

Pox & Human Papilloma Viruses

This lecture will discuss two viruses, or rather a family of viruses (*Poxviridae*) and a single virus (*HPV*).

Poxviridae

Properties

- **dsDNA** viruses which replicates in the **cytoplasm**¹ (*Unique feature*)
- **Large**² viruses ($100 \times 200 \times 300$)nm
- **Brick-shaped** envelope.
- **Complex** capsids (*Not icosahedral or helical*) = Not normal capsids
- Envelope (*Double membrane*) is **neither** acquired from Golgi Apparatus or cell membrane but is **virus-made**.
 - Made by the virus during the replication in the cytoplasm.
 - **Upon exit**, it acquires another membrane from Golgi which **surrounds the whole virus**.
 - Upon exit, the Golgi membrane is lost so we can say that it exits by **exocytosis**.
- Infects humans, mammals, birds & insects.
- Has 3 or 4³ genera⁴.

Epidemiology

- **Dropped** from national (*Regular*) vaccine programs in some countries in **1972**.
- Completely eradicated in **1977**.
- Last reported case was in **Somalia**.

Viruses

(*All of these are seen in animals.*)

- Smallpox
- Vaccinia (*Used in vaccines*)
- Molluscum contagiosum (*MCV, associated with cutaneous lesions but no systemic involvement*)
- Orf
- Cowpox
- Pseudocowpox
- Milker's Nodules⁵

Pox **can be removed** from national vaccine programs if it is eradicated in a country and not the world because it has **no asymptomatic carriers**. The same cannot be said for Polio.

Polio is almost eradicated from all parts of the world except for:

- Subcontinent India
- India
- Afghanistan
- Pakistan
- Nigeria

But that does not mean you can drop it from vaccine programs because it **has** asymptomatic carriers.

¹ Remember that most DNA viruses replicate in the nucleus.

² Used to be the biggest virus before Mimivirus was discovered.

³ Wikipedia says there are 4 genera which can infect humans.

⁴ You don't have to know which virus belongs to which genus but you must know the viruses and their properties.

⁵ This is actually a disease caused by the virus but the professor mentioned it here as a virus.

Variola (Smallpox)

Properties

- Has (*Used to have*) two types
 - Variola Major ⁶
 - Death rates range between **3% to 35%**
 - Variola Minor (*Alastrim*) ⁷
 - Death rates less than **1%**.
- Lesions are characterized by **uniform** papulovesicles which pustulate then heal slowly.
- Incubation period is around **two weeks** but can be shorter.
- Prolonged survival in extracellular environment.
- Zoonotic (*Transmitted between species; monkeys and cows*) and causes mild disease in humans.⁸
- **Highly contagious** in humans through:
 - Respiratory Route
 - Direct contact with a lesion
 - Fomites infected with the virus
- Why do we still study about this virus? (*Some previously mentioned points will be repeated*)
 - It can survive well in extracellular environment.
 - Very stable in its freeze-dried⁹ form for long periods (*Structure, function, and infectivity don't get affected*).
 - Pox in this form can be found in two institutions in: **Moscow, Russia** and the **United States of America** (*Center for Disease Control; CDC*).
 - **High infectivity**¹⁰ in humans.
 - **Limited** supply of vaccines.
 - **No** specific anti-viral therapy.
 - Can be used in **warfare** and **bioterrorism**¹¹.

Pathogenesis

- Once the virus is in the cytoplasm, it **shuts off synthesis of host cell proteins** in favor of its own.
- It also changes the cell's **permeability**, eventually leading to death.
- Produces **eosinophilic cytoplasmic inclusion bodies** known as **Guarnieri bodies**¹².

⁶ More severe and most common form of smallpox, with a more extensive rash and higher fever.

⁷ Less common and much less severe.

⁸ These viruses are still monitored because they can mutate and become more virulent.

⁹ To freeze-dry something is to preserve it by rapidly freezing it and then subjecting it to a high vacuum that removes ice by sublimation.

¹⁰ The frequency with which an infection is transmitted when contact between a virus and host occurs (*Catching the virus*)

¹¹ Stable in aerosol form and very small dose is needed for infection thus making it a Class A Bioterrorism agent.

¹² Appear as pink blobs in cytoplasm of affected epithelial cells stained with eosin. Characteristic of Poxviruses.

Clinical Manifestations

- Sudden onset of fever
- Chills
- Myalgia (*Pain in muscles*)
- Rash
 - Develops **3 to 4 days** after the last 3 symptoms
 - Starts as a **maculopapular** rash which turn into **vesicles, pustulate** then heal slowly **without** leaving any scars.
- Hemorrhagic rash (*Sledge hammer*)
 - Happens in certain cases when **bleeding into lesions** occurs.
- Bacterial superinfections
 - Happen as a result of lesions breaking skin which is the body's first defense barrier.
 - Can be **fatal** if it leads to development of **sepsis**.
- Refer to slide 6 for notes about the pictures
 - Smallpox can be widespread all over the body.
 - All lesions are in the same stage of development (*Uniform*) in contrast to chickenpox¹³.

Diagnosis

- **Scraping** of vesicle for
 - Virus culture
 - Polymerase Chain Reaction¹⁴ (*PCR*)
 - Electron microscopy

Prevention

Edward Jenner was a scientist who noticed that most milkmaids¹⁵ developed cowpox (*Usually presented as a solitary lesion on their hands*) and were immune to smallpox. This observation inspired him to come up with the idea of vaccines.

- Vaccinia virus is used as a **vector** for the vaccine.
- The vaccine includes a **recombinant of smallpox and cowpox** (*Or horse-pox, as it is sometimes called*).
- Vaccine for smallpox follows the **usual course of normal smallpox** and causes a **localized lesion** at the site of injection.
- Vaccination does **not** provide life-long immunity but wanes after around **3 years**.
- Despite the relatively short duration of its effect, it was successful in achieving eradication by vaccination within short periods.

¹³ Another difference worth knowing is that Smallpox starts from the periphery while chickenpox starts from the trunk.

¹⁴ Biochemical test to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

¹⁵ Women who milk cows.

Molluscum Contagiosum

Properties

- Spreads by:
 - Direct contact
 - Towels
 - Sex
- Incubation period is **longer** than smallpox, ranging from **2 to 8 weeks**.
- Characterized by **painless nodules** (*Pearl-like lesions with cheesy material center*)
- **No systemic** involvement, **only** cutaneous lesions.

The professor will **not** ask about incubation periods but you **must** know the **contagiousness periods** for:

- Rubella (*German Measles*)
- Rubeola (*Measles*)
- Chickenpox

Diagnosis

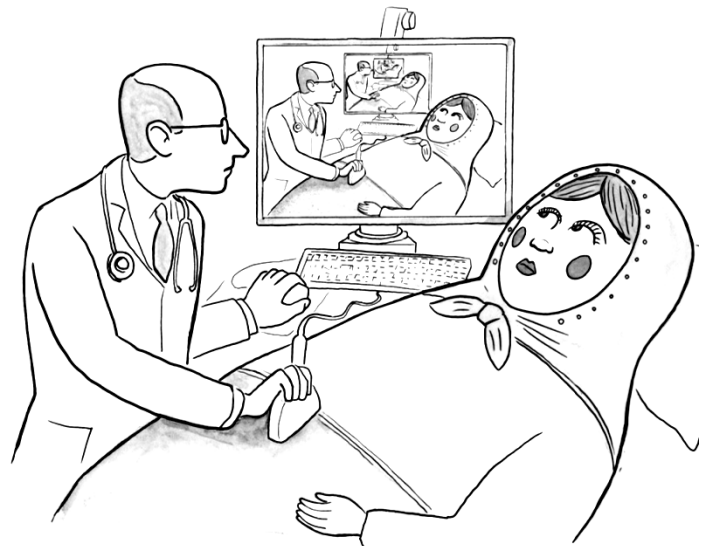
Clinical picture, which can be confirmed by the presence of **eosinophilic inclusions in cytoplasm** of epithelial cells (*Molluscum bodies*).

Treatment

- **No** specific treatment.
- Lesions usually disappear in **2 to 12 months**.
- Can be removed **surgically** or by **curettage**¹⁶ (*Picture in slides*) for cosmetic reasons.

Please refer to slide 9 for some notes regarding the pictures in it

- The top picture shows MC (*Refer to its characteristics above*).
- The two bottom pictures show Orf and Cowpox which infect sheep & goats and cows respectively.
 - When they infect humans, they are usually **solitary** or single lesions.
 - Begin as a **vesicle** which enlarges inside and then starts to become **necrotic in the middle**.
 - Healing of the lesions in Orf takes **about a month** while the one in Milker's Nodules takes a little bit **longer**.

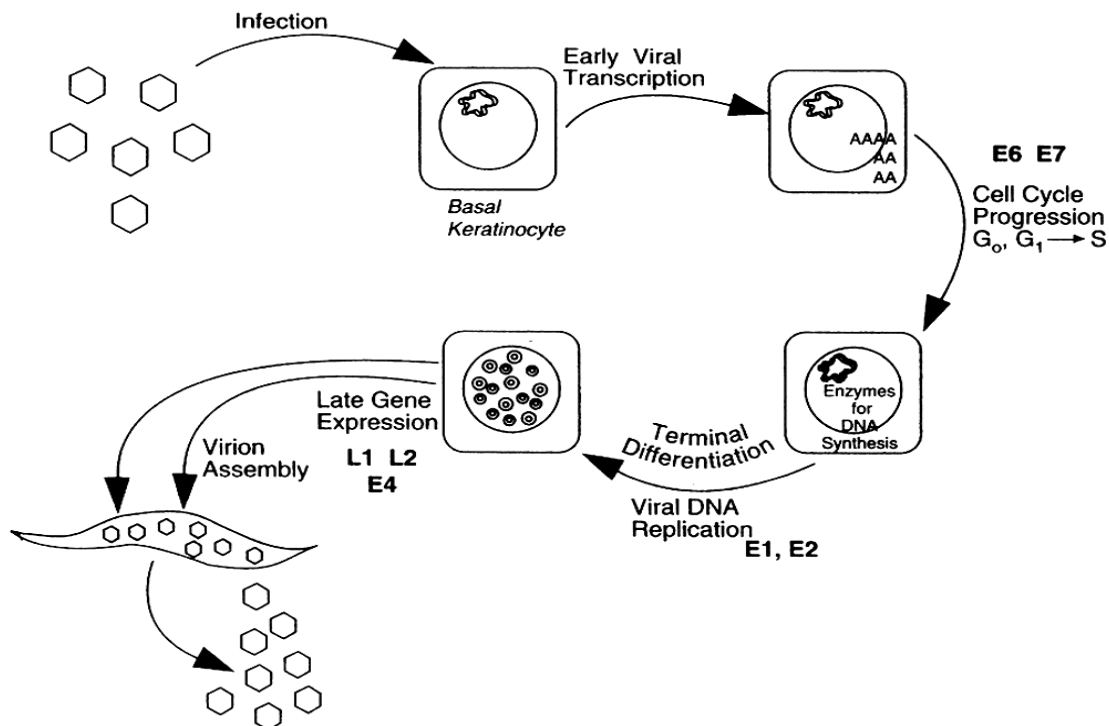


¹⁶ Curettage is the use of a curette (*French, meaning scoop*) to remove tissue by scraping or scooping.

Human Papilloma Virus

Properties

- Does **not** encode its own polymerases but **depends on cellular machinery** for its replication (*Unique Property*).
- Small naked dsDNA virus
- Genome encodes **8 early genes** (*E1-E8*) and **2 structural proteins** (*L1 & L2*).
- **Icosahedral** capsid which is composed of two proteins (*L1 & L2*)
- More than 100 serotypes (*Most of which are not associated with disease*).
- **Cannot** be grown in cell culture, which limits our knowledge about its replication cycle and pathogenesis.
- Associated with **malignancy** (*Proteins E6 & E7*) such as cellular dysplasia or precancerous lesions
 - Just like **herpesviridae** and **Adenovirus**
 - Adenovirus was **not** associated with malignancy in humans, only in animals so theoretically speaking, it *could* be associated with malignancy in humans but the relation hasn't been established yet.
 - Herpesviridae, Adenovirus and HPV all have one thing in common which is **capability of producing a latent infection**.
 - Herpes' latency can last for **years if not decades**.
 - Adenovirus's shedding and production can last for a **year and a half** with **no symptoms** (*Temporary latency*).
 - HPV is capable of latency but the latent infection is usually cleared within **18 to 24 months** (*Temporary latency*).
 - **Most men and 97% of women** clear the virus within a **year and a half**.



Replication

Not much is known about it but we do know that:

- Infects **basal layers of squamous epithelium** or has affinity for **junctions** between squamous and columnar epithelium such as seen in the **anus and cervix**.
- The virus is **internalized¹⁷ uncoated** and then enters the **nucleus** where its replication takes place like a typical DNA virus.
- Host RNA polymerase transcribes E genes followed by early protein synthesis.
- **E6 and E7** play a role in cellular **transformation** leading to **excessive cell division**.
 - E6 bind to **p53** and E7 to **p105RB** proteins **disrupting cell cycle regulation** because both previously-mentioned **genes¹⁸** are **tumor-suppressor genes** with active roles in regulation.
 - p53 repairs DNA damage by **stopping the cell cycle at the G₁** phase to give the cell enough time to repair it or by **inducing apoptosis** if the damage is too great.
 - So binding to p53 causes the cell to divide **continuously without control**.
 - Retinoblastoma gene plays a role in regulating the cell cycle by **preventing the cell from entering the division state** until it's ready in order to minimize faults.
 - This protein has a '**pocket**' and E7 was found to **attach** to that pocket and prevent its activity.
- The dividing cell carries viral genome as **extracellular DNA**.
 - Most of the time, it's in the form of **episomes¹⁹** (*Like Herpesviridae*).
 - **Occasionally**, it was **integrated** within the host's genome.
- Viral DNA synthesis occur at two levels directed by cellular DNA polymerase
 - Latent Infection
 - Virus lies latent in the **lower epidermis**
 - Vegetative DNA Replication
 - **Active** replication of the virus occurs in **differentiated epithelial cells**.
- Epithelial cells differentiate into keratinocytes where capsid proteins are synthesized and DNA replicated.
- DNA replication and synthesis **peak** at a certain time and then the virus **assembles in the nucleus** and virus is released by cell **lysis** since it's a naked virus.

Epidemiology & Prevalence

- **Most common** sexually transmitted disease (*STD*).
 - An estimated 9.2 million sexually active adults (*15 - 24 years*) are infected with genital HPV.
 - An estimated **5% to 30%** of infected people might be infected with multiple serotypes.

¹⁷ The entering of cells by viruses following virus attachment.

¹⁸ Genes encoding the proteins

¹⁹ Closed circular DNA molecules that are replicated in the nucleus.

Pathogenesis

Transmission through:

- **Direct skin-to-skin contact** (*Primary route*), more specifically **sexual contact** with infected:
 - Penis
 - Scrotum
 - Vagina
 - Vulva
 - Anus
 - Anal involvement is seen mostly in **homosexuals**, especially ones with HIV.
- Contact with infected lesion can also lead to disease development.
- **Perinatal** (*During the passage of the baby through the birth canal*)
 - Baby usually develops **oral or pharyngeal** papilloma.

Infectivity is **60%** but most infections are **asymptomatic**.

Risk Factors

- Young age (*Less than 25 years*)
- Multiple sex partners
- Early age at first intercourse (*Best time to give vaccine is before becoming sexually active*).
- Male or female partner has (*or has had*) multiple sex partners.

Average incubation period is **long**, varying from **3 weeks to 1 year** or more so a patient can get infected but not develop symptoms for years.

Clinical Manifestations (*In a wide range of vertebrae including humans*)

- Papilloma²⁰
- Cutaneous Warts
 - Usually occur in **children and young adults**.
 - Cause the body to develop **specific protective immunity** against the serotype it has been infected with.
 - Vaccines might not be effective with people already infected with one or more of the serotypes in the vaccine since they already developed immunity.
 - Serotypes **6 and 11** were associated with warts **only**.
- Serotypes **16, 18, 31, 45 and 56** are associated with **malignancy and wart lesions**.
 - Serotypes **16 and 18** are **most commonly** associated with malignancy.

²⁰ Benign epithelial tumor growing exophytically (*outwardly projecting*) in nipple-like and often finger-like fronds.

Please refer to slide 18 for some notes regarding the pictures in it

- Genital warts are **unsightly cauliflower-like growths**, usually caused by serotypes **6 and 11**.
- Serotypes **16 and 18** are associated with **malignant** genital warts (*Can lead to cervical or penile cancer, especially 16 for penile carcinoma*)
 - It was noted that **uncircumcised males** are **more prone** to malignancy so maybe cell transformations occurs in that part.
- Symptoms (*May recur from time to time*)
 - Single or multiple fleshy growths around the penis, scrotum, groin, vulva, vagina, anus, and/or urethra in males.
 - Itching
 - Bleeding
 - Burning
 - Pain
- Locations of lesions (*As seen in pictures*)
 - Penis
 - Thigh
 - Anus and perianal area
 - Vulva

Diagnosis

- Pap smear for females (*Looking for precancerous transformation or cervical dysplasia*)
- PCR (*Rarely used*)
- Immunofluorescence Tests (*Rarely used*)
- **No** screening test for males.

Treatment

- Surgical excision of lesion
 - There's a great chance for recurrence because removing the lesion **doesn't** remove the virus from the body.
- Medical Treatment
- Cryotherapy (*Lesion can be removed with liquid nitrogen*)
- Electrosurgery (*Using an electric current to remove warts*)
- Radical surgery and radiotherapy are a **must** in case of carcinoma.

Remember that anti-viral drugs do **not** work on latent infections because they need actively-replicating viruses.

Prevention

- Vaccine
 - Relatively **new** vaccine²¹ so the exact coverage period of the vaccine is still being studied but thought to be **5 to 7 years**, maybe longer.
 - **First** vaccine to **prevent cervical cancer**.
 - There are two types:
 - **Gardasil**
 - Includes serotypes **6, 11, 16 and 18**.
 - **Recombinant** vaccine which has an **inactive** capsid protein (*L1*)
 - We have a virus like particle (*VLP*) and within is the inactivated *L1*.
 - There is **no** chance of developing symptoms because this is **not** a live attenuated vaccine and does not have the whole components of the virus.
 - Approved for use in **females and males** as well from **9 to 21 or 26** (*Most importantly before sexual activity*)
 - Given on **3 doses** in the period of **6 months** (*0, 1 or 2 months then 6 months²²*)
 - **Cervarix**
 - Includes only serotypes **16 and 18** (*The most oncogenic serotypes*).

Please note that the footnotes are extra information for explanation and not included in the exam.

Please accompany this sheet with the slides, the professor mentioned at least 90% of all information in the slides but it's better to skim through them at least after the sheet.

College in a nutshell

Goes to class: Teacher repeats the same damn thing again. Nothing important happens.

Misses one class: The cure to cancer is created, Waldo is found, AIs took over, the second coming of Jesus Christ took place and the Fire Nation attacked.

²¹ Approved in 2009 in the USA and 2007 in Australia and some other European countries

²² Months at which doses should be given