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Cytopathogenic Effects and Growth Characteristics of Herpes Simplex Virus-II among Women Attending Selected Hospitals in Kaduna State, Nigeria

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Abstract

The Herpes Simplex Virus Type-II is a double-stranded DNA virus responsible for infections characterized by fever, rashes, and blisters/sores in the genital area. It has a very complex life cycle and stands out as one of the most common pathogens in the aetiology of sexually transmitted infections worldwide. This study determined the cytopathogenicity HSV-2 among women of reproductive age attending selected Hospitals in Kaduna State, Nigeria. A total of 390 vaginal swab samples were collected from women attending Hajiya Gambo Sawaba General Hospital, Major Yusuf Dantsoho Memorial Hospital, and Sir Patrick Ibrahim Yakowa General Hospital, Kafanchan. Serologically confirmed samples were cultured on the chorioallantoic membrane of embryonated eggs. The virus demonstrated cytopathic effects (53%; 208/390), including plaque formation, pocks, egg bleeding, and embryo death, as evidence of viral growth and activity. Viral infectivity was demonstrated by the production of morphological changes on the chorioallantoic membrane of eggs, suggesting that the virus is highly active and rapidly replicating in the genital tract of these participants. The findings demonstrate that HSV-2 isolates from the study population exhibited detectable cytopathic activity in embryonated eggs, suggesting active viral replication and highlighting the need for improved diagnostic surveillance.

Key words: HSV-2, Cytopathogenicity, Plaques, Pocks, Viral culture, Kaduna

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is a globally prevalent sexually transmitted pathogen and a major public health concern, particularly in low- and middle-income countries (Jama *et al.*, 2024; Owen *et al.*, 2024). It belongs to the family *Herpesviridae* and is characterized by its ability to establish lifelong latency in the host following primary infection, with periodic reactivation that may result in symptomatic or asymptomatic viral shedding (Pillay *et al.*, 2024). HSV-2 is the principal etiological agent of genital herpes, a chronic infection associated with significant morbidity, psychosocial burden, and increased risk of transmission of other sexually transmitted infections, including Human immunodeficiency virus (Jama *et al.*, 2024).

The burden of HSV-2 infection remains disproportionately high in sub-Saharan Africa, where seroprevalence rates among women are significantly elevated due to a combination of biological, socio-cultural, and economic factors (Alareeki *et al.*, 2022). Women are biologically more susceptible to HSV-2 acquisition, and the infection often presents with more severe clinical manifestations compared to men (Enitan, 2023). Moreover, HSV-2 infection has been strongly implicated in facilitating the acquisition and transmission of HIV by disrupting mucosal barriers and enhancing viral entry through inflammatory processes (AlMukdad *et al.*, 2022). Despite its high prevalence, HSV-2 infection is frequently underdiagnosed, largely because many infections are asymptomatic and advanced diagnostic tools are limited in resource-constrained settings (Enitan, 2023; Kolb *et al.*, 2023).

A critical aspect of HSV-2 pathogenesis lies in its cytopathogenic effects on infected host cells (Sureram *et al.*, 2022). Following infection, the virus induces characteristic morphological alterations in epithelial cells, including cell rounding, ballooning degeneration, syncytium formation, and eventual cell lysis (Dass *et al.*, 2025). These cytopathic effects (CPE) are essential markers in virological studies and are

widely used in laboratory-based virus isolation and characterization (Lim *et al.*, 2023). Understanding the cytopathogenicity of HSV-2 not only provides insight into viral replication dynamics but also aids in evaluating viral virulence and host–pathogen interactions (Zhang *et al.*, 2024).

In Nigeria, and specifically in Kaduna State, data on the cytopathogenic characteristics and growth dynamics of HSV-2 among infected populations remain scarce (Hudu and Hamal, 2022; Alshrari *et al.*, 2024). Most existing studies have focused primarily on seroprevalence and epidemiological patterns, with limited emphasis on laboratory-based virological characterization (Diamreyan *et al.*, 2023; Dangoggo *et al.*, 2025). This knowledge gap hinders a comprehensive understanding of the biological behaviour of circulating HSV-2 strains and their potential impact on disease severity and population-level transmission dynamics.

Given the increasing burden of sexually transmitted infections among women of reproductive age in North-West Nigeria, there is a pressing need for detailed virological investigations into HSV-2 (Okonko *et al.*, 2023). Women attending healthcare facilities represent a critical population for such studies, as they are often at heightened risk of infection and may serve as reservoirs for ongoing transmission (Onu *et al.*, 2023; Dangoggo *et al.*, 2025). Investigating the cytopathogenicity and growth patterns of HSV-2 in this population will provide valuable insights into viral behavior, inform clinical management strategies, and contribute to the broader body of knowledge needed for effective control measures.

Therefore, this study aimed to evaluate the cytopathogenic effects and growth characteristics of HSV-2 among women attending selected hospitals in Kaduna State, North-West Nigeria.

MATERIALS AND METHODS

Study Area

The study area for this research was Kaduna State, located in the Northern part of Nigeria's High Plains and situated at coordinates $10^0$20`N$7^0$45`E, $10^0$20`$7^0$`N45`E. The vegetation cover is Sudan Savannah type, characterized by scattered short trees, shrubs and grasses. Soil type is mostly loamy to sandy type. The state was created on 27th May, 1967 and has a total land area of 46,053 km^2, a population of 6,066,562 people (Abubakar *et al.*, 2024), consisting of about 23 Local Government Areas (L.G.A), and is also nicknamed the centre of learning, thereby making it cosmopolitan in nature. It is highly populated due to several educational institutions and serves as a gateway to many other states in the country by air, land, and rail.

The hospitals selected for this study include: Hajiya Gambo Sawaba General Hospital, Kofan Gaya, Zaria, located in the Northern senatorial zone, Yusuf Dan Tsoho General Hospital, Ugwuan Rimi, located in the central senatorial zone, and Sir Patrick Ibrahim Yakowa General Hospital, Kafanchan, in the southern senatorial zone of Kaduna.

Study Population

The study population included women of all ages and parity attending the selected hospitals at the time of the study.

Ethical Considerations

Ethical approval (Number: ADM/MOH744/VOL1/519) was obtained from the Research and Ethics Committee of Kaduna State Ministry of Health and also from the ethical committees of the selected Hospitals. Informed Consent was also obtained from participants prior to sample collection.

Study Design

The study was a cross-sectional study, in which women who consented to participate were selected consecutively from the participating hospitals and recruited at each visit.

Inclusion and Exclusion Criteria

Inclusion criteria included consenting women of all categories who were attendees of the selected hospitals. The women attending the hospitals who did not give their consent were not included in the study.

Sample Size Determination

The sample size was determined using the equation by Naing *et al.* (2006) and a reported 57.8% HSV-2 IgM prevalence among women of reproductive age in Zaria, Kaduna State, Nigeria (Ameh *et al.*, 2016), with a 95% confidence interval.

$n = z^2 pq/d^2$

Where, n= no of samples.

P= prevalence rate of previous study= 57.8%

z= standard normal distribution at 95% confidence limit=1.96

d= absolute desired precision of 5%= 0.05

q= 1-p=1-0.578=0.422

$n= (1.96)^2 x0.578x0.422/ (0.05)^2 = 374.856$

The calculated sample size was 374.9

However, 390 vaginal swab samples were collected.

Collection of Samples and Processing

Vaginal swab samples were collected with the assistance of qualified and authorized medical personnel who determined if the women had fever and other clinical features.

Vaginal swabs were also collected alongside the blood samples of the participants. This was collected by the nurse in the ward or a female Laboratory staff by using a sterile sealed cotton swab stick to insert into the vagina in order to obtain the vaginal discharge or fluid present in the vesicles/blisters present on the genitals of the respondents. The swab stick was immediately transferred into a viral transport medium (2ml of Balanced Salt Solution) in Bijou bottles and transported in ice packs to the Virology Laboratory of the Department of Microbiology, A.B.U-Zaria, for further analysis.

Sample Analysis

Samples of vaginal swabs were analyzed for HSV-2 using the enzyme-linked immunosorbent assay. The vaginal swabs were also cultured in egg culture.

Determination of the Cytopathic Effect of the HSV-2 Using Egg Culture

This was done in line with the standard set by Nozawa *et al*. (2014).

Processing of Egg

Fertile day old eggs were obtained from the Hatchery at the National Animal Production Research Institute, Shika, Zaria. The eggs were transported to the Virology Laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, and further incubated at 37 ^oC for about 10-12 days, preparatory to egg inoculation.

Egg Candling

Prior to inoculation, the eggs were candled in a process where the eggs were placed at the orifice of a candling box, and the airspace as well as the veins were marked to determine the route of inoculation and to prevent piercing the veins, which may lead to bleeding and death of the egg (Nozawa *et al*., 2014).

Sample Preparation and Egg Inoculation

The vaginal swabs were placed in 2 ml of Balanced Salt Solution (BSS) to suspend the viral particles. These were stored at -20 $^\circ C$ and brought to room temperature before inoculation into the whole egg.

The chorioallantoic membrane route was employed for the egg inoculation. Briefly, the egg was placed on its side on a plate; the egg surface was disinfected with 70% alcohol and iodine tincture before inoculation. Using an egg borer, the airspace was punctured, as was the route for the chorioallantoic membrane. A slight pressure was placed on the airspace to allow for the collapse of the chorioallantoic membrane, at the region of the punch of the chorioallantoic membrane, a total of 0.1ml of the already prepared viral suspension was placed on the chorioallantoic membrane of the egg using a sterile needle and syringe and the punch was sealed, using candle wax. The inoculated eggs were incubated in a humid incubator at 37 ^oC for ten days. The eggs were observed for the formation of plaques, pocks, egg bleeding and death as evidence of viral growth and activity. One egg was inoculated per sample, and 2 eggs were incubated without inoculation to serve as a negative control.

Data Analysis

Tables and plates were used to present the results obtained after observing the inoculated eggs for ten days of incubation. Results are also presented in percentages.

RESULTS

Viral growth was demonstrated by production of plaques, bleeding of the entire egg; pocks and death of the embryo. This was observed in 53.3% (208/390) of the eggs that were inoculated with the viral suspension and incubated (Figure 1).

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Tables 1: Percentage occurrence of different Cytopathic Effects

Cytopathic Effects	No of Occurrence	% Occurrence
Haemorrhage	67	32.2
Pocks	55	26.4
Embryo Death	42	20.3
Other Effects	44	21.2
Total	208	100

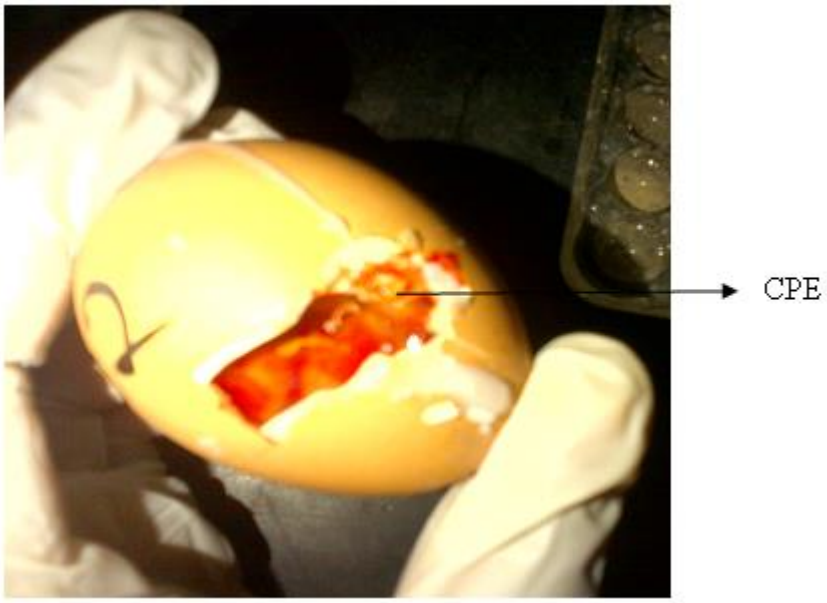

Plate I: Cytopathic Effect of HSV-2 cultivated in 10-day-old embryonated eggs

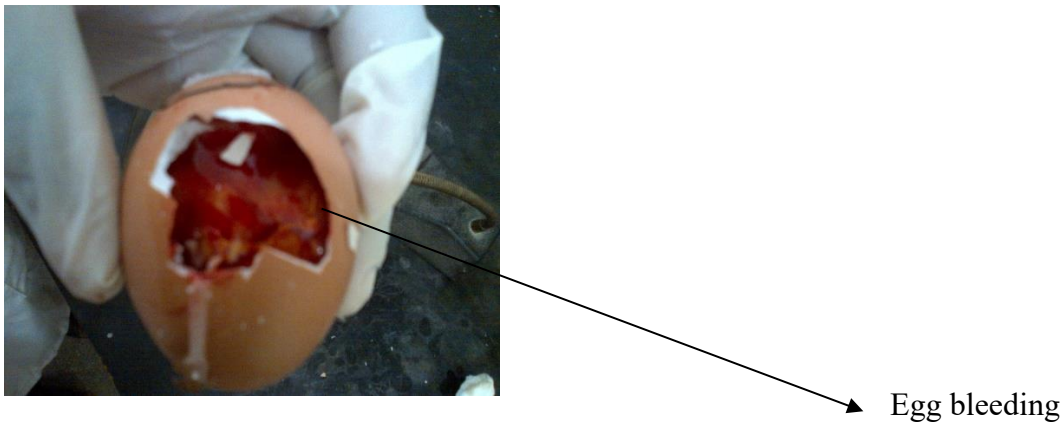

Plate II: Viral growth demonstrated by bleeding/ haemorhage of the egg

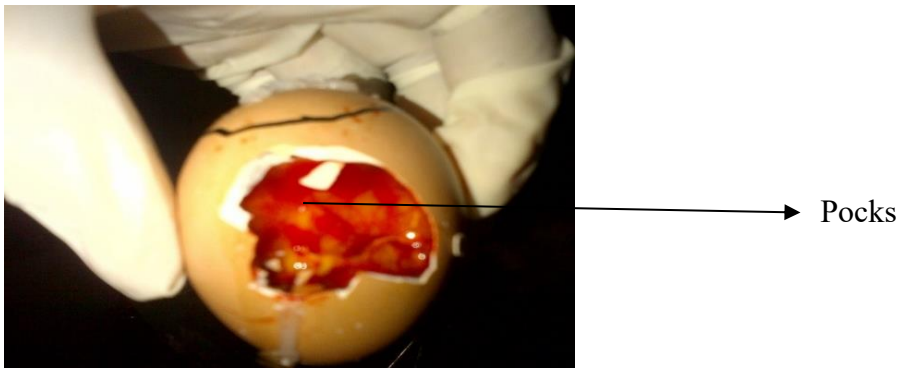

Plate III: Viral growth Demonstrated by Production of pocks on the Chorioallantoic Membrane of the egg

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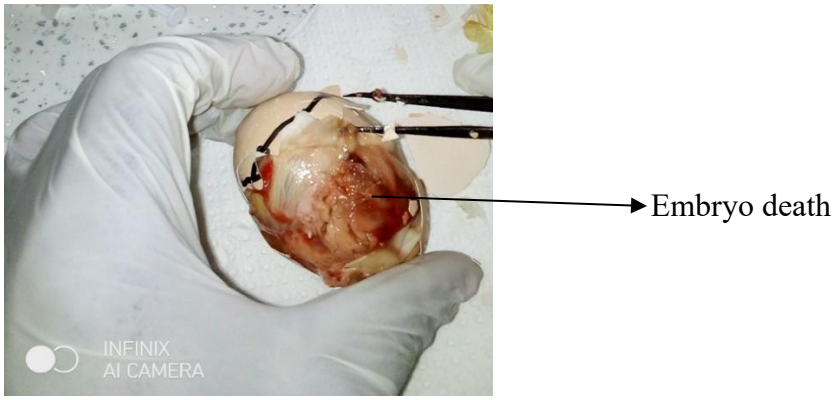

Plate IV: Demonstration of viral growth with attendant death of embryo

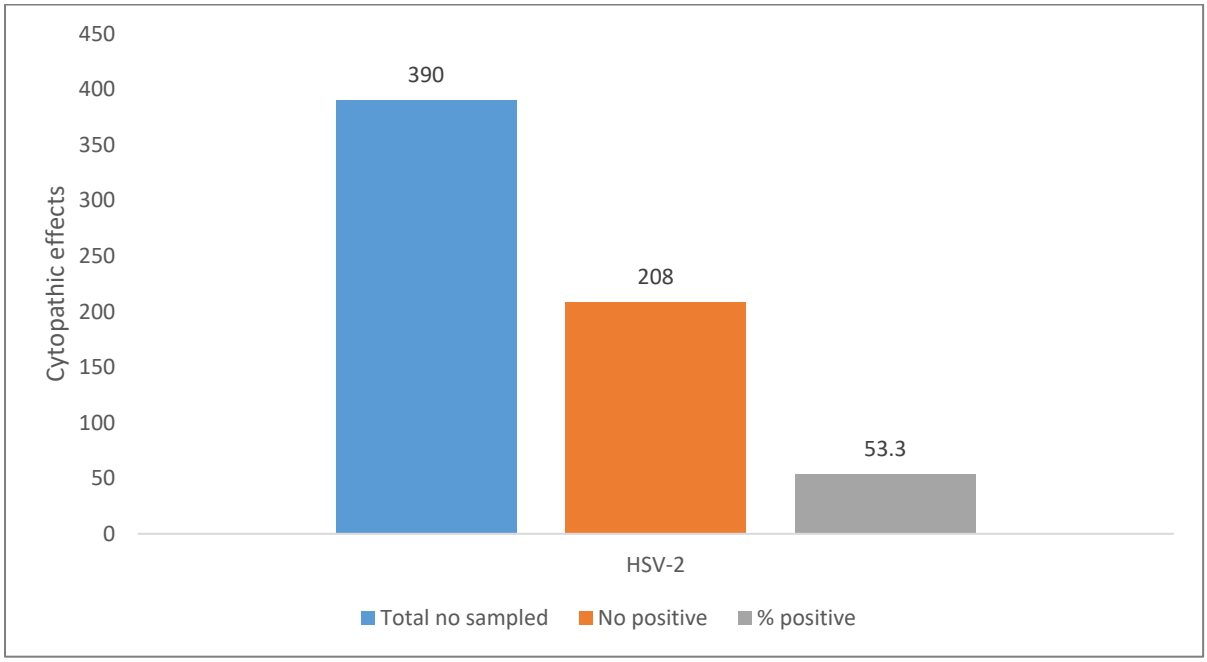

Figure 1: Detection of HSV-2 in Samples as Demonstrated in Egg Culture

Table 1 shows the percentage occurrence of the different types of cytopathic effects. Haemorrhage, pocks, embryo death and other cytopathic effects had percentage occurrences of 32.2%, 26.4%, 20.2% and 210.2%, respectively.

Plates I, II, III and IV show pictographs of inoculated eggs that produced viral growth. Here, the virus produced morphological changes (plaques) at the site of inoculation, resulting in cytopathic effect, bleeding/haemorrhage of the entire egg, the formation of visible lesions known as "pocks", and the death of the egg embryo.

DISCUSSION

The observation that 53.3% (208/390) of inoculated embryonated eggs demonstrated evidence of viral growth, manifested by plaque formation, generalized hemorrhage ("bleeding") of the embryo, pock lesions, and eventual embryo death, provides important insight into the infectivity, replication efficiency, and cytopathogenic characteristics of the virus under investigation. Embryonated egg inoculation remains a classical and highly sensitive method for the isolation and propagation of viruses, particularly those capable of replicating in chorioallantoic membranes and embryonic tissues (Banda and Yan, 2022). The constellation of findings observed in this study is consistent with active viral replication. Plaque formation indicates localized destruction of infected cells due to viral cytopathic effects, while the presence of pock lesions suggests focal areas of viral multiplication, often associated with viruses that exhibit tropism for epithelial or membrane tissues (Laleye *et al.*, 2022). The occurrence of diffuse hemorrhage within the embryo reflects severe tissue damage, likely resulting from viral-induced disruption of

vascular integrity and cellular lysis. Ultimately, embryo death represents the culmination of extensive viral replication and systemic pathogenic effects (Banda and Yan, 2022; Nuñez *et al.*, 2024). The relatively high proportion of eggs showing these changes (over half of the inoculated samples) suggests that a substantial number of the clinical specimens contained viable and infectious viral particles. This finding supports active viral shedding in the study population and indicates that the virus has a strong ability to adapt to and replicate within the embryonated egg system (Banda and Yan, 2022). However, the fact that 46.7% of the eggs did not exhibit observable viral growth may be attributed to several factors. These include low viral load in some specimens, degradation or inactivation of the virus during sample handling and transport, or the presence of inhibitory substances (Wang *et al.*, 2023). Additionally, variability in inoculation technique or differences in the susceptibility of individual embryos could have contributed to this outcome (Wang *et al.*, 2023). From a virological perspective, the observed cytopathogenic features align with the known behavior of highly lytic viruses, including members of the Herpes simplex virus type 2 family, which are capable of inducing cell death and tissue destruction in infected host (Dass *et al.*, 2025). The ability to produce visible lesions, such as plaques and pocks, further reinforces the pathogenic potential of the isolates and supports their suitability for downstream analyses, including viral titration, pathogenicity assessment, and molecular characterization (Kun-Varga *et al.*, 2023; Rashidi *et al.*, 2024).

This observations in this study is in line with Rodgers (2015) who reported infection, haemorrhage and death of chick embryos after inoculation of HSV-2 onto the chorioallantoic membrane with a high degree of virulence of the virus. Nath *et al.* (2021) also reported changes in cell morphology, such as cytoplasmic granulation, cell rounding or lysis, indicating the presence of HSV-2.

Le Goff (2014) reported that viral culture remains the test of choice in the diagnosis of HSV genital infection. Laboratory confirmation of clinically suspected genital herpes is necessary, as it helps identify persons at risk of transmitting the infection (Glass *et al.*, 2002). Lui *et al.* (2015) also reported that viral culture is a classical method for the diagnosis of genital herpes, though it is time-consuming. However, in developing countries like Nigeria, egg culture may be valuable, as it is relatively inexpensive and readily available. The cultivation method could be a promising approach for vaccine production, as viral attenuation can be easily achieved through multiple passages.

Other methods used in the diagnosis of HSV-2, such as direct immunofluorescence assay using fluorescein-labelled monoclonal antibodies on smears or enzyme immunoassay on swabs, have shown high specificity and sensitivity (Guzman *et al.*, 2014), though most researchers continue to hold the view that culture remains valuable.

CONCLUSION

Herpes Simplex Virus-2 grew and replicated in more than half (53.3%; 208/390) of the embryonated eggs, causing cytopathic effects such as plaques, pocks, egg bleeding or haemorrhage, and embryo death.

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