

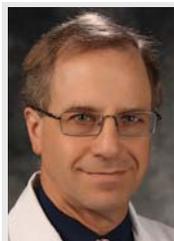
A risk for non-sexual transmission of human papillomavirus?

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Human papillomaviruses (HPVs) are estimated to be the most common sexually transmitted virus in humans. The virus is of great interest as it is the etiological agent of cervical cancer. Sexual transmission of HPV is generally accepted, however, non-sexual transmission of the virus is often debated. Here, we review the evidence from basic research and clinical studies that show HPV can survive well outside of its host to potentially be transmitted by non-sexual means. In doing so, we hope to discover problems in current prevention practices and show a need for better disinfectants to combat the spread of HPV.

Human papillomaviruses (HPVs) are small DNA tumor viruses and are estimated to be the most common sexually transmitted viruses in humans. To date, over 200 types have been identified. All HPV types are epitheliotropic, however, they are subdivided on their ability to infect either mucosal or cutaneous keratinocytes. Of the types that infect mucosal keratinocytes, further subdivisions can be made as to whether the virus causes benign neoplasms such as warts (low risk) or malignant neoplasms such as cervical cancer (high risk) [1,2]. Typically associated with genital disease and cancer, HPV can also lead to head and neck cancers. These viruses are stable and able to remain infectious on surfaces, even when treated with common disinfectants. Vaccinations against HPV infection has increased public awareness about how HPV is sexually transmitted to cause cervical cancer [3]. However, there is a certain level of risk to encounter HPV infection through non-sexual means. In light of this information, we explore the potential risks and current findings of vertical and non-sexual transmission of HPV to help prevent future spread of this virus and highlight gaps in current prevention practices.

HPV life cycle

HPV virions are non-enveloped, contain histone-associated dsDNA and have

icosahedral capsids. The viral genome is circular and approximately 8 kb in length, replicating within the nuclei of the host cell. The viral genome has an average of 8 open reading frames (ORFs), which are expressed from polycistronic mRNAs transcribed from a single DNA strand [4]. The ORFs can be divided into early and late genes. The early ORFs encode the E1, E2, E4, E5, E6 and E7 proteins [4]. The E1 and E2 genes have been shown to regulate viral replication as well as coordinate the expression of other early genes [5]. The E4 protein is thought to be involved in disrupting intermediate filaments in the keratin cytoskeleton facilitating virion release into the environment [6]. The oncogenic E5, E6 and E7 proteins that are encoded by the high-risk types are able to transform and stimulate cell growth [7]. The late ORFs encode the L1 and L2 proteins. L1 is the major capsid protein and L2 is the minor capsid protein.

The life cycle of HPV is dependent on the host cell differentiation program. Initial infection by HPVs is thought to occur through microabrasions of the epithelium, thus allowing entry of the HPV particle into cells of the basal layer. Infection of the basal layer is the first step to the potential development of HPV-related disease. There is a very strong correlation between malignant

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progression with certain HPV types such as HPV16, HPV18, HPV31 and HPV45 [1,2]. These types are labeled as 'high risk'. The most significant risk of the development of cervical cancer is an infection with any high-risk HPV type [8]. Other HPVs labeled as 'low risk' such as HPV6 and HPV11 cause benign growths. More recently, HPVs have been found to also contribute to a growing percentage of oropharyngeal cancers [9]. Even though sexual transmission is well documented as the primary transmission route of HPV, there is the potential for non-sexual transmission.

The stability of HPV

Viruses that infect through non-direct methods such as fomites often have to survive great environmental stresses in order to reach their host. As stated previously, HPV requires its capsid to be assembled in differentiated epithelium. This restriction is based on HPV's need for capsid gene expression, but is also required for an intricate process of disulfide bonding that takes place to properly assemble the virus through a redox gradient that naturally occurs in the human epithelium [5,7,10-16].

Many viruses undergo structural changes, called maturation, in order to complete their life cycle [17,18]. Research on recombinant HPV particles, grown from expression vector systems in monolayer, showed dependence on the redox potential of their environment, as their capsids become more ordered after an overnight incubation in an oxidizing solution as viewed under electron microscopy [19]. Also, exposure of the capsid to oxidizing agents such as oxidized glutathione can reduce the time of capsid maturation [20]. This change is characterized by an increased resistance of the capsid to proteolysis and chemical reduction [19]. For both HPV16 and HPV18 recombinant particles, capsid maturation produces an increase in the amount of dimeric and trimeric L1 species [19]. It was shown that the change in morphology during maturation is driven by the formation of inter-pentameric L1 disulfide bonds that condense the capsid and increase its stability [19]. Native HPV16 virions isolated from differentiating epithelium also share a dependence on the redox potential. When comparing virions harvested from 15- and 20-day organotypic tissues, virions were more resistant to breaking apart during ultracentrifugation when compared with virions from 10-day tissues, suggesting a capsid that could withstand a more stressful environment [12]. Upon testing infectivity, it was found that 20-day virions were twice as infectious per particle than 10- and 15-day virions. An increase in infectivity is one commonality that usually occurs when other viruses have undergone a maturation step [18]. Analyses of 10- and 20-day tissue sections show that 10-day virions were mostly localized within the nuclei of the suprabasal epithelial layers, whereas 20-day virions were mostly found in the top cornified layers of the epithelium. This change in localization within the tissue coincides with a natural redox gradient that occurs within human tissue, with the lower part of the tissue being a reducing environment and the upper part of the tissue being an oxidizing environment [12]. Because of this, it is now hypothesized that the virion's maturation occurs as it

moves from a reducing environment at 10 days to an oxidizing environment at 20 days. This redox gradient seems to be responsible for the creation of disulfide bonding found within the capsid, creating a more stable structure.

As discussed previously, disulfide bonds formed within the oxidizing environment of the cornified layer of the epithelium are thought to be important in completing the maturation of the HPV virion [12]. Previous studies with HPV in monolayer culture using recombinant particles, as well as in differentiating tissue systems using native virus, have shown dependence on L1 inter-pentameric disulfide bonding at three conserved cysteines: C175, C185 and C428 [11,21-23]. Three other cysteines, C161, C229 and C379, function as transient disulfide bonds, potentially guiding the virion through the assembly process to a mature capsid conformation [24]. Other non-enveloped viruses, such as polyomaviruses, have cysteines located within their capsid protein that function in this way. All evidence points to the importance of these L1 cysteines during the formation of native HPV in the context of a differentiating epithelium.

The HPV capsid is highly stable by itself, however, it is important to note that this virus is transmitted along with sloughed off epithelial cells. It is unknown whether free virions or infected keratinocytes are deposited in microabrasions to cause an infection. Clearly, surfaces can become contaminated with both virus and cellular debris. At the end of a keratinocytes differentiation program, the cell is little more than a bag of keratin fibrils. Keratin is a strong, insoluble protein that gives the outer layer of skin its strength and flexibility. This network of keratin, along with the protection of the intact cellular membrane may act as a secondary structure to protect the viral capsid from environmental stress, allowing it to survive longer than it would by itself.

HPV & disinfectants

Viruses that can be spread through non-sexual means are typically stable and able to be spread via fomites. A study using recombinant HPV16 particles and BPV-1 virions was performed to test the ability of papillomaviruses' resistance to desiccation, which would be crucial in determining their ability to survive long-term on surfaces [25]. BPV-1 is already implicated in non-sexual transmission in cattle and is known to be spread by contact with fomites [26]. It was discovered that both viruses had similar resistance to desiccation, retaining 50% infectivity after 3 days at room temperature [27]. This information provides the foundation for a risk of HPV to be spread via non-sexual means.

Even though HPV is resistant to desiccation, in a society that values cleanliness, evident by the abundance of hand sanitizers, the viral stability will be challenged often by disinfectants. What little is known about HPV's resistance to virucides has been taken from studies using recombinant particles or hospital screenings of potentially infected equipment. Current protocols use the outcomes of disinfectant studies from surrogate viruses thought to have similar stability and resistance as HPV.

To date, only one study has been performed to test whether HPV16 can survive treatment of common disinfectants. Using HPV16 virions, the susceptibility to 11 common clinical disinfectants was tested [28]. This study produced some interesting findings. First, HPV was shown to be resistant to inactivation by gluteraldehyde (GTA). GTA is used primarily in hospitals and is used as a broad-spectrum anti-microbial. Its ability to cross-link DNA and protein provides great killing ability among many microbial pathogens. Non-enveloped viruses are notoriously hard to neutralize, but GTA has been shown to be effective against some including adenoviruses, parvoviruses, caliciviruses and many enteroviruses [29–31]. HPV also showed resistance to *ortho*-phthalaldehyde (OPA), a new alternative to GTA [28]. HPV was found to be sensitive to hypochlorite and to a *para*-acetic acid-Silver-based disinfectant [28]. Like most non-enveloped viruses, HPV was resistant to alcohol-based disinfection, including ethanol and isopropanol [28]. This resistance to alcohol provides evidence that the often relied upon hand sanitizer systems do not effectively control the spread of HPV. Also, the desiccating qualities of alcohols may work in the virus's favor, as it has been shown that disinfectants work less effectively after a contaminant has dried on a surface [32]. Combined, the identification of HPV as an extremely stable virus as well as its ability to be resistant to common disinfectants provides grounds that this virus is available to be transmitted via inoculated fomites and surfaces.

Potential routes & evidence of non-sexual transmission

As discussed, HPV is a stable virus and has the ability to survive an onslaught of chemical treatment and still able to be infectious. While the typical route of infection occurs through sexual contact, other possibilities, albeit at lower rates, can occur to transmit HPV from one human to another. The following are potential routes of non-sexual transmission of HPV, which have been clinically described or suspected based on available evidence and current knowledge about HPV.

One set of evidence highlighting a potential non-sexual route of self-inoculation of HPV infection is the detection of HPV positive female virgins. This study showed that 51.1% of women claiming not to have a history of sexual intercourse to be HPV positive, compared with 69.1% of sexually active women [33]. However, another study indicated that high-risk HPV was most likely only contracted via sexual contact, after following 100 virgins pre- and post-sexual contact [34]. Female virgins are often included in studies of sexually transmitted disease to serve as negative controls for subjects with no prior sexual contact. With any study, it is possible that the state of the female participants' virginity may not be accurate. Low-risk HPV infection by self-inoculation has been documented in children in cases where no signs of sexual abuse can explain the transmission of genital warts [26,35]. Of equal importance, two separate studies, one in a hospital and another at a university, found abundant detection of HPV DNA on the fingers of infected individuals [36,37]. This can be attributed to inadequate or non-existent hand washing by the infected individual. It was

not tested if intact, infectious virions were present on hands, but clearly if DNA can be found there, that possibility does exist. As previously discussed, those relying on hand sanitizers to disinfect their hands would not be effectively killing HPV. It should be noted that in either case, this evidence potentially signals the ability for individuals to self-inoculate with HPV via their hands due to their own improper hygiene or that of others they come in physical, but non-sexual contact with.

Vertical transmission from mother to child is another route of transmission. Potential options include *in utero*, during delivery or spread through contact with relatives or mother post-birth. The most probable transmission of mother to child is that of vaginal delivery, with the child passing through an infected birth canal [38–43]. It explains why HPV DNA is detected on infants [38]. Studies have shown that there is a correlation between a mother's HPV DNA load and their ability to transfer that DNA to their infant [41]. Sequencing of the detected genomes as well as typing analysis showed that mothers are directly responsible for their infant's inoculation. While an infected birth canal is a risk most notably for infants who are delivered vaginally, those delivered non-vaginally could potentially still have a risk. HPV DNA has been detected in both placenta and amniotic fluid from women with cervical swab samples that are HPV positive and there may be a risk for congenital infection [44–46]. However, the data indicate that the highest risk is coming in contact with an infected birth canal [38]. Viral DNA is often found in the newborn's oral cavity. Even though high-risk HPV_s appear to be linked to oral cancers, these incidences of vertical transmission of HPV are labeled as transient infections, as infants' oral cavities are HPV negative 1–2 months after being inoculated at birth, suggesting viral clearance [43,47]. In the case of vertical HPV transmission, however, non-oncogenic types create a potential health risk to infants [38,41]. Juvenile-onset recurrent respiratory papillomatosis (JORRP) is a rare disease of young children caused by infection with HPV types 6 and 11. As discussed, infants acquire JORRP at birth or potentially *in utero* from their mother's infected genital tract. Onset of disease occurs between ages 2 and 5. Cases after age 5 are fewer, but do occur. All cases below the age of 14 are classified as JORRP, with cases older than age 14 are considered caused by sexual contact [48]. This disease is rare, with only 820 confirmed cases annually [49]. The papillomas are not cancerous, but grow rapidly. Often repeated surgeries are required to clear the airway and as many as 100 may be needed before reaching the age of 10 years old [48]. The disease is type dependent, as HPV11 is associated with more advanced symptoms, with less occurring during infection of HPV6 [50]. There is also the potential for the growths to transfer to the trachea and lungs or for the disease to become malignant [48,51]. In an attempt to link risk factors to JORRP, the association between condylomas and pregnant women was evaluated in a population-based study in Denmark for a time period of 20 years [48,52]. They found that women with condylomas had a greater than 200-fold risk for their children developing JORRP than women who did not [52].

Hospital-acquired, or noscomial, infections are common for many viruses to gain a route to their host. A potential route for HPV noscomial infection are transvaginal ultrasound probes, which are highly used in the emergency and gynecology departments in healthcare settings to perform endovaginal cavity ultrasounds. This harmless procedure is used in the detection of biliary tract diseases, intrauterine pregnancy and abdominal aortic aneurysms. Though harmless, there is close contact between the probe and the cervix or vaginal wall, which provides a potential pathway for HPV infection. Probes are not disposable and are disinfected after each use with mild disinfectants to avoid damaging the sensitive equipment. Between patients, probes are also sheathed with a barrier to physically protect cross-infection. However, these barriers have a perforation rate of 1–9% [53–56]. One study collected 217 samples before and 200 samples after the ultrasound examination [57]. After the ultrasound procedure, 3% of the samples contained high-risk HPV types (16, 31, 53, 58) [57]. Similarly, HPV was detected in six pre-examination samples. This study, as well as others, showed that a large amount of ultrasound probes were contaminated with high-risk HPV [57–60]. As discussed, most clinical disinfectants are inadequate to neutralize HPV. Other studies looked at transvaginal ultrasound probes further and concluded that not only are they contaminated by HPV DNA, but also by free virions [59]. They determined this by treating swab samples with an exonuclease, which destroys all unprotected DNA. HPV DNA that is protected inside capsids is not destroyed and can be detected after liberating it from the capsid. This assay has been used reliably in many studies of HPV in basic research as well as with other viruses [11,12,24]. It is possible that more reusable medical equipment that comes in contact with areas infected with HPV could become contaminated. These studies indicate that noscomial infection of HPV is a real risk.

Regardless of how HPV is acquired, a breach in the epithelium is still thought to be a major commonality among all routes of transmission [12,13,24]. Epithelial microabrasions allow the virus access to the basal layer of the skin. Sexual activity can cause such breaches, and may also be responsible for allowing non-

sexual transmission to occur in the genital region. The oral cavity can also be infected by HPV. Here, activities including eating and tooth care cause damage to the epithelium and could potentially be involved in non-sexual transmission of HPV [61].

Conclusion

HPV is a major viral pathogen, as discussed, causing more diseases than just cervical cancer. Much work has been done identifying these viruses as the most common sexually transmitted disease. Here, we educate that HPVs are very stable viruses, able to survive on fomites and surfaces for days. This is based on multiple studies both in the laboratory and in real-world healthcare scenarios [11,13,24,25,28]. We also inform that many of the current disinfectants used to protect us from environmental or noscomial infection of microbes are ineffective at neutralizing HPVs. Again, there is evidence for this in comprehensive basic research studies as well as well-documented cases involving contaminated hospital equipment [28]. Because of these two factors, HPV is available to be transmitted through non-sexual means, either by way of mother to child, fomites, self-inoculation or noscomial infection. As discussed, some transmission avenues work better than others, as some result in transient infections with little risk to the individual. It is also possible that other less documented forms of transmission of HPV infection may be possible [62]. However, more research is necessary to determine the rate and consequences of non-sexually transmitted HPV. At the very least, new disinfectants or new disinfection protocols for healthcare equipment are in need to limit noscomial infections and public awareness needs to be increased about the potential of HPV infection through non-sexual means.

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