-vous le résultat de la biopsie?_____1 = Non

3.5 Avez-vous déjà eu des condylomes (verrues) au niveau génital?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.5.1 Si oui: date approximative: mmm aaaa ☐☐☐ ☐☐☐☐

3.5.2 Avez-vous reçu un traitement pour éliminer les condylomes? 1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

3.5.2.1 Si oui, vous souvenez-vous du nom du médicament?_____ 1 = Non

3.6. Combien de temps cela vous a-t-il pris pour devenir Enceinte de votre grossesse actuelle? Mois ☐☐☐

3.7 Est-ce qu'un médecin ou autre professionnel de la santé a diagnostiqué chez vous et/ou chez votre partenaire un problème de fertilité? 1 = Oui 2 = Non

Si oui, indiquez la raison : (cochez toutes les réponses qui s'appliquent)

Causes féminines :

3.7.1 Facteurs tubaires (trompes bloquées ou dysfonctionnelles)

3.7.2 Dysovulation / anovulation

3.7.3 PCOS (syndrome des ovaires polykystiques)

3.7.4 Endométriose

3.7.5 Réserve ovarienne réduite

3.7.6 Insuffisance ovarienne prématurée (spontanée ou après traitement)

3.7.7 Anomalie du mucus cervical (mucus cervical hostile, insuffisance du mucus cervical)

3.7.8 Malformation de l'utérus

3.7.9 Autre cause féminine, veuillez préciser : _____.

3.7.10 Raison inconnue ☐

Causes masculines :

3.7.11 Absence de sperme

3.7.12 Incapacité à déposer le sperme (dysfonction érectile/éjaculatoire)

3.7.13 Anomalie des spermatozoïdes (peu de spermatozoïdes ou spermatozoïdes de mauvaise qualité)

3.7.14 Autre cause masculine, veuillez préciser : _____.

3.7.15 Raison inconnue

3.8 Avez-vous eu recours à des méthodes de procréation assistée ou avez-vous utilisé des médicaments déclenchant l'ovulation afin d'être enceinte de votre grossesse actuelle?

1 = Oui 2 = Non, (Si non, allez à la section 4) Si oui, précisez, (cochez toutes les réponses qui s'appliquent) 3 = Refuse de répondre

3.8.1 Stimulation ovarienne : 1 = Oui 2 = Non Si oui, précisez (cochez toutes les réponses qui s'appliquent)

3.8.1.1 Stimulation ovarienne par voie orale (ex. Clomid ®, Serophene ®)

3.8.1.2 Stimulation ovarienne par voie injectable (ex. Repronex ®, Follistim ®, Gonal-F ®, Menopur ®, Bravelle ®)

3.8.1.3 Médicament injectable pour déclencher l'ovulation (ex. Ovidrel ®, Profasi ®, Pregnyl ®, Novarel ®, hCG-endo ®)

3.8.1.4 Autre médicament facilitant la conception (ex. Metformin ®, Lupron ®)

3.8.2. Insémination intra-utérine (IIU) : 1 = Oui 2 = Non Si oui, précisez :

3.8.2.1 Avec sperme du partenaire

3.8.2.2 Avec sperme du donneur

3.8.3. Fécondation in-vitro (FIV) : 1 = Oui 2 = Non Si oui précisez :

3.8.3.1 Avec ICSI (Injection intra-cytoplasmique du spermatozoïde)

3.8.3.2 Sans ICSI

3.8.4. Maturation In Vitro (MIV) 1 = Oui 2 = Non

3.8.5. Autres : 1 = Oui 2 = Non Si oui précisez (cochez toutes les réponses qui s'appliquent)

3.8.5.1 Transfert d'embryons congelés (TEC)

3.8.5.2 Don de sperme

3.8.5.3 Don d'ovules

3.8.5.4 Don d'embryons

3.8.5.5 Éclosion embryonnaire assistée

4. ACTIVITÉ SEXUELLE

4.1 Âge à la première relation sexuelle (avec pénétration vaginale) ☐☐

☐ Ne sait pas ☐ Refuse de répondre

4.2 Nombre de partenaires sexuels au cours de votre vie ☐☐☐

☐ Ne sait pas ☐ Refuse de répondre

4.3 Nombre de partenaires sexuels au cours de la dernière année ☐☐☐

☐ Ne sait pas ☐ Refuse de répondre

4.4 Parmi les partenaires sexuels que vous avez eus au cours de la dernière année, combien d'entre eux étaient des NOUVEAUX partenaires ? ☐☐☐

☐ Ne sait pas ☐ Refuse de répondre

4.5 Avez-vous eu une relation sexuelle avec pénétration dans les dernières 24 heures (24 heures avant la prise de votre frottis vaginal)? 1 = Oui 2 = Non

5. TABAGISME

Je vais maintenant vous poser des questions sur la consommation de cigarettes. Par cigarettes, nous entendons les cigarettes prêtes à l'usage et celles que vous roulez vous-même, sauf les cigares, les cigarillos, la marijuana et la pipe.

5.1 Avez-vous déjà fumé?

1 = Oui 2 = Non 3 = Refuse de répondre

Si non ou refuse de répondre, passez à la question 6

5.1.2 Si oui, avant votre grossesse, combien de jours avez-vous fumé par

1 = semaine ☐☐ 3 = Ne ☐☐ 4 = Refuse de répondre

5.1.2.1 Les jours ☐☐ez fumé, combien d☐☐s avez-vous fu☐☐

1 = jour 2 = semaine 3 = mois 4 = Ne sait pas 5 = Refuse de répondre

5.1.3 Depuis le début de votre grossesse, avez-vous fumé?

1 = Oui 2 = No 3 = Refuse de répondre

Si non, passez à la question 6

5.1.3.1 Si oui, combien de jours avez-vous fumé par

1 = jour ☐☐emaine 3☐☐ 4 = Ne s☐☐ Refuse de répondre

5.1.3.2 Les jours ou vous fumez, combine☐ cigarettes fumez-vous par

1 = jour ☐☐emaine 3☐☐ 4 = Ne ☐☐ = Refuse de répondre

6. ALCOOL

J'aimerais maintenant vous poser quelques questions sur votre consommation d'alcool. Lorsqu'on parle d'un « verre », on entend : - une bouteille ou une canette de bière, ou un verre de bière en fût, un verre de vin ou de boisson rafraîchissante au vin (« cooler »), un verre ou un cocktail contenant une once et demie de spiritueux.

6.1 L'année précédant votre grossesse, combien de fois avez-vous consommé des boissons alcoolisées?

SVP encerclez la bonne réponse

1 = Tous les jours 2 = 4 à 6 fois par semaine 3 = 2 à 3 fois par semaine 4 = Une fois par semaine 5 = 2 à 3 fois par mois 6 = Une fois par mois 7 = Moins d'une fois par mois 8 = Jamais (Passez à la question 6.7) 9 = Ne sait pas 10 = Refuse de répondre

6.2 Depuis le début de votre grossesse, combien de fois avez-vous consommé 5 boissons alcoolisées ou plus en une même occasion?

1 = Plus d'une fois par semaine 2 = Une fois par semaine 3 = 2 à 3 fois par mois 4 = Une fois par mois 5 = Moins d'une fois par mois 6 = Jamais 7 = Ne sait pas 8 = Refuse de répondre

6.3 Depuis le début de votre grossesse, c.-à-d. depuis la première journée de vos dernières menstruations, votre consommation d'alcool a-t-elle été supérieure, à peu près la même ou inférieure à la quantité que vous consommiez habituellement?

1 = Supérieure 2 = À peu près la même 3 = Inférieure 4 = Jamais (Passez à la question 6.7) 5 = Ne sait pas 6 = Refuse de répondre

6.4. Depuis le début de votre grossesse, à quelle fréquence avez-vous consommé des boissons alcoolisées ?

1 = ☐☐ Nombre de jours par semaine 2 = ☐☐ Nombre de jours par mois

3 = ☐☐ Nombre total de jours depuis le début de la grossesse
4 = Ne sait pas 5 = Refuse de répondre

6.5 Les journées où vous avez consommé de l'alcool, depuis le début de votre grossesse, combien de verres buviez-vous habituellement ?

1 = ☐☐ rres 2 = Ne sait pas 3 = Refuse de répondre

6.6 Depuis le début de votre grossesse, combien de fois avez-vous consommé 5 boissons alcoolisées ou plus en une seule occasion ?

1 = ☐☐ Nombre de jours par semaine 2 = Nom☐☐s par mois

3 = ☐☐ Nombre total de jours depuis le début de la grossesse
4 = Ne sait pas 5 = Refuse de répondre

6.7 Je comprends que vous ne consommez généralement pas d'alcool, car vous êtes enceinte, mais vous est-il arrivé de consommer de l'alcool au cours d'occasions spéciales, tel des anniversaires ou rassemblements familiaux?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

6.7.1 Si oui, combien de consommations d'alcool avez-vous prises lors de ces occasions?

1 = ☐☐ 2 = Refuse de répondre 3 = N☐☐

6.7.2 Combien de fois est-ce arrivé? 1 = occasions
2 = Ne sait pas 3 = Refuse de répondre

7. DROGUES ILLICITES

Je vais vous poser quelques questions au sujet de la consommation de drogues. Encore une fois, j'aimerais vous rappeler que tout ce que vous dites demeurera strictement confidentiel.

7.1 Avez-vous déjà pris ou essayé des drogues (ex.. marijuana, cannabis, haschisch, cocaïne, speed, hallucinogènes, LSD, PCP, etc.)?

1= Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non**, **Ne sait pas** ou **Refuse de répondre**: *passez à la fin à la signature*

7.2 Avez-vous déjà pris ou essayé de la marijuana, du cannabis ou du haschisch?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 7.3*

7.2.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = Jo☐☐is

3 = ☐☐ Nombre total de jours pendant la grossesse
4 = Ne sait pas 5 = Refuse de répondre

7.3 Avez-vous déjà pris ou essayé de la cocaïne ou du crack?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 7.4*

7.3.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = Jo☐☐is

3 = ☐☐ Nombre total de jours pendant la grossesse
4 = Ne sait pas 5 = Refuse de répondre

7.4 Avez-vous déjà pris ou essayé du speed (amphétamines)?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 7.5*

7.4.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = Jo☐☐is

3 = ☐☐ Nombre total de jours pendant la grossesse

4 = Ne sait pas 5 = Refuse de répondre

7.5 Avez-vous déjà pris ou essayé des hallucinogènes tels que le LSD, le PCP, l'ecstasy (MDMA), la mescaline, le buvard ou autres drogues semblables?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

➢ Si **Non:** *passez à Q 7.6*

7.5.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = ☐☐ Jours par mois

3 = ☐☐ Nombre total de jours pendant la grossesse

4 = Ne sait pas 5 = Refuse de répondre

7.6 Avez-vous déjà inhalé de la colle, de l'essence ou d'autres solvants?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

➢ Si **Non:** passez à *Q 7.7*

7.6.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = ☐☐ Jours par mois

3 = ☐☐ Nombre total de jours pendant la grossesse

4 = Ne sait pas 5 = Refuse de répondre

7.7 Avez-vous déjà pris ou essayé de l'héroïne?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

➢ Si **Non:** passez à *la signature*

7.7.1 Si oui, combien de fois depuis que vous êtes devenue enceinte, c'est-à-dire depuis la première journée de vos dernières menstruations? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = ☐☐ Jours par mois

3 = ☐☐ Nombre total de jours pendant la grossesse

4 = Ne sait pas 5 = Refuse de répondre

SECTION RÉSERVÉE

1. Initiales:_____ 2. Durée de l'entretien _____min

3. Signature _____4. Date ☐☐ **I** ☐☐☐ ☐☐☐☐ **A**

A A A

Aide-mémoire: Information regardant cette visite doit être à la coordonnatrice du projet VPH (HERITAGE), Tel : 514-345-4931, ext. 7031 ou pagette: 514-415-7600

Transmission périnatale du virus du papillome humain (VPH) et persistance du VPH chez les enfants (projet HERITAGE: une cohorte prospective) Q1 recrutement, projet HERITAGE.

Annex II. Sociodemographic questionnaire at birth

1. ANTÉCÉDENTS MÉDICAUX

1.1 Avez-vous été vaccinée pour le VPH en cours de grossesse?

1 =Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.1.1 Si oui: date approximative: mmm aaaa | | | | | | | |

1.1.2 Nom du vaccin:

1 = Gardasil (Quadrivalent) (4 types)

2 = Cervarix (Bivalent) (2 types)

1.2 Avez-vous eu un test pour le VPH depuis le recrutement?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.2.1 Si oui: date approximative: mmm aaaa | | | | | | | |

1.2.2 Connaissez-vous le résultat?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.2.2.1 Si oui: 1 = Positif 2 = Négatif

1.3 Depuis votre recrutement, avez-vous eu un test Pap anormal?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.3.1 Si oui, date approximative : mmm aaaa | | | | | | | |

1.3.2 Lieu où ce test Pap a eu lieu : _____

2 = Ne sait pas 3 = Refuse de répondre

1.3.3 Connaissez-vous le résultat du test Pap?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.3.3.1 Si oui, spécifiez_____

1.3.4 Avez-vous eu une colposcopie?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.3.4.1 Si oui, avez-vous eu une biopsie? 1 = Oui 2 = Non

1.3.4.2 Si oui, connaissez-vous le résultat de la biopsie?_____1 =Non

1.4 Depuis le recrutement, avez-vous eu des condylomes (verrues) au niveau génital?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.4.1 Si oui, date approximative: mmm aaaaa | | | | | | | |

1.4.2 Avez-vous reçu un traitement pour éliminer les condylomes?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

1.4.2.1 Si oui, vous souvenez-vous du nom du médicament?_____ 1 = Non

2. ACTIVITÉ SEXUELLE

2.1 Nombre de partenaires sexuels durant votre grossesse? | | |

1 = Ne sait pas 2 = Refuse de répondre

2.2 Nombre de nouveaux partenaires pendant votre grossesse? | | |

1 = Ne sait pas 2 = Refuse de répondre

2.3 Combien de fois par semaine en moyenne avez-vous eu des relations sexuelles pendant la grossesse? | | |

1 = Ne sait pas 2 = Refuse de répondre

2.4 Avez-vous eu une relation sexuelle avec pénétration dans les 24 dernières heures (24 heures avant la prise de votre frottis vaginal)

1 = Oui 2 = Non

3. TABAGISME

Je vais maintenant vous poser des questions sur la consommation de cigarettes. Par cigarettes, nous entendons les cigarettes prêtes à l'usage et celles que vous roulez vous-même, sauf les cigares, les cigarillos, la marijuana et la pipe.

3.1 Depuis votre recrutement, avez-vous fumé?

1 = Oui 2 = Non 3 = Refuse de répondre

Si non, passez à la question 4

3.1.1 Si oui, combien de jours avez-vous fumé par?

1 = semaine ☐☐ 2 = mois ☐☐ 3 = Ne sait pas 4 = Refuse de répondre

3.1.2 Les jours où vous ☐☐ mbien de cigarettes ☐☐ us par?

1 = jour ☐☐ 2 = semaine ☐☐ 3 = mois ☐☐ 4 = ne sait pas

5=Refuse d☐☐

4. ALCOOL

J'aimerais maintenant vous poser quelques questions sur votre consommation d'alcool. Lorsqu'on parle d'un « verre », on entend : - une bouteille ou une canette de bière, ou un verre de bière en fût, un verre de vin ou de boisson rafraîchissante au vin (« cooler »), un verre ou un cocktail contenant une once et demie de spiritueux.

4.1 Depuis votre recrutement, votre consommation d'alcool a-t-elle été supérieure, à peu près la même ou inférieure à la quantité que vous consommiez habituellement?

1 = Supérieure 2 = À peu près la même 3 = Inférieure 4 = Jamais (Passez à la question 4.5)

5 = Ne sait pas 6 = Refuse de répondre

4.2 Depuis votre recrutement, combien de fois avez-vous consommé des boissons alcoolisées?

1 = ☐☐ Nombre de jours par semaine ou 2 = ☐☐ Nombre de jours par mois ou

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

4.3 Depuis votre recrutement, les journées où vous avez consommé de l'alcool, combien de verres buviez-vous habituellement ?

1 = verres 2 = Ne sait pas 3 = Refuse de répondre

4.4 Depuis votre recrutement, combien de fois avez-vous consommé 5 boissons alcoolisées ou plus en une seule occasion ?

1 = ☐☐ Nombre de jours par semaine ou 2 = ☐☐ Nombre de jours par mois ou

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

4.5 Je comprends que vous ne consommez généralement pas d'alcool, car vous êtes enceinte, mais vous est-il arrivé de consommer de l'alcool au cours d'occasions spéciales, tel des anniversaires ou rassemblements familiaux?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

4.5.1 Si oui, combien de consommations d'alcool avez-vous prises lors de ces occasions?

1 = ☐☐ verres 2 = Refuse de répondre 3 = Ne sait pas

4.5.2 Comb☐☐ est-ce arrivé? 1 = ☐☐ occasions 2 = Ne sait pas 3=Refuse de répondre

5. DROGUES ILLICITES

Je vais vous poser quelques questions au sujet de la consommation de drogues. Encore une fois, j'aimerais vous rappeler que tout ce que vous dites demeurera strictement confidentiel.

5.1 Avez-vous pris ou essayé des drogues (ex. marijuana, cannabis, haschisch, cocaïne, speed, hallucinogènes, LSD, PCP, etc.)?

1= Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non**, **Ne sait pas** ou **Refuse de répondre**: *passez à la fin à la signature*

5.2 Avez-vous pris ou essayé de la marijuana, du cannabis ou du haschisch?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 5.3*

5.2.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2 = ☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

5.3 Avez-vous pris ou essayé de la cocaïne ou du crack?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 5.4*

5.3.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

5.4 Avez-vous pris ou essayé du speed (amphétamines)?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 5.5*

7.4.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

5.5 Avez-vous pris ou essayé des hallucinogènes tels que le LSD, le PCP, l'ecstasy (MDMA), la mescaline, le buvard ou autres drogues semblables?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** *passez à Q 5.6*

5.5.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

5.6 Avez-vous inhalé de la colle, de l'essence ou d'autres solvants?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** passez à *Q 5.7*

5.6.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

5.7 Avez-vous pris ou essayé de l'héroïne?

1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

- Si **Non:** passez à *la signature*

5.7.1 Si oui, combien de fois depuis votre recrutement? (Inscrire la fréquence ci-dessous)

1 = ☐☐ Jours par semaine 2☐☐ Jours par mois

3 = ☐☐ Nombre total de jours depuis votre recrutement

4 = Ne sait pas 5 = Refuse de répondre

QUESTION BÉBÉ

6. Date de naissance : ☐☐ ☐☐☐ ☐☐☐☐

J J M M M A A A A

7. Sexe : F☐ M☐

8. Âge gestationnel : ☐☐ s jours /7 ☐

9. Poids (en kg) : ☐☐ ☐☐☐ (l'enfant doit être nu et sans couche)

10. Taille (en cm & mm) : ☐☐ ☐☐☐

11. Allez-vous allaiter votre enfant : 1 = Oui 2 = Non 3 = Ne sait pas 4 = Refuse de répondre

SECTION RÉSERVÉE

1. Initiales:_____ 2. Durée de l'entretien _____min

3. Signature _____4. Date ☐☐ **D** ☐☐☐ ☐☐☐☐ **A**

A A A

Aide-mémoire: Information regardant cette visite doit être à la coordonnatrice du projet VPH (HERITAGE), Tel : 514-345-4931, ext. 7031 ou pagette: 514-415-7600

Transmission périnatale du virus du papillome humain (VPH) et persistance du VPH chez les enfants (projet HERITAGE: une cohorte prospective) Q2 accouchement, projet HERITAGE..

Annex III. Case report form

SECTION MÈRE

1. 1.1 Le diagnostic d'hypertension gravidique a-t-il été posé par le médecin de la participante **avant** son admission pour l'accouchement (après 20 semaines) ?

   1 = Oui 2 = Non

   1.1.2 Date du premier diagnostic (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   1.2 Le diagnostic d'hypertension gravidique a-t-il été posé par le médecin **au cours de ou après** l'admission pour accouchement?

   1= Oui 2 = Non

   1.2.1 Date du diagnostic (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   1.3 Le diagnostic de diabète de grossesse a-t-il été posé par le médecin au cours de la grossesse?

   1= Oui 2= Non

   1.3.1 Date du diagnostic (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   1.4 Le diagnostic de pré-éclampsie a-t-il été posé par le médecin au cours de la grossesse?

   1= Oui 2= Non

   1.4.1 Date du diagnostic (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

ISSUES DE GROSSESSE

2. 2.1 Quel a été l'issue de la grossesse? (Encerclez l'issue)

   1. Naissance vivante 2. Fausse couche/avortement spontané

   3. Avortement électif 4. Grossesse molaire

   5. Interruption thérapeutique 6. Mort-né (si oui répondre Q 19, 20, 21)

   2.2 Date (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   2.3 Âge gestationnel : ☐☐ sem ☐☐ jrs

SECTION TRAVAIL ET ACCOUCHEMENT

3. Admission pour accouchement :

   3.1 Date (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   3.2 Heure (sur 24h) : _ _ h _ _ min

4. La participante a-t-elle débuté son travail? (Note l'induction n'inclut pas la stimulation d'un travail déjà en cours par Ocytocine)

   1 = Aucun travail (ex : césarienne planifiée sans travail) Allez à la question 5

   2 = Spontané, Complétez les questions 4.1 et 4.2

   3 = Induit, Complétez les questions 4.3 à 4.6

   4.1 Date de début : (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   4.2 Heure de début (sur 24h) : _ _ h _ _ min

   4.3 Méthode maturation du col : 1 = Oui 2 = Non

   4.3.1 Si oui, précisez (plusieurs réponses possibles)

   1 = Ballonnet de Foley 2 = Tiges laminaires 3 = Cervidil

   4 = Prévidil 5 = Prostin

   6 = autres, précisez :_____

   4.4 Méthode d'induction (plusieurs réponses possibles)

   1 = Ocytocine 2 = Prostin 3 = Rupture artificielle des membranes

   4 = Misoprostol

   5 = Autres, précisez :_____

   4.5 Date de début de l'induction : (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

   4.6 Heure du début de l'induction (sur 24h) : _ _ h _ _ min

5. 5.1 Rupture des membranes :

1 = Spontanée 2 = Artificielle 3 = N/A (césarienne planifiée)

5.1.1 Date de la rupture des membranes : (jj/mmm/aaaa) : _ _ / _ _ / _ _

5.1.2 Heure de la rupture des membranes (sur 24h) : _ _ h _ _ min

5.2 Épisiotomie : 1 = Oui 2 = Non

NAISSANCE DU BÉBÉ

6. Date : (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

7. Heure (sur 24h) : _ _ h _ _ min

8. Présentation du bébé (si césarienne, référez au protocole opératoire)

1 = Céphalique 2 = Siège 3 = Autre 4 = Inconnue

9. Type d'accouchement (une seule réponse possible; indiquez la méthode qui a permis de sortir le bébé)

1 = Spontané 2 = Césarienne, précisez 3 = Vaginal – ventouse 4 = Vaginal – forceps

Si césarienne, précisez l' (les) indication(s) : (plusieurs réponses possibles)

a. Dystocie b. mauvaise présentation c. Suspicion de souffrance fœtale basée sur un tracé anormal d. Saignements e. suspicion de macrosomie

f. pathologie hypertensive sévère (incluant prééclampsie) g. Placenta abruption

h. placenta prævia i. Échec de ventouse ou forceps j. échec d'induction

k. antécédent de césarienne l. demande maternelle

m. autre :_____

10. Abruption Placentaire (Décollement placentaire)

1 = Oui 2 = Non

CONDITION MATERNELLE

11. 11.1 Dépistage du Streptocoque B effectué

1 = Oui 2 = Non

11.1.1 Si oui, précisez le résultat :_____

11.2 Infection maternelle avant l'accouchement (autre que chorioamnionite)

1 = 0ui 2 = Non

11.2.1 Précisez laquelle :_____

INFORMATION BÉBÉ

12. Poids à la naissance : _ _ _ _ g

13. Taille à la naissance : _ _ . _ cm

14. Périmètre crânien : _ _ . _ cm

15. Sexe : 1 = fille 2 = garçon

16. Âge gestationnel : _ _ semaines _ jours /7

17. Le bébé présente-t-il une (des) anomalie (s) congénitale (s)?

1 = Oui, précisez 2 = Non, allez à la question 18

17.1.1 Inscrivez le code CDC de cette/ces anomalie(s)

_ _ _ . _ _ _ _ _ _ . _ _ _ _ _ _ . _ _ _ _ _ _ . _ _ _

18 Le bébé présente-il un traumatisme lié à la naissance ?

1 = Oui, précisez 2 = Non, allez à la question 19

18.1 Précisez les résultats :

0=paralysie faciale, 1=paralysie brachiale, 2=fracture clavicule, 3=fracture crâne, 4=hémorragie sous-galéale, 5=lacérations cutanées, 6=blessure colonne vertébrale, 7=blessure organes internes (foie, rate, etc.) 8=céphalohématome, 9=autre_____

19. NÉONATAL

19.1 Si le bébé est vivant, allez à Q 22

19.2 Le bébé est-il décédé à l'hôpital de naissance?

1 = Oui 2 = Non

20. Précisez la date du décès : (jj/mmm/aaaa) : _ _ / _ _ _ / _ _ _ _

21. Y-a-t-il eu autopsie?

1= Oui, allez à la question 21.1 et 21.2 2 = Non, allez à la question 21.3

21.1 Quelle était la cause première du décès selon l'autopsie?

a. prématurité

b. RCIU sévère

c. malformation congénitale

d. asphyxie

e. septicémie

f. trauma à la naissance

g. autre

21.2 Précisez toute autre cause particulière qui aurait pu contribuer au décès selon l'autopsie? _ N/A

_____

21.3 Précisez la cause première du décès

_____

21.4 Précisez toute autre cause de décès : _ N/A

_____

22. Le bébé a-t-il présenté une détresse respiratoire néonatale?

1 = Oui 2 = Non, allez à la question 23

22.1 Si oui, le bébé a-t-il reçu un ou plusieurs des traitements suivants?

0=oxygénothérapie, 1= VANI, 2=VAI

23. Score APGAR

23.1 _ _ (1 min) _ non fait 23.2 _ _ (5 min) _ non fait 23.3 _ _ (10 min) _ non fait

SECTION RÉSERVÉE

1. Initiales:_____ 2. Durée _____min

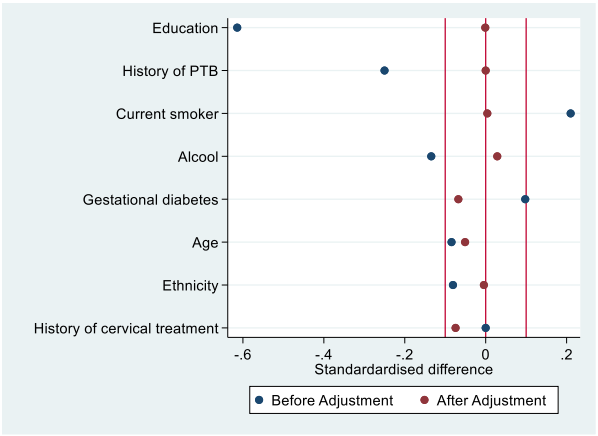
3. Signature _____4. Date

_	_	_	_	_	_	_	_	_	_

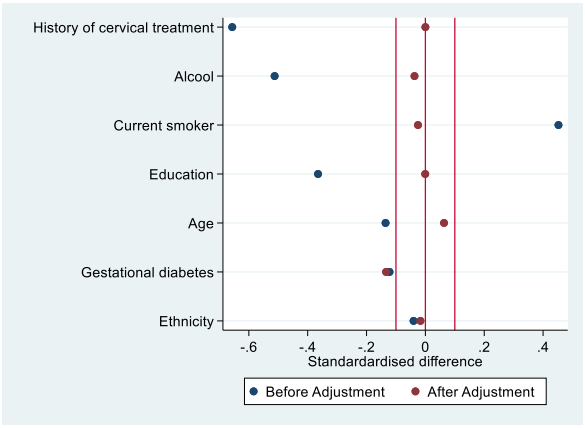
Aide-mémoire: Information regardant cette visite doit être à la coordonnatrice du projet VPH (HERITAGE), Tel : 514-345-4931, ext. 7031 ou pagette: 514-415-7600

Annex IV. Standardised differences of covariates before and after weighting with IPTW for each model

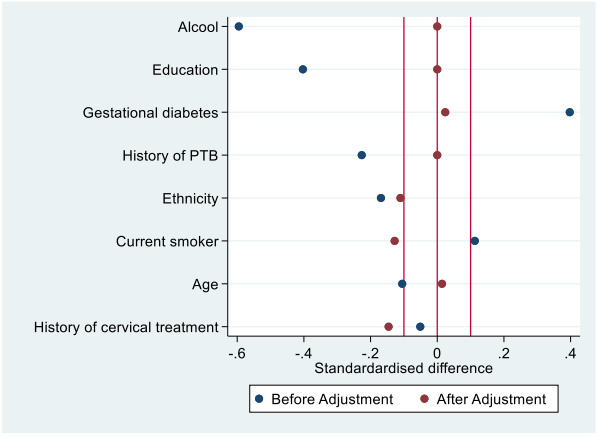
Cutoff = 0.05 copy/cell, first trimester



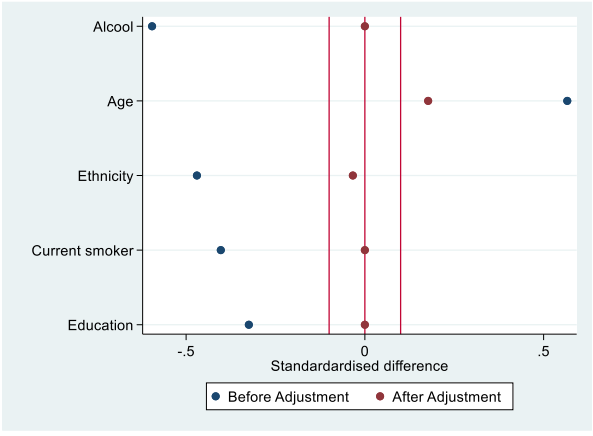
Cutoff =0·05 copy/cell, third trimester



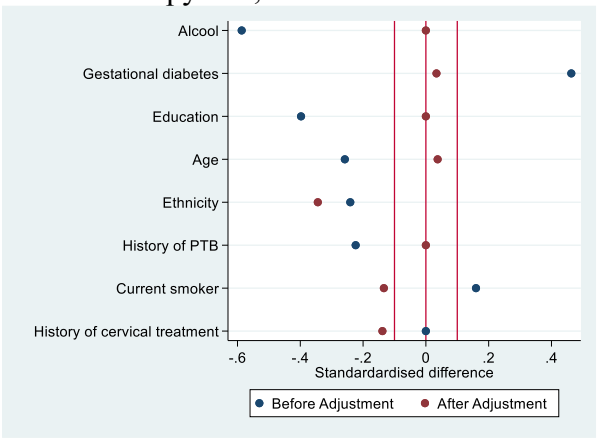
Cutoff= 0.5 copy/cell, first trimester



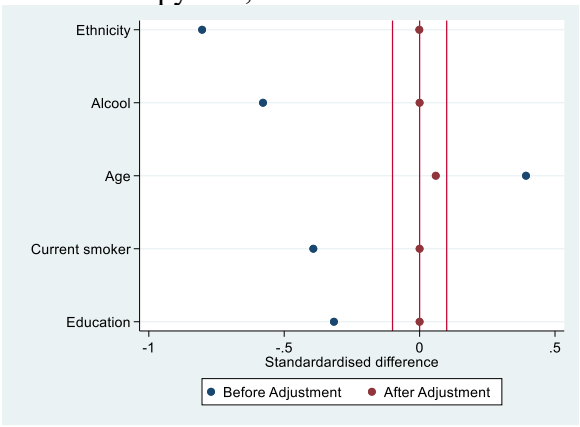
Cutoff= 0.5 copy/cell, third trimester



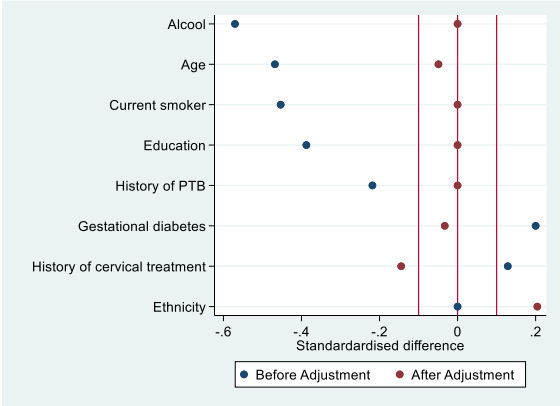
Cuttoff=1 copy/cell, first trimester



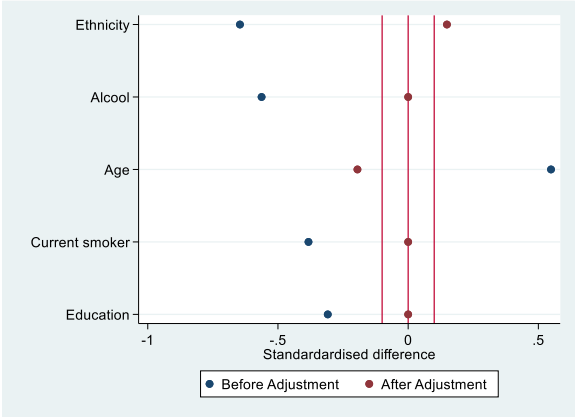
Cutoff=1 copy/cell, third trimester



Cuttoff=2 copies/cell, first trimester



Cutoff=2 copies/cell, third trimester



Annex V. Initial Ethics Approval

Le 12 mars 2010

Madame Helen Trottier
Centre de recherche
Étage A Bloc 7

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

Comité d'éthique de la recherche

OBJET: Titre du projet: Étude pilote de la transmission périnatale du virus du papillome humain (VPH) et persistance du VPH chez l'enfant (HERITAGE)

No. de dossier: 3043

Responsables du projet: Helen Trottier Ph. D., Chercheur responsable au CHU Sainte-Justine et chercheur principal. Collaborateurs: Marie-Hélène Mayrand, M.D., CHUM-St-Luc, Patricia Monnier, M.D., CHU Sainte-Justine, William Fraser, M.D., CHU Sainte-Justine, Ana Maria Carceller, M.D., CHU Sainte-Justine, François Cloutlée, M.D., CHUM-Notre-Dame, Diane Francoeur, M.D., CHU Sainte-Justine

Chère Madame,

Votre projet cité en rubrique a été approuvé par le comité d'éthique de la recherche en date d'aujourd'hui. Vous trouverez ci-joint la lettre d'approbation du Comité, la liste des documents approuvés ainsi que vos formulaires d'information et de consentement estampillés dont nous vous prions de vous servir d'une copie pour distribution.

Tous les projets de recherche impliquant des sujets humains doivent être réexaminés annuellement et la durée de l'approbation de votre projet sera effective jusqu'au **12 mars 2011**. Notez qu'il est de votre responsabilité de soumettre une demande au comité pour que votre projet soit renouvelé avant la date d'expiration mentionnée. Il est également de votre responsabilité d'aviser le comité dans les plus brefs délais de toute modification au projet ainsi que de tout effet secondaire survenu dans le cadre de la présente étude.

Nous vous souhaitons bonne chance dans la réalisation de votre projet et vous prions de recevoir nos meilleures salutations.

Président du Comité d'éthique de la recherche

JMT/nh

3175, Côte-Sainte-Catherine
Montréal (Québec)
H3T 1C5

Annex VI. Ethics Renewal 2022-2023

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

Formulaire de demande de renouvellement annuel de l'approbation d'un projet de recherche

Titre du protocole : **Étude de cohorte prospective de la transmission périnatale du virus du papillome humain (VPH) et persistance du VPH chez l'enfant (HERITAGE)**

Numéro(s) de projet : **2010-265, 3043**

Identifiant Nagano : **3043**

Chercheur principal (au CER Éval) : **Helen Trottier**

Date d'approbation du projet par le CER : **2010-03-12**

Formulaire : **F9H-45402**

Date de dépôt initial du formulaire : **2022-09-08**

Date de dépôt final du formulaire : **2022-09-08**

Statut du formulaire : **Formulaire approuvé**

Décision finale du CÉR

1. **Décision de la demande**
   Approuvé - comité restreint

2. **Commentaires concernant la décision**

   Bonjour,

   Les membres du comité délégué du Comité d'éthique de la recherche du CHU Sainte-Justine ont examiné votre demande de renouvellement de l'approbation éthique de votre projet à leur réunion du 14 octobre 2022. L'approbation éthique de votre projet a été renouvelée par le Comité.

   Les formulaires d'information et de consentement n'ont pas été réévalués puisque votre rapport annuel indique que le recrutement des participants est terminé.

   En vous souhaitant une bonne poursuite de votre projet.

3. **Période couverte par ce renouvellement:**

   Du 14 octobre 2022 au 14 octobre 2023.

4. **Date de la décision**
   2022-10-14

Signature

Samira Akrah
Responsable administrative des projets de recherche en cours
Bureau de l'éthique de la recherche
CHU Sainte-Justine
Pour Geneviève Cardinal, présidente du Comité d'éthique de la recherche

Annex VII. Mini protocol and comments

Background

Preterm birth accounts for nearly two thirds of infant deaths in Canada, where almost 8% of babies are born prematurely each year, many of which suffer from serious health complications and have an increased risk of chronic diseases[1]. Genital infections have been linked to preterm birth [2-4]. HPV is one of the most common sexually transmitted infection: 75% of sexually active Canadians will have HPV at some point in their life [5]. HPV infection has also been associated with adverse pregnancy outcomes such as preterm birth [6-17]. Findings from *in vitro* experiments have suggested that HPV can infect the placenta and cause changes in trophoblast physiology [2, 18-20], inducing cell replication and production of cytokines which in turn could compromise gestation and explain HPV-related pregnancy complications [10]. However, clinical studies have yielded inconsistent results regarding the association between HPV and preterm birth; some have found significant associations [7-10, 13, 15, 16], while others reported no association [19, 21-23], or loss of association after controlling for important confounders [24]. However, most studies did not differentiate between individual HPV genotypes, but instead provided a measure of the presence/absence of HPV or of a cluster of HPV genotypes. The few studies that did consider specific HPV genotypes were not sufficiently powered to reach firm conclusions[19, 21]. A recent study indicates that persistence of HPV 16/18 infection throughout pregnancy may by a key risk factor for preterm birth [9]. Although explaining the relationship between HPV-16 persistence during pregnancy and preterm delivery remains difficult, some answers may lie in the viral load of this infection given that higher viral loads may favor HPV persistence [25, 26].

Objective

The aim of this study is to investigate the association between HPV-16 viral load in pregnant women and preterm birth.

Study design and Methods

Study Design and Participants Data for this study comes from a prospective cohort study on perinatal HPV transmission, called HERITAGE. The HERITAGE cohort included 1050 pregnant women, recruited between 2009-2012 and 2015-2016 from three academic hospitals in Montreal, Quebec, Canada. Participants were eligible for inclusion into HERITAGE if they were 1) at least 18 years of age, 2) pregnant at 14 weeks or earlier of gestation 3) able to provide written consent, and 4) negative for HIV. For this analysis, we will include HERITAGE participants who tested positive for HPV-16 in the first trimester, and who did not have 1) multiple pregnancies (twins or more), 2) spontaneous or induced abortions and 3) a history of cervico-isthmic incompetency with a prophylactic cerclage in the first trimester. We will thus include 49 participants.

Sample and data collection Participants self-collected vaginal samples for genotype-specific HPV DNA testing at the first recruitment visit (1st trimester), and those positive collected an additional sample at the third trimester visit (32 to 35 weeks). Sociodemographic information, medical and sexual history, as well as alcohol and tobacco consumption were collected at both recruitment and follow-up visits.

HPV genotyping and viral load testing Linear array (LA) assay (Roche Diagnostics®) was used for HPV genotyping testing. HPV viral load was measured with this real-time PCR assay that allow for the precise quantitation of HPV DNA over a wide range of concentrations. Results are recorded as copy numbers/cell.

Exposure The exposure is HPV-16 viral load among mothers positive with HPV-16 at first and/or third trimester. The exposed group will include HPV-16 positive participants with high viral load (> 2 copies

of HPV-16/cell), which will be compared to those with lower viral load (<2 copies of HPV-16/cell). Sensitivity analysis will allow to analyse the impact of different threshold for categorization and for the exposure considered continuously. The associations will be measured between viral load measured at visit 1 (n=49 HPV-16 positive) as well for visit 2 (n=35 HPV-16 positive). The association between difference in viral load between the two visits and preterm birth will also be explored.

Outcome The outcome of interest is preterm birth, defined as a birth between 20 weeks and 0 days and 36 weeks and 6 days of gestation. Gestational age was based on date of last menstrual period and confirmed by ultrasound dating in the first trimester. This will be coded as a dichotomous variable.

Statistical analysis The association between HPV-16 viral load and preterm birth will be measured using logistic regression. Odds ratios and 95% CIs will be computed, and confounders will be adjusted for using propensity score with inverse probability treatment weighting (IPTW). Propensity scores will be estimated using known risk factors associated with preterm birth [27-29] including age, urinary tract or other genital infections (bacterial vaginosis, cystitis, active herpetic lesion and non-specified infections), and smoking at enrollment. Tests will be two-sided, and p values will be considered statistically significant at $p< 0.05$. Analysis will be done using Stata/SE version 14.0.

Power Given 49 HPV-16 positive women at visit 1 (18 exposed as having high viral load and 31 non-exposed / low viral load), the minimum detectable odds ratio (OR) would be 5,7 with a 5% alpha level and power of 80%. We will have a power of 30% and 40% to detect ORs of 2.5 and 3, respectively. We acknowledge that the power of the study is limited. Since this association has never been studied, this exploratory study would be a stepping-stone to understanding the overall effect of HPV-16 viral load in preterm birth, and thus, it is worthwhile despite its low power. In addition, different sensitivity analysis strategies will be implemented to increase the power of the study, such as the use of different cut-offs to categorize viral load exposure as well as the inclusion of HPV-negative women in pregnancy from the cohort (n=550), which could serve as a reference group.

Potential bias

Information bias Non differential misclassification in viral load measurement is possible. However, this is minimized by the robust laboratory method used to measure viral load, as the number of copies of HVP-16 per cell is being considered. Non-differential misclassification of outcome is possible when defining preterm birth. However, given that information on number of weeks of pregnancy was taken from echography tests for all women and data was collected in a meticulous manner with multiple verifications for outliers and missing values, this bias is reduced. There is also possibility of social desirability bias for an important confounder-smoking at enrollment, as this was self-reported by participants; yet since this questionnaire was auto administered, this bias might be reduced. Nevertheless, residual confounding is still possible. *Selection bias* It is unlikely as there were no HPV-16 positive women lost to follow-up.

Ethical Considerations

Ethical approval was obtained from participating institutions in HERITAGE and participants signed a consent form. Ethical approval from CERES (Ethics Committee for Health Research at University of Montreal) for this analysis will also be obtained.

Feasibility

All data from this cohort is readily accessible for analysis.

Calender

Tasks	Deadline
Finalize mini protocol *(submission deadline 29 August 2022)*	22 August 2022
Finalize protocol presentation *(presentation date: 9 September 2022)*	2 September 2022
Literature Review	30 September 2022
Write Introduction + Methods	14 October 2022
Analysis	14 October- 2 December 2022
Write Results + Discussion	1 February 2023
Finalize + Revise	1 May 2023
Submission *(submit avis de dépôt 2 months prior)*	1 June 2023

References

1. Government of Canada. Preterm Birth Initiative **2019**.
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Nom de l'étudiant: Pranamika Khayargoli

	Exceptionnel – 5	Excellent – 4	Compétent – 3	Nécessite améliorations – 2	Incomplet - 1	Note sur 5
Contexte, revue de la littérature et justification	Le contexte et la revue de la littérature sont pertinents, informatifs et présentée de façon logique, incluant une critique des recherches antérieures et une identification des lacunes justifiant l'objectif proposé.	Le contexte et/ou la revue de la littérature sont assez pertinents; les lacunes dans les connaissances et une critique des recherches antérieures sont p X entés, mais ils ne sont pas t ement liés aux objectifs proposés.	Le contexte et/ou la revue de la littérature sont moins clairs; les lacunes dans les connaissances doivent être déduit ou une critique des recherches antérieures justifiant l'objectif manque.	Le contexte et/ou la revue de la littérature ne sont pas clairs et ne permettent pas de situer l'objectif de la recherche dans leur contexte.	La revue de la littérature ne fournit pas les informations pertinentes.	
Objectif	L'objectif est clair et concis, informe le lecteur du but exact de l'étude, utilise l'approche PICOT, et n'inclut pas les méthodes.	L'objectif est assez clair et informe le lecteur du but exact de l'étude, mais om X **rtaines** informations nécessaires (ex. population, l'exposition, l'issue).	L'objectif omet **plusieurs** informations nécessaires (ex., la population, l'exposition, l'issue) mais le but de l'étude peut être déduit.	L'objectif n'est pas clair et n'inclut pas les éléments permettant de comprendre le but de l'étude.	L'objectif est manquant.	
Devis d'étude	Le devis du projet est approprié et clairement énoncé et décrit; les participants inclus sont bien spécifiés. X	Le devis est approprié, bien énoncé mais sa description manque de clarté **ou** les participants ne sont pas clairement spécifiés ou décrits.	Le devis est approprié, bien énoncé mais sa description manque de clarté **et** les participants ne sont pas clairement spécifiés ou décrits.	Le devis est énoncé mais n'est pas du tout décrit **et** il manque de précisions importantes sur les participants à l'étude.	Le devis est inapproprié ou manquant.	
Méthodes	La description des méthodes est bien organisée et concise tout en fournissant suffisamment d'information pour permettre la reproduction des analyses; les variables primaires sont clairement définies et l'analyse statistique proposée est appropriée ; les considérations de tailles d'échantillons sont bien justifiées et étayées.	Idem au niveau 5, mais contient quelques informations inutiles et/ou des descriptions moins claires. X	Présente des méthodes reproductibles, avec des informations pertinentes, mais certaines informations sont manquantes et/ou les informations sont désorganisées.	Présente des méthodes qui sont légèrement reproductibles ; certaines parties des méthodes doivent être déduites par le lecteur ; les variables et les considérations de taille d'échantillon ne sont pas bien décrites.	Les méthodes ne sont pas cohérentes avec le devis ou sont décrits de manière qu'elles ne peuvent pas être reproduites; les variables et les considérations de taille d'échantillon sont inadéquatement décrites.	
Considérations de biais potentiels	Les principales sources de biais (sélection, information, confusion) sont évaluées ; une proposition est fournie sur la façon dont l'étude aborde chacune d'entre elles.	Les principales sources de biais sont reconnues ; une proposition est fournie sur la manière dont l'étude abordera la plupart d'entre elles. X	Certaines sources de biais majeurs sont reconnues mais au moins une limite principale est manquante ; une proposition est fournie sur la manière dont l'étude abordera la plupart d'entre elles.	Plusieurs sources de biais majeurs sont omises et/ou une réponse vague est fournie sur la façon dont l'étude les abordera.	Le potentiel pour le biais n'est pas adressé.	

Commentaires sur le titre, les considérations éthiques, la faisabilité, et le calendrier proposé

titre clair, considération éthiques adéquates et faisabilité convaincante. Très bonne présentation qui a fourni des réponses à certaines interrogations soulignées dans mon évaluation.

Autres commentaires pertinents

- Quelles différences entre les études peuvent expliquer que la litérature a des conclusions qui ne sont pas unanimes? A été bien expliqué dans la présentation, moins clair dans le protocole.
- on ne sait pas depuis quand l'infection s'est installée - limite?
- sachant que l'échantillon est très petit, pourquoi catégoriser l'exposition et l'issue?
- l'idée d'analyse de sensibilité pour augmenter la puissance statistique est inquiétante, car pour moi c'est du datadredging? A été bien clarifié dans la présentation.