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 x 200 x 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 4\(^3\) genera\(^4\).

**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 Nodules\(^5\)

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\(^1\) 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
    - Death rates range between 3% to 35%
  - Variola Minor *(Alastrim)*
    - Death rates less than 1%.
- Lesions are characterized by **uniform** papulvesicles 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 bioterrorism11.

**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.

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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 chickenpox\(^\text{13}\).

Diagnosis

- **Scraping** of vesicle for
  - Virus culture
  - Polymerase Chain Reaction\(^\text{14}\) (**PCR**)
  - Electron microscopy

Prevention

Edward Jenner was a scientist who noticed that most milkmaids\(^\text{15}\) 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.

\(^\text{13}\) Another difference worth knowing is that Smallpox starts from the periphery while chickenpox starts from the trunk.
\(^\text{14}\) 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.
\(^\text{15}\) 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.

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 (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.

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

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

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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 **internalized**\(^{17}\) **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 genes\(^{18}\) are **tumor-suppressor genes** with active roles in regulation.
    - p53 repairs DNA damage by **stopping the cell cycle at the G\(_1\)** 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 **episomes**\(^{19}\) (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.

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\(^{17}\) 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)*

- Papilloma
- 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.

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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 vaccine\(^{21}\) 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 months\(^{22}\))
    - **Cervarix**
      - Includes only serotypes 16 and 18 (The most oncogenic serotypes).

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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.

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

22 Months at which doses should be given

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**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.