

HPV Genotypes and Epidemiology in Women With Cervical Cancer in Senegal

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Abstract

Background

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

Methods

The aim of the study is to analyze the distribution of HPV among Senegalese women with cervical cancer. Main objectives of this study are to identify the HPV types associated or “co-associated” with cervical oncogenesis in Senegal. The correlations with risk factors of cervix carcinogenesis, with risk factors, were analyze too. Cervical biopsies were performed on women hospitalized at Aristide Hospital Le Dantec-Julio Curie Institute. Three methods has been used to detect HPV genotypes - SANGERsequencing 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@chippron.com).

Results

It this study, patients had multiple infections (co-infections) at all, and the majority of coinfections was 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 co-infections detected in different regions of Senegal among 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 noted.

Conclusion

Polygamy represents a cofactor in the occurrence of cervical cancer in Senegalese women. No association between HPV-High Risk co-infections and cancer stages.

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 Senegal, 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 Senegal, a study conducted in general population of women from 18 years old and focused in many areas (Mbaye et al. 2014) showed that the risk of HPV infection was estimated at 22.8%. Mbaye et al, (2014) also demonstrated that genotypes HPV 31,52 and 53 were the most prevalent in the general women population in Senegal. Here, we set ourselves the goal of studying the distribution of HPV

genotypes among Senegalese women with confirmed cervical cancer in order to see if the introduction of the new vaccine Gardasil-9® in Senegal will provide a better preventive barrier.

Methods

//Study population //

This was a cross-sectional study on cervical cancer surveillance conducted between 2013 and 2017. The HPV genotypes screening was performed on 120 specimens of cancerous tissue. These tissues were biopsies performed at the Joliot Curie Institute of the Aristide Le Dantec Hospital in Dakar on women with cervical cancer. These patients came from different areas of Senegal and had a pathology of the cervix. All mentally and physically competent women from 18 years age, diagnosed for invasive cervical cancer (ICC) are included in the study after due counseling and obtaining consent to be involved in the study. Patients were free to refuse the inclusion. For each consenting woman, data related to the age, sex, place of birth, place of residence, year of tumor diagnosis, tumor site, histology, clinic, treatments received and disease evolution, 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 (Refs.....+ refs avec les genotypes ciblés...?). 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 the 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 (CNER) of Senegal 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 areas of Senegal, diagnosed with cervical cancer were included in the study. Fresh cervical biopsies were collected from each patient and screened for HPV. Regarding their histopathological aspects, the lesions were divided in three groups: 40 samples

(33.3%) were classified as CIN II (Cervical Intraepithelial Neoplasia type II), 53 patients (44.2%) as CIN III grade and 27 patients (33.5%) as invasive cervical cancer grade (Table I). Patient age ranged from 27 to 85 years with a mean age at diagnosis of 27 years and a median age of 50.62 years. Regarding age groups, 35 biopsies (29.2%) were obtained from women younger than 45 years of age, 51 samples (42.5%) from women between 45 and 55 years of age, and 34 (2.7%) from women above 55 years old.

//HPV types prevalence //

At least one HPV were detected in each sample. 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 the kaffrine area we have only 2 genotypes on the 39 and 59

//HPV types coinfections and cofactors. //

We found 29 unique cases of single genotype infection in our patients. In fact, co-infections with at least 2 genotypes accounted for 33.3% (40/120) of the cases. In 49 samples (40.83%), more than two viruses were detected. Co-infections with 3 dominated 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 0.028) compared to patients with monogamous status (OR of 0.30). For gestationality criterion, coinfections with multiple genotypes (up to 8 genotypes) were more frequent in patients who had 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 Senegal, CC is the first most common cancer of women (Refs...), 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 (54.16%), HPV18 (33.33%), HPV45 (26.66%), HPV33 (20.83%), HPV59 (18.33%), HPV35 (11.66%) and HPV31 (10%). 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) had been reported as the second commonest.

//Infections and cofactors //

We also noted that most of women had multiple infections (70 %) with 2 or more types co-detected. 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. 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).

Other cofactors, such as environment and smoking were also known to modulate the coinfection (Ref - Brotman, RM et al., Association between cigarette smoking and the vaginal microbiota: a pilot study. BMC Infect. Dis. 14,471) and has been correlated to HPV persistency; although women in our study with a high grade disease were likely no smokers.

// Link with Vaccines //

Based on the new Gardasil–9® vaccine which operates against 9 HPV genotypes (6, 11, 16, 18, 31, 33, 45, 52 and 58), only 5 ICC cases were exclusively associated with non-vaccine types. So regarding the results obtained in the present study, the use of this nonavalent vaccine might provide an effective prevention outcomes for cervical cancer in Senegalese adolescent girls or young women, with the expectation to prevent up to 95% of ICC. However, further studies including more patients are needed for a better assesment of the real impact of this nonavalent vaccine.

Regarding the vaccination perspectives, the relatively high prevalence of the non vaccine types HPV59 (18.33%) and HPV35 (11.66%) could be a public health issue in Senegal, but once again additional studies are needed to confirm these findings.

However some limitations can be pointed out of this study. Firstly, the modest sample size included in this study. Indeed, infortenately only few cases were sampled and tested, and the HPV genotypes prevalence reported here should be considered as trends which need confirmation through additional more exhaustive studies. Secondly, the high prevalence of multiple infections, sometimes with more than 3 genotypes, complicates the establishment of the carcinogenic value of some HPV types or group of HPV types. Genotypes viral load determination by quantitative methods would probably help to have an idea about the more carcinogenic types or combinations 'types' associated with (ICC) in Senegal.

Conclusion

In conclusion, the overall frequency of HPV types detected in cervical cancerous lesions was high for HPV16 and HPV18 in Senegal. 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 Senegal. 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 Senegal.

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 conceived 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 management 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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Competing interests

The authors declare that they have no competing interests.

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Tables

Table I: **Demographic and clinical characteristics.**

CINII^o: Cervical Intraepithelial Neoplasia type II., *CINIII*: Cervical Intraepithelial Neoplasia type III.

Number of patients	Age			Types of lesions		
	<45	45-55	>55	Cervical Intraepithelial Neoplasia type II (<i>CINII</i> ^o)	Cervical Intraepithelial Neoplasia type III (<i>CINIII</i> ^o)	invasive cervical cancer grade
	35 (29.16%)	51 (42.5%)	34 (28.33%)	40 (33.3%)	53 (44.2%)	27 (33.5%)

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

		≤2 HR-HPV	>2 HR-HPV	<i>p-value</i>	OR
Matrimonial situation	Poly	32	71	0.02	0.30
	Mono	9	6		
gestity	≤6	24	37	0.22	1.60
	>6	17	42		
age	<45	15	25	0.77	1.10
	>55	12	22		
	45-55	14	32		
Stade	II	12	28	0.49	0.75
	III_&_IV	29	51		

Figures

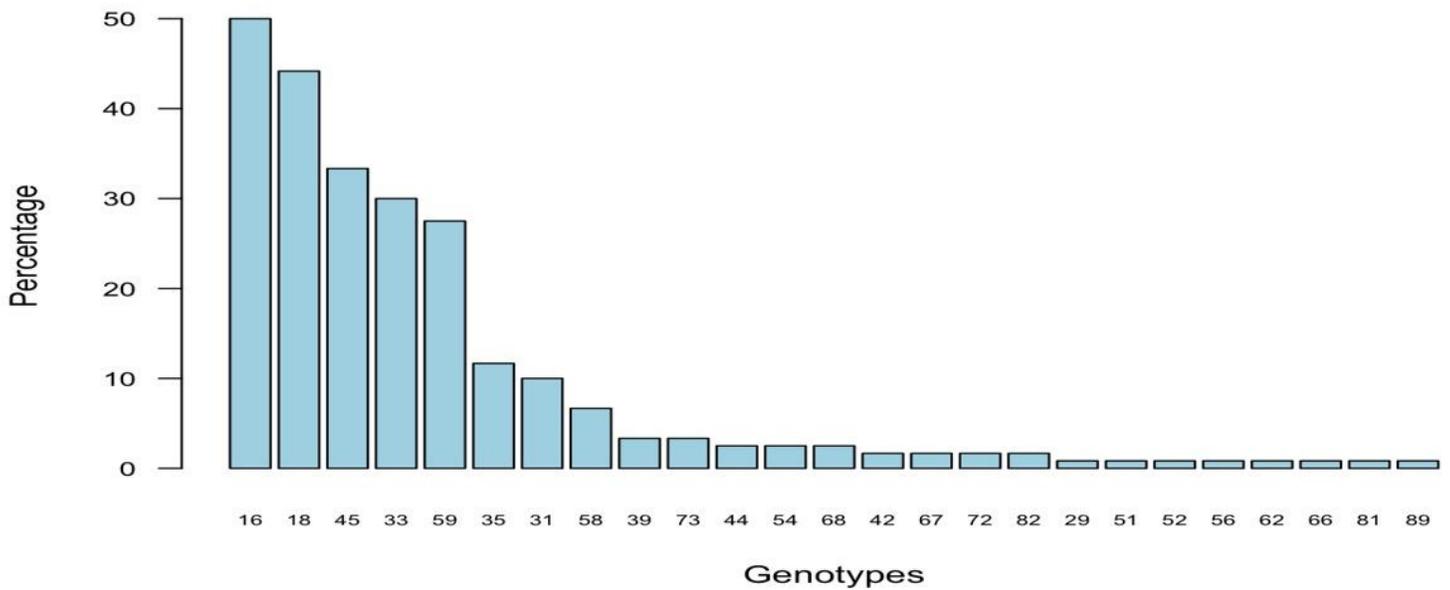


Figure 1

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%.

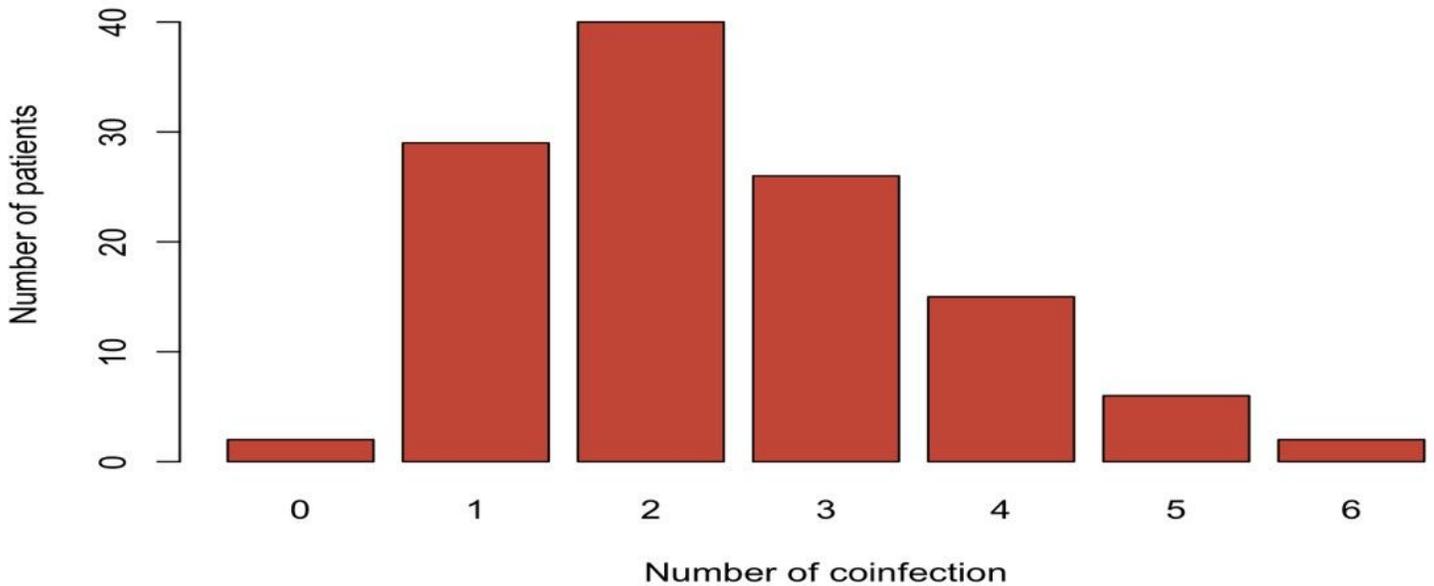


Figure 2

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) .

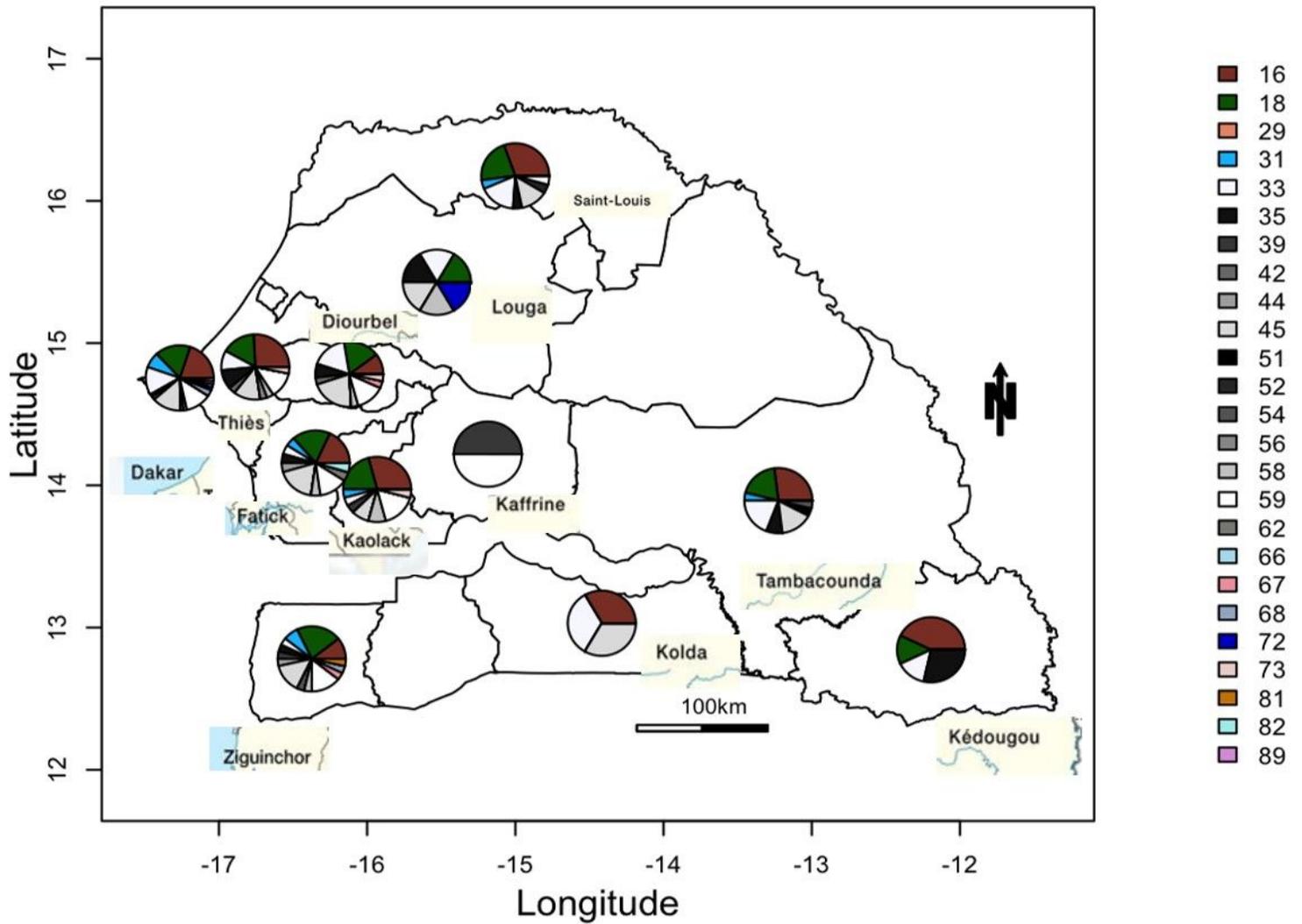


Figure 3

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.