

HPV Genotypes and Epidemiology in Women With Cervical Cancer in Senegal.

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Research article

Keywords: cervical cancer, Human papillomavirus, Senegal

Posted Date: February 26th, 2020

DOI: <https://doi.org/10.21203/rs.2.15323/v3>

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Abstract

Background Cases of cervical cancer are increasing steadily in sub-Saharan Africa, with over 75,000 new cases and nearly 50,000 deaths a year (Mboumba et al., 2017). In Senegal, pathologies such as cervical cancer are one of the top causes of death and the Human papillomavirus (HPV) is its aetiological agent (Steenbergen et al., 2005).

Methods The aim of the study is to analyse the distribution of HPV among Senegalese women with cervical cancer. The main objective of this study is to identify the HPV types associated or “co-associated” with cervical oncogenesis in Senegal. The correlations with the risk factors of cervical carcinogenesis were analysed as well. Cervical biopsies were performed on the women admitted to Aristide Hospital Le Dantec-Julio Curie Institute. Three methods were used to detect HPV genotypes: SANGER sequencing genotyping (Applied BioSystems), PCR real-time approach technique (HPV 16 & 18 RealTime PCR kit) (www.bioneer.co.kr) and the genotyping approach from Chippron (HPV kit 3.5 LCDArray) (info@chipron.com).

Results In this study, the sample had multiple infections (co-infections), and a majority of the coinfections were high-risk types (HR-HPV types). The most common type of HPV in our study were 16 (34.37%), 18 (23.29%), 45 (10.75%), 33 (9.94%), 59 (9.09%), (3.97%) and 31 (3.69%). Among the co-infections detected in different regions of Senegal in women with cervical cancer, we found that HPV types 16 and 18 had the highest prevalence. In the Dakar region, which had the highest number of cases, a prevalence of 17.89% of HR-HPV co-infections was found.

Conclusion Polygamy could represent a cofactor in the occurrence of cervical cancer in Senegalese women. No association was found between high-risk HPV co-infections and cancer stages. However, an increase of our cohort would be necessary to affirm these hypotheses.

Background

Cervical cancer is the fourth commonest cancer affecting female population in the world, and the seventh most common cancer in the general population worldwide (Bray et al, 2018). The disease is also the fourth leading cause of cancer death among women with 311,000 associated deaths in 2018. The highest regional incidence and mortality rates are seen in Africa, especially in Eastern (Malawi, with the highest mortality rate ; and Zimbabwe) and Western Africa (Guinea, Burkina Faso, and Mali). Globally, it was previously admitted that low- and middle-income countries account for almost 90% of the burden of cervical cancer (WHO, 2014) due to insufficient awareness, lack of effective screening programs, and late clinical presentation. In addition, reports of trends in cervical cancer mortality in these countries have been limited by poor data quality and inaccurate estimates of population (Bailey et al, 2016). Additionally, in most of these countries, especially in sub-Saharan Africa, there is no cancer registry. Human papillomavirus (HPV) is a causative agent of cervical cancer that has been detected in 99.7% of cervical squamous cell carcinoma and in 94-100% of cervical adenocarcinoma (Steenbergen et al, 2005). HPV is transmitted through sexual intercourse or skin-to-skin genital contact (Hernandez et al, 2008), and persistent infection with high-risk HPV (HR-HPV) is the major cause of cervical intraepithelial neoplasia and invasive cervical cancer (Munoz et al, 1992 ; Schiffman et al, 1993 ; Walboomers et al, 1999). In general, most infections resolve on their own, as the immune response controls infection and prevents progression to precancerous lesions (Rodriguez et al, 2008). Papillomaviruses are circular, nonenveloped double-stranded DNA viruses with a genome length of 8 kb. More than 200 HPV genotypes have been reported and grouped into cutaneous and mucosal types according to their site of infection, and then subdivided into high risk (HR) and low risk (LR) types, depending on their association with a particular infection. Malignant disease (IARC, 2011). Based on epidemiological studies conducted mainly in developed countries, HPV16 and HPV18 are the two most common types of HR in cervical cancer, accounting for about 70% of all cases worldwide (Ramakrishnan et al, 2015). Sensitivity to risk factors for cervical cancer may facilitate public participation in screening campaigns, including young age at first intercourse, (Jimenez et al., 1999, Louie et al., 2009), multiple sexual partners, (Brinton et al., 1989) multiparity, (Castellsague et al., 2003, Brinton et al., 1989) human papillomavirus infections, (Walboomers et al., 1999, Bosch et al. Al, 2007) the first term pregnancy (Appleby et al 2006). Similarly, early research for help may be encouraged if women in middle-income countries are more aware of the symptoms of cervical cancer – menopausal vaginal bleeding, postcoital vaginal bleeding, superficial vaginal discharge, and lower abdominal pain. (Petignat et al., 2007, Lea et al., 2012)

In Sénégal, a West African country, a high-incidence and cervical cancer mortality area (Bray et al, 2018 ; IARC, 2018), data on cervical cancer and related genotypes are scarce. While genotype HPV-16 is the most common cervical cancer genotype in the

world, the prevalence of other genotypes varies geographically (Crow et al, 2012). These data are highly variable and incomplete in Africa. Thus, the prevalence and distribution of HPV genotypes in Africa among women with invasive cervical cancer is necessary. Until now, two vaccines were available : the « old » Gardasil®, which contains two oncogenic HPV genotypes (HPV 16 and 18) and two other genotypes of HPV responsible for condyloma acuminata, and Cervarix®, which contains HPV 16 and 18. Both vaccines provide 70% protection against cervical cancer. To cover a broader spectrum of oncogenic HPV, a new Gardasil-9® vaccine was developed in 2016 with additional genotypes included (HPV 31, 33, 45, 52 and 58) and an expected protection against 90% of HPV-induced cervical cancers.

In Sénégal, a study of a general population of women aged 18 and over in many regions (Mbaye et al., 2014) showed that the risk of HPV infection was estimated at 22.8% . Mbaye et al. (2014) also demonstrated that the HPV 31.52 and 53 genotypes were the most prevalent in the general population of women in Sénégal. Here, we have set ourselves the goal of studying the distribution of HPV genotypes in Senegalese women with confirmed cervical cancer in order to see the decrease in the percentage of these cases of cervical cancer with introduction of the Gardasil vaccine. -9®.

Methods

//Study population //

This was a cross-sectional study on cervical cancer surveillance conducted between 2013 and 2017. HPV genotype screening was performed on 120 samples of cancerous tissue. These tissues were biopsies made at the Joliot Curie Institute of the Aristide Le Dantec Hospital in Dakar on women with cervical cancer. These patients came from different parts of Sénégal and hadj a pathologie of the Cervin. All mentale and physically competent women aged 18 or older diagnosed with invasive cervical cancer (ICC) are included in the study after receiving the necessary counseling and consent to participate in the study. All patients with HIV infection were excluded. Patients were free to refuse inclusion (although all the patients we met agreed to participate in the study). For each consenting woman, data relating to age, sex, place of birth, place of residence, year of tumor diagnosis, tumor site, histology, clinic, received and the evolution of the disease, are collected on a dedicated form.

//DNA extraction, HPV L1 gene and PCR-Sanger Sequencing //

For each sample DNA was extracted from tissue using the Qiagen DNeasy® Blood & Tissue Kit

(QIAGEN, Valencia, CA, USA) according to the manufacturer's recommendations. A 450 bp long fragment of the HPV L1 gene was targeted using the MY09/MY11 consensus primers previously described (Bauer et al, 1991). The PCR reactions were performed using a Gotaq ®Green Master Mix (Promega, Germany) in a total volume of 25 µl containing 5 µl of genomic DNA (5 ng/µl), 1.5 µl of each primer (10 µM), 12.5 µl of Master Mix, and 4.5 µl H2O DNase free. Cycling conditions were as follow: initial denaturation step at 95 °C for 5 min, 35 cycles including each 95 °C for 30 s, 62 °C for 45 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. For positive samples, amplicons were purified using BioGel P100 gels (Bio-Rad) and nucleic acid quantified for Sanger sequencing. The corresponding genotypes were determined using Blast software and compared with the set of genomic sequences available in the GenBank database.

//HPV Genotyping Technique using the Chippron Approach (HPV 3.5 LCD-Array Kit) (www.chipron.com) //

It consists of a specific identification of 32 HPV genotypes, with a single hybridization on a combination of independent robust PCR amplifications in a matrix field. Combined PCR allows parallel and robust detection of HPV types at 5 target copies per reaction for HPV 16, 18, and 31 types and at 50 target copies for all others. Both, primer mixtures are directed against highly conserved motifs in the genome. Primer mixture 'A' generate fragments of about 450 bp in length; while mixture 'B' generate amplicons around 165 bp long (depends on the type of HPV). Generated PCR product of small amplicons were biotinylated, labeled and hybridized to species-specific capture probes immobilized on the surface of the LCD chip. A cascade of enzyme substrates allows short and highly

stringent visualization of the bound amplicon and each LCD chip contains eight identical microarrays separated into individually addressable small reaction chambers.

//Real-time PCR-genotyping using the Bionner approach //

The BIONEER team has designed a kit to detect HPV16 and HPV18 genotypes by real-time PCR approach (rRT-PCR). Bionner tests are performed in a closed system in three steps: DNA extraction, RT-PCR steps and Data analysis.

//Statistical analysis //

Statistical analysis was performed to evaluate the global distribution and the association between Cervix Cancer clinical status and HPV genotypes via R software. HPV Genotypes estimate were obtain using a simulation algorithm via R logistic software (R Development Core Team (2010)). Differences in HPV genotypes frequencies among stages of the disease (Figo classification groups), and other factors (age, marital status, gesture, parity, number of partners and profession), were examined using the chi-squared test, and differences were considered statistically significant for p-values <0.05. Nominal p values were corrected under listed cofounders' factors such as age, marital status, gesture, parity, number of sexual partners and profession. For age, the women were classified into 3 age groups (<45, 45-55, >55) and 2 groups for the oncogene HPV genotypes (<or=2 and >2).

//Ethical approbation of the study //

Our protocol has been reviewed according to the rules issued by the National Committee for Ethics for Health Research (CNERS) of Sénégal and in accordance with the procedures established by the University Cheikh Anta Diop Dakar (UCAD) for the ethical approval of any research involving human participants. Based on the information provided in the protocol, UCAD's Committee on Research Ethics (CER) considers that the research proposed, respects the appropriate ethical standards and, as a result, approves its execution under "Protocole 0224/2016/CER/UCAD".

Results

//Demographic and clinical characteristics //

Between 2013 and 2017, a total of 120 women from different regions of Sénégal diagnosed with cervical cancer were included in the study. Fresh cervical biopsies were taken from each patient and screened for HPV. Regarding their clinical aspects, 40 of our patients or 33.3% were in stage II (figo class), 53 or 44.1% in stage III and 27 (22.5%) in stage IV. The age of the patients varied from 27 to 85 years with an average age at diagnosis of 50.62 years and a median age of 50 years. Regarding age groups, 35 patients (29.2%) were under 45 years of age, 51 (42.5%) between 45 and 55 years of age and 34 (28.33%) of patients were over 55 years.

//HPV types prevalence //

Several types of HPV were detected in this study. Gathering the results obtained with the 3 technical approaches, a total of 23 HPV genotypes were detected in our patients. The most common genotypes in our population were HPV16 systematically detected in more than half of our patients, 18 (44%), 45 (33%), 33 (31%), 59 (28%), 35 (12%), 31 (11%), 58 (8%), 39 and 73 (4%), 44, 54 and 68 (3%) and the rest less than 1%.

// HPV 16 and 18 distribution//

The cartography of genotypes by area, showed that the genotypes 16 and 18 co-dominated in Dakar, Thiès, Diourbel, Saint-Louis, Fatick, Kaolack, Tambacounda, Kedougou and Ziguinchor areas, while in Kolda genotype 16 was the most prevalent. In kafrine we note the absence of these two genotypes.

//HPV types coinfections and cofactors. //

We found 29 unique cases of single genotype infection in our patients. In fact, co-infections with 2 genotypes represented 33.3% (40/120) of the cases. In 49 samples (40.83%), more than two viruses were detected. Co-infections with 3 dominant HPV genotypes (26 patients) followed by 4 genotypes (15 patients), 5 genotypes (6 patients) and 6 genotypes (2 patients).

We noted that co-infections with several HR-HPVs were predominant in polygamous patients (p value of 0.02) compared to patients with monogamous status (OR of 0.30). For the gestity criterion, coinfections with multiple genotypes (up to 6 genotypes) were more frequent in patients who hadj 6 pregnancies or more (OR = 1.60). We also noted that co-infections with multiple genotypes were significantly associated to patients older than 45 years (OR> 1). The association analysis between HRHPV coinfections and age showed that HR-HPV co-infections were more prevalent among "young" patients (<45 years old) with 35 cases and those aged between 45 and 55 years (41 cases). In both groups, co-infection with more than 2 HR-HPV genotypes dominated (79 cases vs 41 with 2 or less).

Discussion

Cervical cancer (CC) is a scourge that imposes a therapeutic emergency. This cancer is increasing steadily in sub-Saharan Africa, with more than 75,000 new cases and nearly 50,000 deaths a year (Steenbergen et al., 2005)(Mboumba et al., 2017). According to the World Health Organization, CC is the fourth most common affecting female population worldwide, and will kill more than 443,000 people worldwide by 2030, nearly 90% of them in sub-Saharan Africa. This high incidence may be justified by the lack of adequate structure for diagnosis, screening and treatment (Castellsague et al., 2007). In Sénégal, CC is the first most common cancer of women (ICO/IARC HPV Information Center, 2019), and here we reported a pilot study exclusively dedicated to the distribution of HPV genotypes among Senegalese women with cervical cancerous lesions.

As expected, all cervical specimens collected in women with histologically confirmed ICC were HPVpositive with at least one genotype detected. The most commonly detected HPV types in women with single or multiple HPV infections were HPV16 (systematically detected in more than half of our patients), 18 (44%), 45 (33%), 33 (31%), 59 (28%), 35 (12%), 31 (11%), 58 (8%), 39 and 73 (4%), 44, 54 and 68 (3%) and the rest less than 1%. The high prevalence of HPV16 and HPV18 reported in our study was expected, since these types are the two most common HPV types across the world (de Sanjose et al, 2010; Li et al, 2011; de Oliveira et al, 2013; Deny et al, 2014; Lagheden et al, 2018; Liao et al, 2018). However in some studies (Mejia et al, 2015; Wang et al, 2018; Schisler et al, 2018; Long et al, 2018), other genotypes (HPV45, 58, 52) hadj been reported as the second commonest. This result agrees with that of Missaoui et al, 2010 hadj found that HPV16 is the most frequent virus among invasive squamous cell carcinomas (47.6%, p = 0.001) and adenocarcinomas (80%, p <0.001) in Africa from the north (Tunisia). On the other hand, in Central Africa more precisely in Cameroon, the prevalence of the HPV 16 and, HPV 18 genotypes represented 30.8% (Tebeu et al., 2018).

//Infections and cofactors //

we also noted that most women were infected with at least one high-risk HPV (70%). This high co-detection rate is especially due to the high sensitivity of the Chippron real-time PCR test which targeted a wide range of genotypes but also of the fact that the almost totality of our patients were under polygamous regime. indeed polygamy can involve the diversity of the noted genotypes as well as the high number of coinfection. Overall, it was noted that the proportion of HPV multiple infections increased in the last years from 4% to 15% (Li et al, 2011), reflecting the use of new tests more prone to detect multiple infections in recent studies.

Multiple HPV infections could, in part,explain the special aggressivity and the rapid progression of the cervical cancer disease noted in the Senegalese women as it was reported as an independent predictor of poorer survival another study (Genta et al, 2017). However the mechanism how multiple HPV infections affects patients survival is not fully understood. We also noted that co-infections with more than 2 HR-HPV types were especially prevalent in patients between 45 to 55 years age, probably due to viral persistence or reactivation of latent HPV (Simon and Poppe 2008; Dufit et al., 1991).

With regard to marital status, multiple HR-HPV co-infections were significantly more prevalent among polygamist. We also noted that women with more than six pregnancies were prone to multiple coinfections. Both results are in line with previous results which clearly indicated that multiple pregnancies and the high number of sexual partners increase the risk of HPV infection and persistence (IARC, 2016).

// Link with Vaccines //

On the basis of the new Gardasil-9® vaccine which operates against 9 genotypes of HPV (6, 11, 16, 18, 31, 33, 45, 52 and 58), only 5 cases of CCI were exclusively associated with non-vaccine types. Thus, with regard to the results obtained in the present study, the use of this non-swallowing vaccine could provide effective prevention results for cervical cancer in adolescent girls or young Senegalese women, in the hope to prevent up to 95% of CHF. However, additional studies including more patients are needed to better assess the real impact of this non-swallow vaccine.

Regarding the prospects for vaccination, the relatively high prevalence of types present in nonavalent vaccine (genotypes 16, 18, 31, 33, 45 and 58), Gardasil 9 shows that the latter could be a clear progress in the prevention of cervical cancer and papilloma virus infection (HPV) but also an additional means screening in Sénégal with the aim of lowering the risk of cervical cancer due to genotypes 16 and 18, which are among the most common known causes of precancerous lesions of the Cervix.

However, some limitations can be highlighted in this study. First, the modest sample size included in this study. Indeed, only a few cases have been sampled and tested, and the prevalence of HPV genotypes reported here should be seen as a trend that needs to be confirmed by more comprehensive additional studies. Second, the high prevalence of multiple infections, sometimes with more than 3 genotypes, makes it difficult to establish the carcinogenic value of certain types or groups of HPV. Determining the viral load of genotypes by quantitative methods would probably help to get an idea of the types or combinations of more carcinogenic associated types (ICC) in Sénégal.

Conclusion

In conclusion, the overall frequency of HPV types detected in cervical cancerous lesions was high for HPV16 and HPV18 in Sénégal. Multiple infections were also very common. Globally, our findings can be beneficial to health policy decision-makers and shows that current HPV vaccines, especially the current 9-valent one, could have great impact to reduce the burden of cervical cancer in Sénégal. However a special attention should be drawn on some non vaccine types (HPV 59 and 35 especially) which could emerge as leading causes of cervical cancer in a context of HPV vaccination in Sénégal. Polygamy could represent a cofactor in the occurrence of cervical cancer in Senegalese women. No association between high-risk HPV co-infections and cancer stages. However the increase of our cahorte would be necessary in order to affirm these hypotheses.

Abbreviations

HPV : Human Papillomavirus

Declaration

*Authors' contributions and details.

(NK), (DCT), (DG), (DN), (TC), (LC), (NM¹), (NM²), (DA), (KS), (DA), (SA), (DA).

NK, DCT, DN and DG concepted the study, the methodology, conducted the study, drafted the manuscript and approved the final version.

TC and LC conducted the data analysis and approved the final version.

CD and KN conducted the methodology, to performing molecular biology, and approved the final version.

NM¹ and DA contributed to the recruitment and managment of the Cervix Cancer Cases and approved the final version.

DA, DN and DA coordinated this study, revised the manuscript.

All authors read and approved the final manuscript.

Availability of data and materials : the data of this study are disponible

Consent to participate : The participants gave their consent in a form and signed

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***Acknowledgements**

The authors are grateful to all the patients and medical staff who have generously collaborated in this study. Authors thanks to the staff of the Cancerology Aristide LeDantec Hospital (Dakar, Sénégal) assistance in inclusion/recruitment. The Sénégal Ministry of Education and Research and Institute Pasteur of Dakar (Sénégal) supported the PhD Candidate NK.

***Funding**

This study was supported by grants from Sénégal Ministry of Research and Institut Pasteur of Dakar.

***Competing interests**

The authors declare that they have no competing interests.

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Tables

Table I A: Demographic and clinical characteristics (Age and Stade-classe figo)

	Age			Figo class stade		
	<45	45-55	>55	Stade II	Stade III	Stade IV
Number of patients						
	35 (29.16%)	51 (42.5%)	34 (28.33%)	40 (33.3%)	53 (44.2%)	27(22.5%)

Table IB: Demographic and clinical characteristics (year of diagnosis, place of residence, Gestity and first sexual rapport)

	year of diagnosis		place of residence			Gestity			first sexual rapport			
Number of patients	2013-2015	2016-2017	West	Center	North	South	<5	5-10	>10	<15years	15years-20years	>20years
	48 (40%)	72 (60%)	58 (48.33%)	25 (20.83%)	11 (9.16%)	26 (21.66%)	24 (20%)	84 (70%)	12 (10%)	15(12.5%)	67(55.83%)	38(31.66)44

West: Dakar , Thies, Diourbel ; Center: Kaolack, Kaffrine, fatick.Louga ; North: Saint-Louis ; South: Kolda, Kédougou, Ziguichor

Table II: Number of percentage of genotypes

Percentage	<10%	10-20%	21-30%	31-40%	41-50%	>50%
Genotypes	58-39-73-44-54 68-42-67-72-82 29-51-52-66-81 89-56	31-35	59	33-45	18	16

Table III: Correlation between risk factors and HR-HPV coinfects. HPV, human papillomavirus; HR, high-risk; Gest, Gestity; Pol, Polygamy; Gest: Gestity ; Mono, Monogamy; P: p-value; OR: Odds Ratio.

		≤ 2 HR-HPV	>2 HR-HPV	p-value	OR (univariable)	OR (multivariable)
Sit	poly	32 (78.0)	71 (92.2)	-	-	-
	mono	9 (22.0)	6 (7.8)	0.028	0.30 (0.09-0.90, p=0.034)	0.30 (0.09-0.93, p=0.039)
Gest	≤ 6	24 (58.5)	37 (46.8)	-	-	-
	>6	17 (41.5)	42 (53.2)	0.224	1.60 (0.75-3.47, p=0.225)	0.63 (0.07-4.57, p=0.648)
Age	<45	15 (36.6)	25 (31.6)	-	-	-
	>55	12 (29.3)	22 (27.8)	0.778	1.10 (0.42-2.88, p=0.844)	0.84 (0.29-2.41, p=0.751)
	45-55	14 (34.1)	32 (40.5)	-	1.37 (0.56-3.39, p=0.490)	0.94 (0.35-2.53, p=0.909)
Stade	II	12 (29.3)	28 (35.4)	-	-	-
	III & IV	29 (70.7)	51 (64.6)	0.496	0.75 (0.33-1.68, p=0.497)	0.77 (0.32-1.78, p=0.547)

Figures

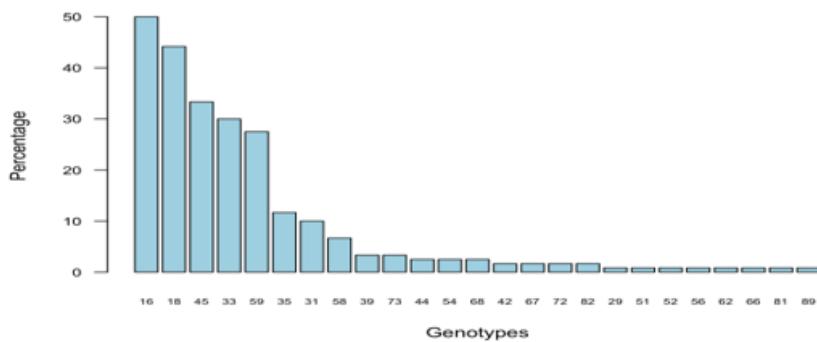


Figure 1

Distribution (prevalence) of genotypes in relation to the total number of patient. This analysis was done to see the prevalence of the different types of HPV detected in our study population. The most common types of HPV in descending order in our population were: 16 (50%), 18 (44%), 45 (33%), 33 (31%), 59 (28%), 35 (12%), 31 (11%), 58 (8%), 39 and 73 (4%), 44, 54 and 68 (3%) and the rest less than 1%.

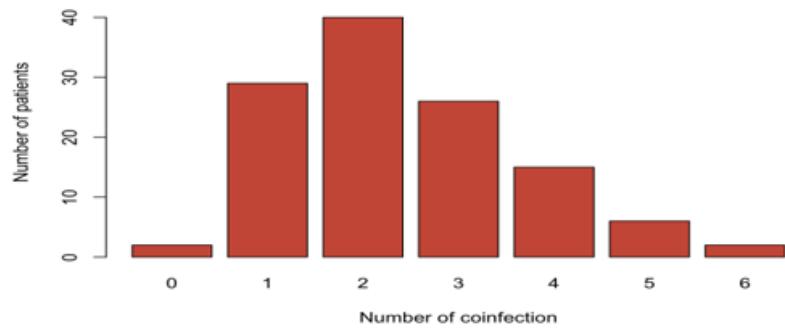


Figure 2

The distribution of coinfects according to the number of patients It was important to see the number of HPV infecting a given sample. we noted 29 monoinfection (1 HPV infection) in our study population. the co-infections with 2 types of HPV dominated (40 patients) followed by those with 3 types (26 patients), those with 4 types (15 patients), those with 5 types (6 patients) and those with 6 types (2 patients).

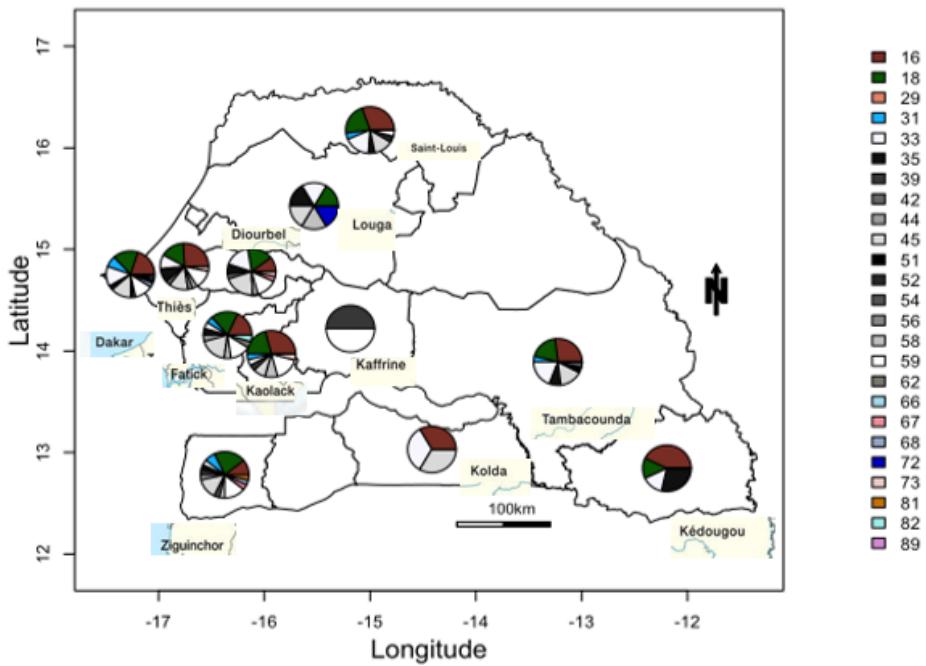


Figure 3

The cartography of HPV genotypes (HPV 16 and 18 co-dominated in Dakar, Thiès, Diourbel, Saint-Louis, Fatick, Kaolack, Tambacounda, Kedougou and Ziguinchor areas, while in Kolda genotype 16 was the most prevalent. In the kaffrine area we have only 2 genotypes on the 39 and 59.