BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

Tamil Nadu, India

Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-V

Molecular Diagnostics

Dr. R. POORNIMA

Guest Faculty

Department of Biomedical Science

Unit V:

Molecular Diagnostics- Diagnosis of Mycobacterim tuberculosis, HCV & HIV; Conventional vs Molecular Diagnostics- Merits & Demerits. Biological warfare: Bacillus anthracis, H5N1, SARS- CoV, Chikunguniya- pathogenesis- diagnostic methods- Molecular diagnosis of single gene disorder- sickle cell anemia- Molecular HLA typing- Sequence Specific PCR (SSP), Sequence Specific oligonucleotide probe (SSOP), Sequence Based Typing (SBT)- Advantages of molecular HLA typing over serological methods- HLA typing and clinical significance- comment on Sensitivity and Specificity of clinical laboratory techniques- Quality assessment Programs (external & internal assessment Programs)

PRESENTATION: 1

Molecular Diagnostics: Diagnosis of Mycobacterium tuberculosis, HCV & HIV

1. Mycobacterium tuberculosis (TB):

- **Molecular Diagnostics:**

  Techniques like Polymerase Chain Reaction (PCR), Xpert MTB/RIF assay, and Line Probe Assay (LPA) are used.

  - **PCR:** Detects TB DNA, offering high sensitivity and specificity. It can be used on various specimens, including sputum.
  - **Xpert MTB/RIF:** An automated PCR test that identifies TB and resistance to rifampicin, a key antibiotic, in less than 2 hours.
  - **LPA:** Detects genetic mutations associated with drug resistance.

Advantages:

- Rapid diagnosis.
- High sensitivity and specificity.
- Detects drug resistance, aiding in appropriate treatment.

Challenges:

- Costly equipment and reagents.
- Requires technical expertise.

2. Hepatitis C Virus (HCV):

- **Molecular Diagnostics:**
  - **PCR:** Used for detecting HCV RNA, allowing early diagnosis and monitoring of viral load during treatment.
  - **Genotyping:** Identifies the HCV genotype, crucial for selecting appropriate antiviral therapy.
- **Advantages:**
  - Detects the virus before antibodies are formed.
  - Helps in monitoring treatment response.
- **Challenges:**
  - Expensive.
  - Limited access in low-resource settings.

3. Human Immunodeficiency Virus (HIV):

- **Molecular Diagnostics:**
  - **Nucleic Acid Tests (NAT):** Detect HIV RNA, allowing early detection even during the "window period" before antibodies develop.
  - **Quantitative PCR:** Measures viral load, which is important for monitoring disease progression and treatment efficacy.
- **Advantages:**
  - Early detection of infection.
  - Accurate monitoring of treatment response and disease progression.
- **Challenges:**
  - High costs.
  - Requires specialized infrastructure.

Conventional vs. Molecular Diagnostics

1. Conventional Diagnostics:

Methods: Include microscopy, culture, serology, and biochemical tests.

- **Merits:**
  - Cost-effective and widely available.
  - Easy to perform in basic laboratory settings.
  - Useful in detecting a wide range of infections.
- **Demerits:**

- Time-consuming (e.g., culture for TB takes weeks).
- Lower sensitivity and specificity.
- Inability to detect early infections or low pathogen load.
- Cannot detect drug resistance directly.

2. Molecular Diagnostics:

- **Methods:** PCR, NAT, genotyping, and other nucleic acid-based techniques.
- **Merits:**
  - High sensitivity and specificity.
  - Rapid results.
  - Can detect low levels of pathogens and early infections.
  - Identifies genetic markers of drug resistance.
- **Demerits:** Expensive and requires specialized equipment.
- Requires technical expertise and quality control.
- Not always accessible in low-resource settings.

BIOLOGICAL WARFARE:

BACILLUS ANTHRACIS, H5N1, SARS COV, CHIKUNGUYA- PATHOGENISS AND DIAGNOSTIC METHODS

Biological warfare:

- Biological warfare involves the use of biological agents such as bacteria, viruses, fungi, or toxins as weapons to cause illness or death in humans, animals, or plants.
- These agents are often chosen for their ability to multiply rapidly, spread easily, and cause significant harm.

Characteristics:

1. Agents:

Common biological agents include ***Bacillus anthracis*** (anthrax), Variola virus (small pox), ***Yersinia pestis*** (Plague), and botulinum toxin.

2. Delivery methods:

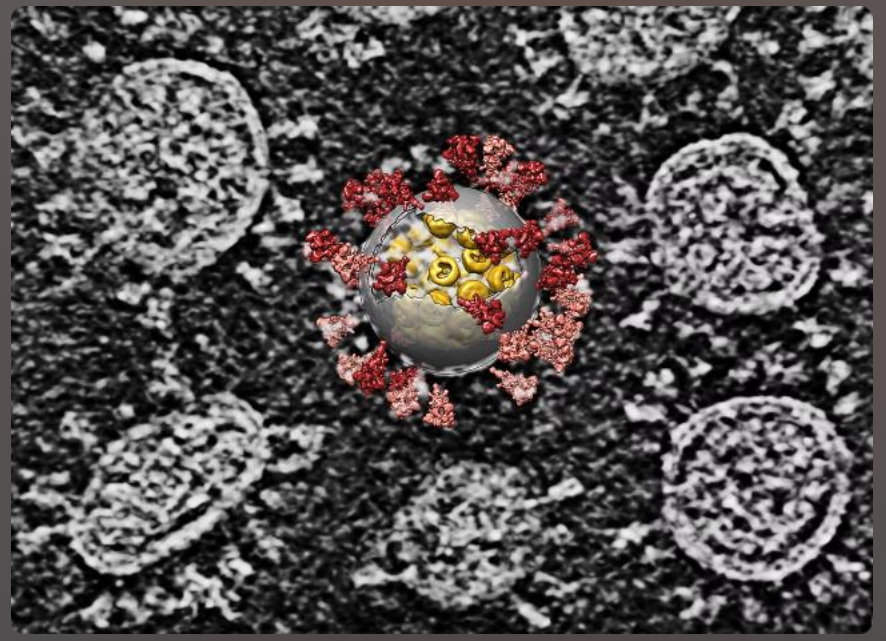
Biological agents can be disseminated via aerosols, food, and water contamination, or infected vectors.

3. Latency and transmission:

Some agents have an incubation period, causing delayed symptoms, and can be transmissible from person to person, leading to widespread outbreaks.

SARS-CoV: The Enigmatic Virus

SARS-CoV, also known as Severe Acute Respiratory Syndrome Coronavirus, is a highly contagious virus that can that can cause a severe respiratory illness in humans. It is a It is a member of the Coronaviridae family and was first first identified during an outbreak in 2003.



Origins and Emergence

1

Animal Reservoir

SARS-CoV is believed to have originated in bats, which serve as a serve as a natural host for the virus.

2

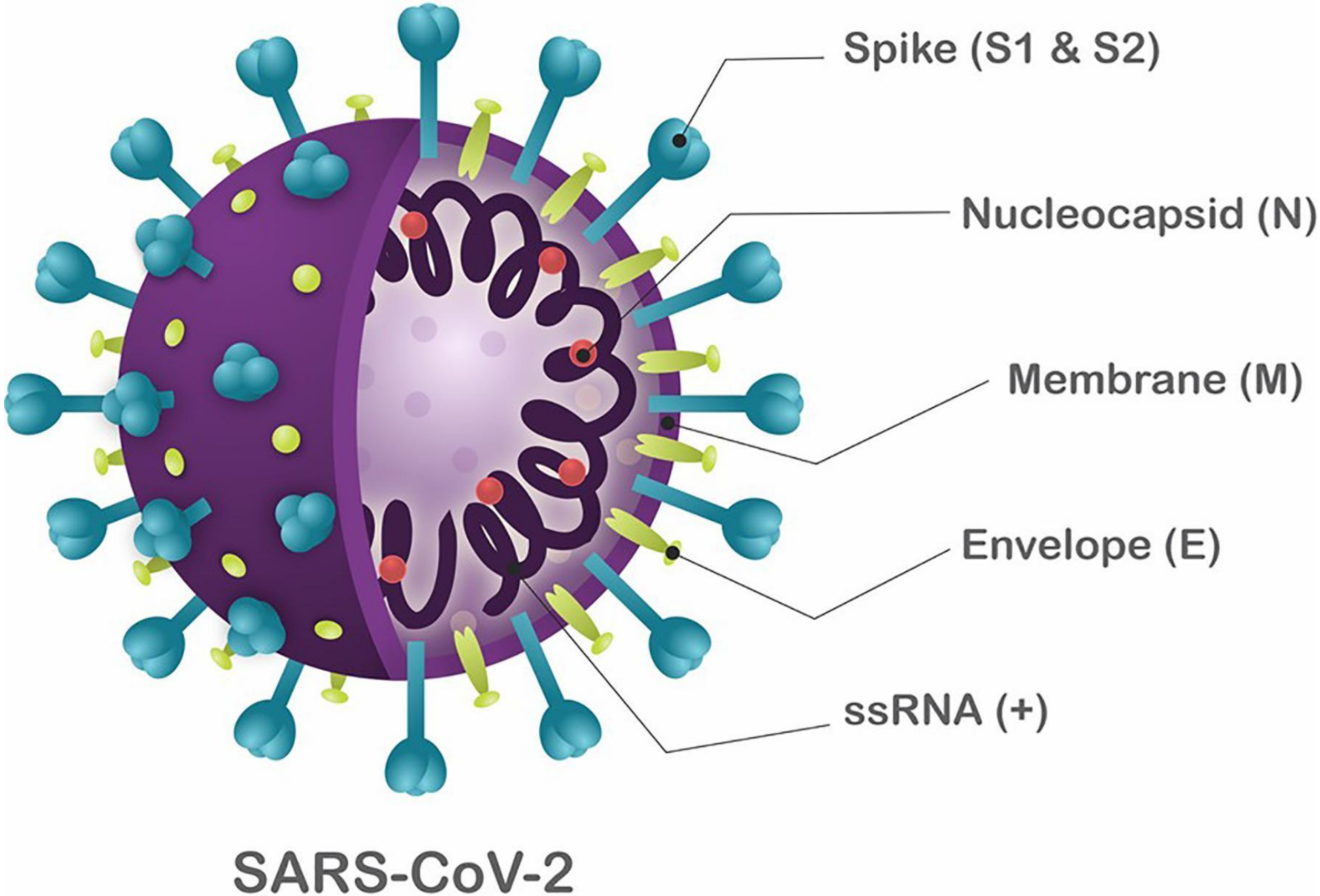
Zoonotic Transmission

The virus likely jumped from bats to humans through an intermediary animal host, such as civets or pangolins.

3

Global Outbreak

The first known human cases of SARS-CoV occurred in China in China in 2002, leading to a worldwide pandemic in 2003.


Spike (S1 & S2)
Nucleocapsid (N)
Membrane (M)
Envelope (E)
ssRNA (+)
SARS-CoV-2

Transmission and Infection

Respiratory Droplets

SARS-CoV spreads primarily through respiratory droplets expelled when an an infected person coughs, sneezes, or or speaks.

Close Contact

The virus can also be transmitted through close personal contact with an infected individual or by touching contaminated surfaces.

Incubation Period

The incubation period for SARS-CoV ranges from 2 to 14 days, during which which time an infected person can spread spread the virus without showing symptoms.

Symptoms and Disease Progression

1 **Fever**

Elevated body temperature is one of the most common symptoms of SARS-CoV infection.

2 **Cough and Shortness of Shortness of Breath**

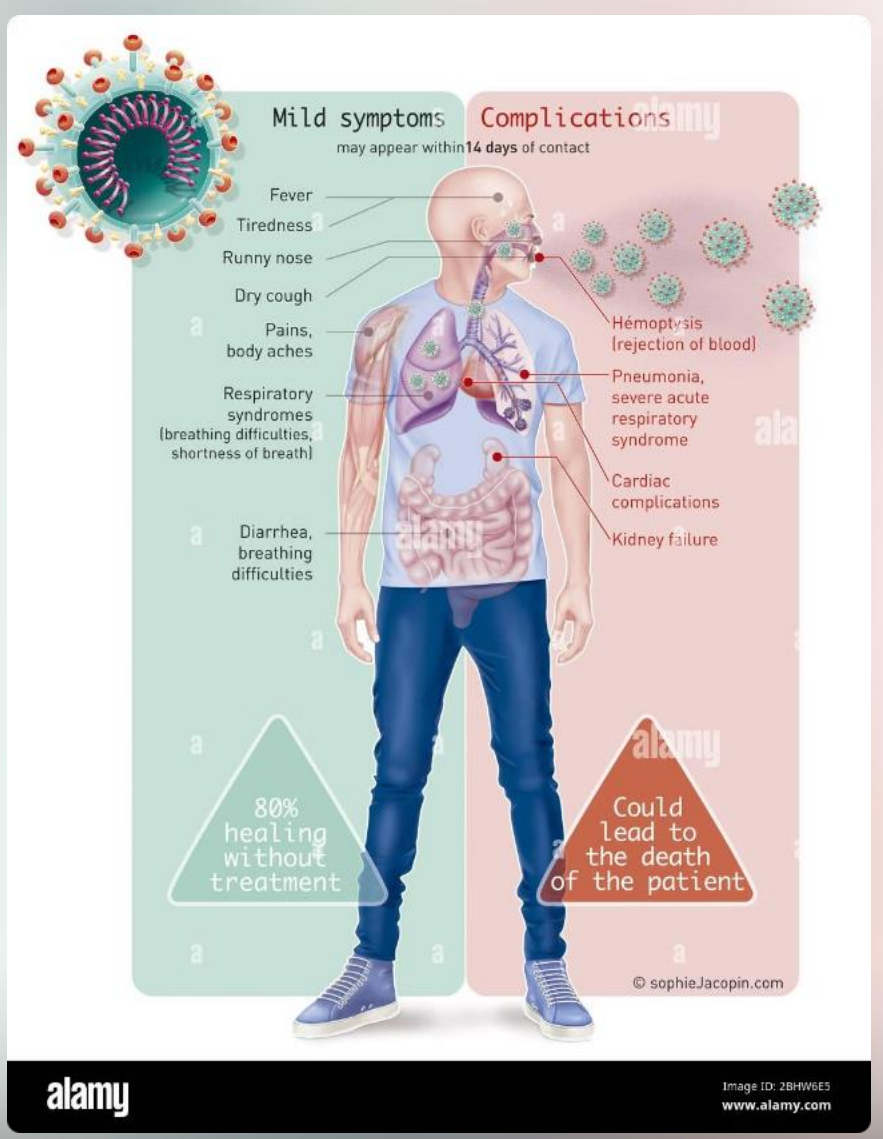
Patients often develop a dry, dry, persistent cough and experience difficulty breathing. breathing.

3 **Pneumonia**

SARS-CoV can cause severe pneumonia, leading to respiratory distress and potentially life-threatening complications.

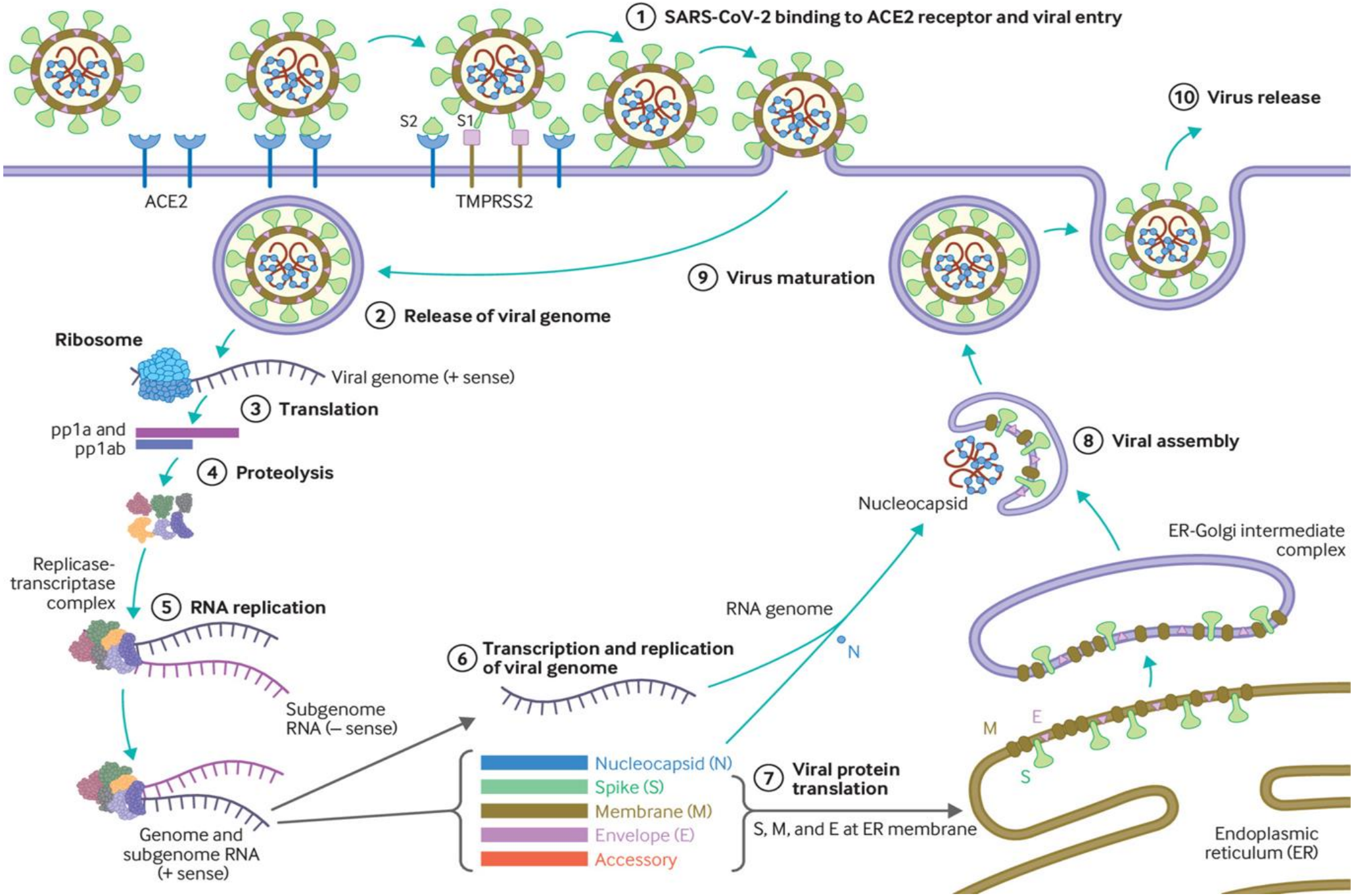
4 **Multiorgan Failure**

In severe cases, the virus can can attack multiple organs, resulting in organ dysfunction dysfunction and failure.



PATHOGENESIS

- SARS-CoV-2 binds to ACE 2, the host target cell receptor.
- Active replication and release of the virus in the lung cells lead to non-specific symptoms such as fever, myalgia, headache, and respiratory symptoms.
- In an experimental hamster model, the virus causes transient damage to the cells in the olfactory epithelium, leading to olfactory dysfunction, which may explain temporary loss of taste and smell commonly seen in covid-19.
- The distribution of ACE 2 receptors in different tissues may explain the sites of infection and patient symptoms. For example, the ACE 2 receptor is found on the epithelium of other organs such as the intestine and endothelial cells in the kidney and blood vessels.


1 SARS-CoV-2 binding to ACE2 receptor and viral entry
S2
S1
ACE2
TMPRSS2
10 Virus release
9 Virus maturation
2 Release of viral genome
Ribosome
Viral genome (+ sense)
3 Translation
pp1a and pp1ab
4 Proteolysis
Replicase-transcriptase complex
5 RNA replication
Subgenome RNA (– sense)
Genome and subgenome RNA (+ sense)
6 Transcription and replication of viral genome
RNA genome
N
Nucleocapsid
8 Viral assembly
ER-Golgi intermediate complex
M
E
S
Nucleocapsid (N)
Spike (S)
Membrane (M)
Envelope (E)
Accessory
7 Viral protein translation
S, M, and E at ER membrane
Endoplasmic reticulum (ER)

Diagnosis and Testing

Molecular Tests

Reverse transcription-polymerase polymerase chain reaction (RT-(RT-PCR) tests are used to detect detect the presence of SARS-CoV CoV genetic material.

Antibody Tests

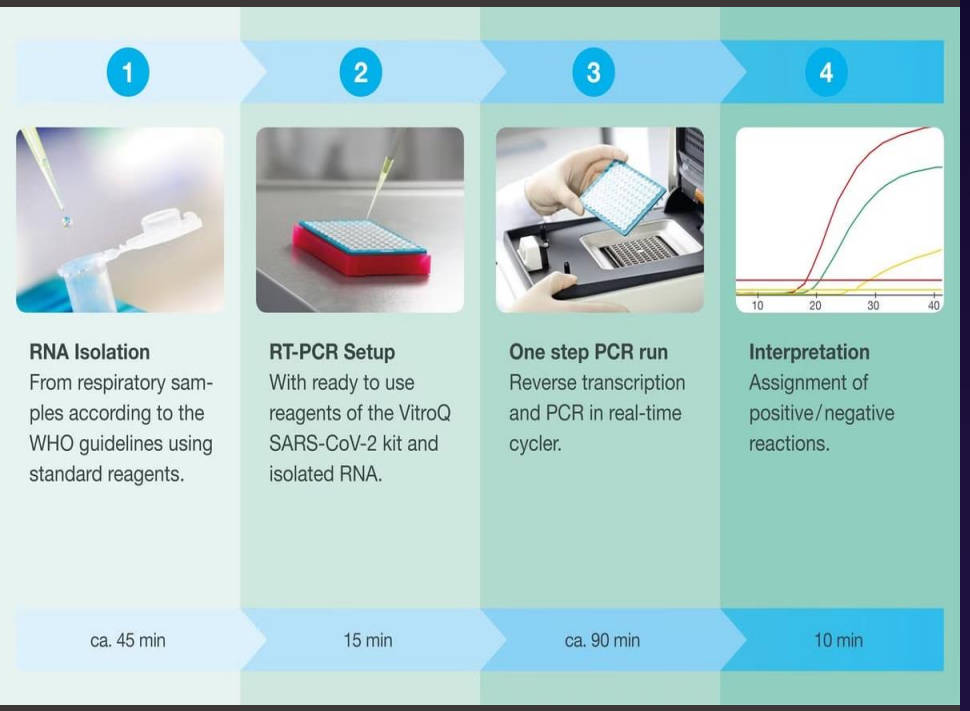
Serological tests can identify antibodies produced by the body body in response to SARS-CoV CoV infection.

Imaging Techniques

Chest X-rays and CT scans can help diagnose pneumonia and other respiratory complications associated with SARS-CoV.

Clinical Evaluation

Healthcare providers may also consider a patient's symptoms, symptoms, travel history, and potential exposure when diagnosing SARS-CoV infection. infection.



Treatment and Management

1

Supportive Care

Treatment for SARS-CoV primarily involves providing supportive supportive care to manage symptoms and complications.

2

Antiviral Drugs

Certain antiviral medications, such as remdesivir, may help reduce help reduce the severity of SARS-CoV infection.

3

Hospitalization

Patients with severe SARS-CoV illness often require hospitalization hospitalization and intensive medical treatment.

Vaccines and Immunity

Vaccine Development

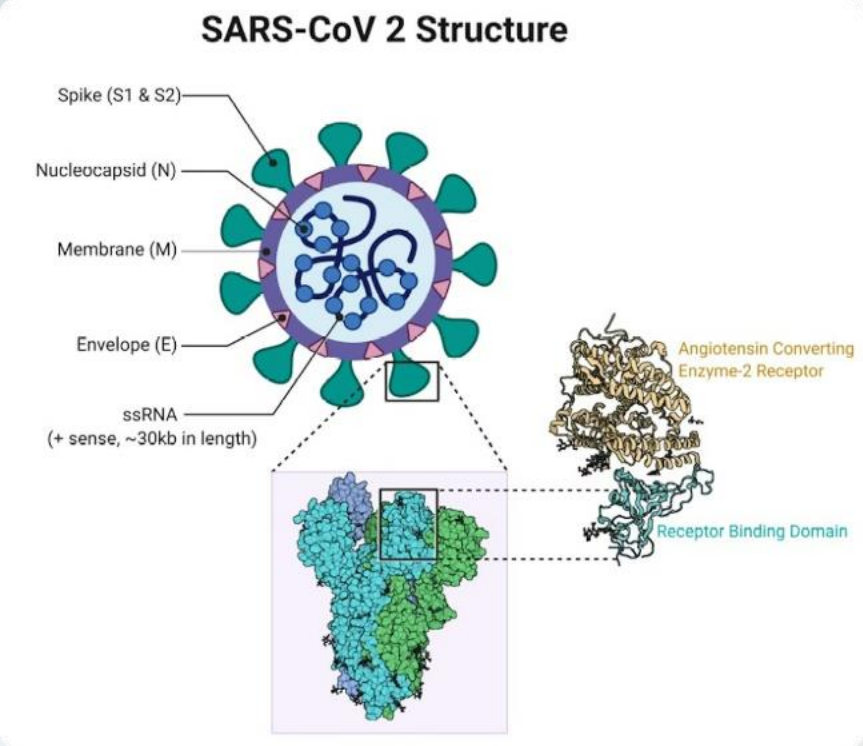
Several effective vaccines have been developed to prevent SARS-CoV infection and reduce the reduce the severity of COVID-19.

Immune Response

Infection with SARS-CoV can stimulate the body's immune system to produce antibodies, providing antibodies, providing some level of natural immunity.

Boosting Immunity

Vaccination and booster shots can further enhance the body's immune defenses against SARS-CoV SARS-CoV and its variants.

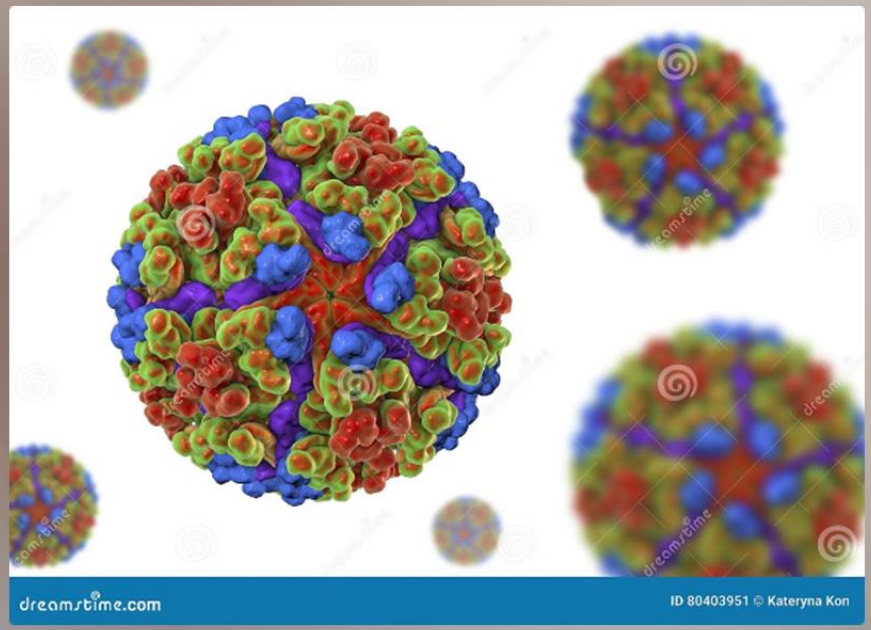


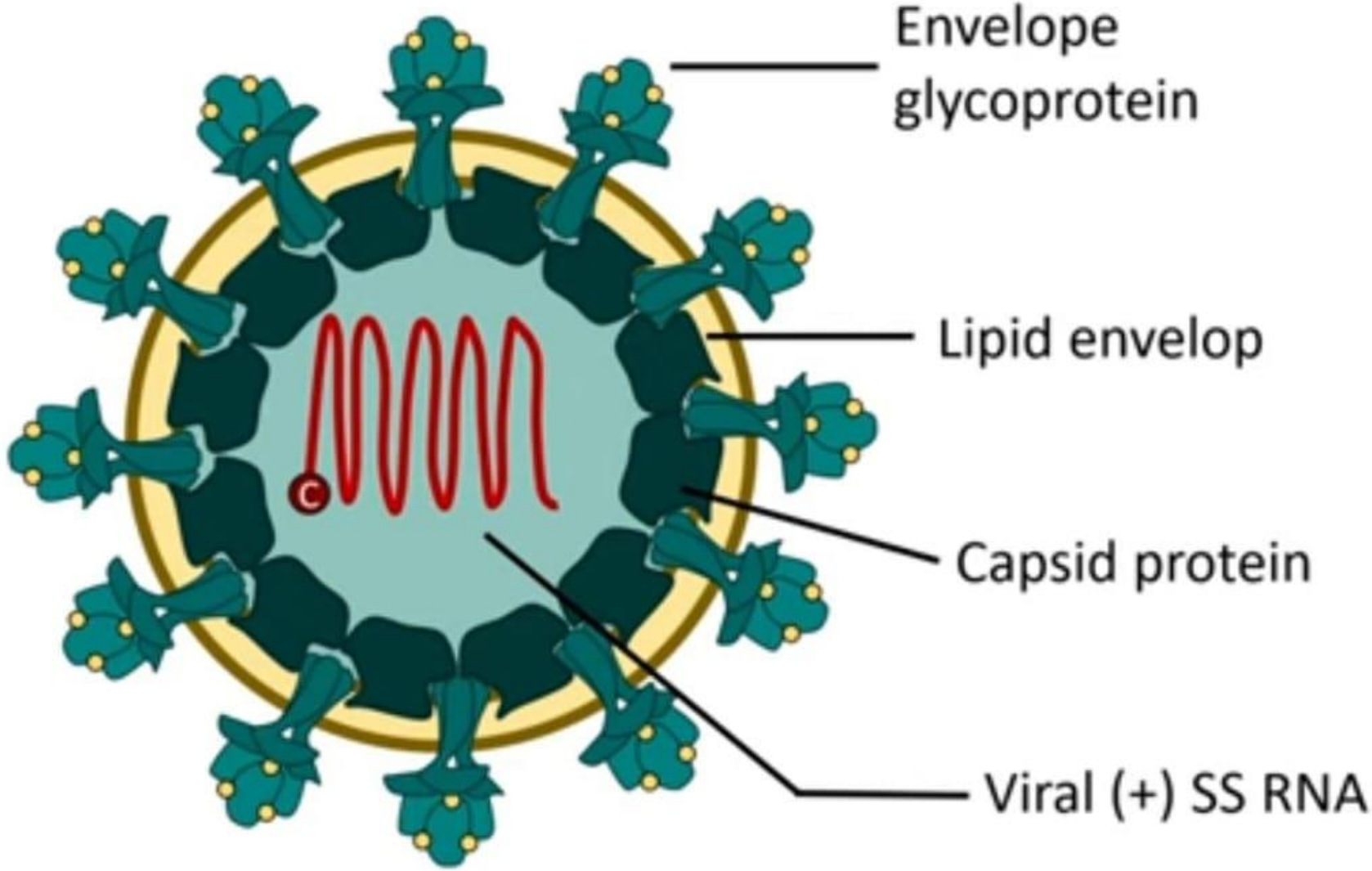
Variants and Mutations

Variant	Characteristics	Impacts
Alpha	More transmissible than original strain	Increased global spread
Beta	Able to evade some some antibody treatments	Reduced vaccine effectiveness
Delta	Highly contagious, more severe disease	Drove major outbreaks worldwide
Omicron	Highly mutated, increased immune evasion	Rapid global spread, breakthrough infections

Introduction to Chikungunya Virus

Chikungunya is a viral disease transmitted by infected mosquitoes. It causes fever, joint pain, and other debilitating symptoms. Understanding the pathogenesis of this virus is crucial for effective prevention and treatment. I is causes by **Aedes aegypti,** belongs to Togoviridae family




Envelope
glycoprotein
Lipid envelop
Capsid protein
Viral (+) SS RNA

Viral Structure and Genome

Enveloped Virus

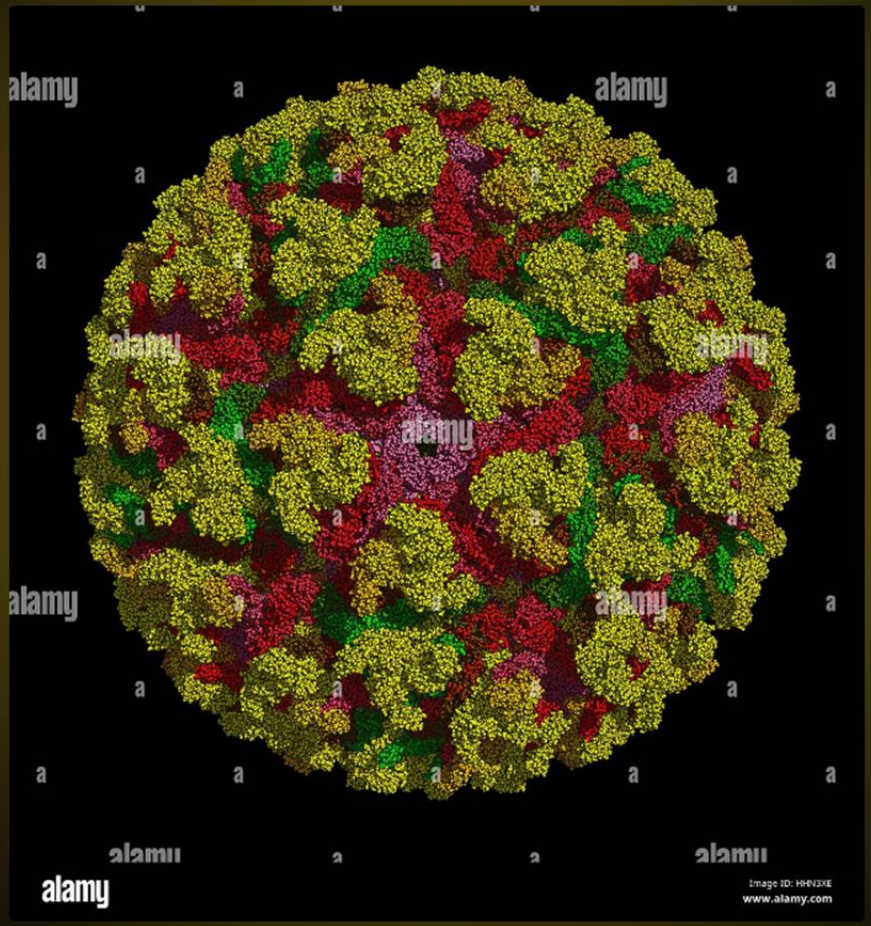
The chikungunya virus is an enveloped, positive-sense, single-stranded RNA virus belonging to the Alphavirus genus.

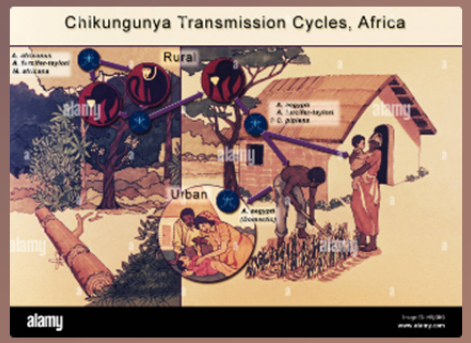
Genome Structure

The viral genome encodes structural proteins for the virus capsid and envelope, as well as non-structural proteins essential for replication.

Surface Proteins

The envelope proteins, E1 and E2, play crucial roles in viral attachment, entry, and fusion with host cells.





Transmission Cycle

Mosquito Vector

The chikungunya virus is primarily transmitted by Aedes mosquitoes, such as Aedes aegypti and Aedes albopictus.

1

Infected Humans

Humans become infected when bitten by a mosquito carrying the virus, which then amplifies the infection.

2

Mosquito Feeding

Infected humans can transmit the virus to uninfected mosquitoes during blood meals, completing the transmission cycle.

3

Host Immune Response

Innate Immunity

The innate immune system recognizes the virus, triggering the production of type I interferons and other inflammatory cytokines.

Adaptive Immunity

The adaptive immune response involves the activation of T cells and the production of neutralizing antibodies that can clear the infection.

Immune Evasion

Chikungunya virus has developed mechanisms to evade the host's immune defenses, contributing to its ability to cause persistent infections.

Viral Attachment and Entry

1

Receptor Binding

The virus envelope proteins bind to specific receptors on the host cell surface, initiating the entry process.

2

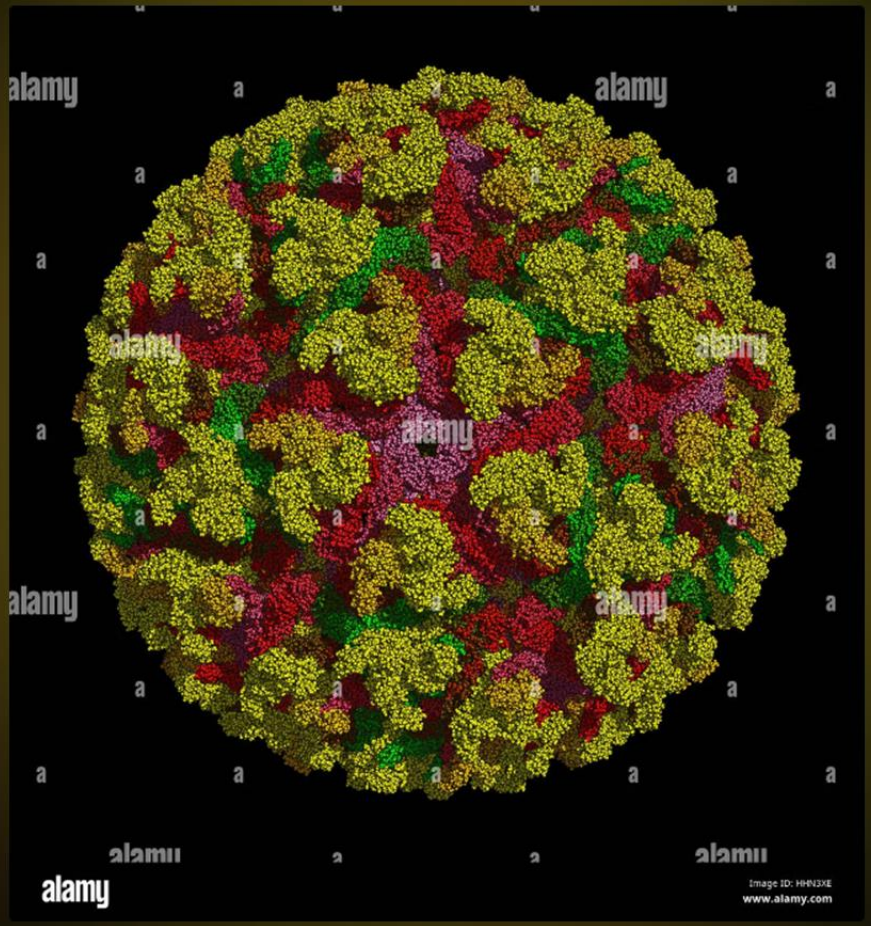
Membrane Fusion

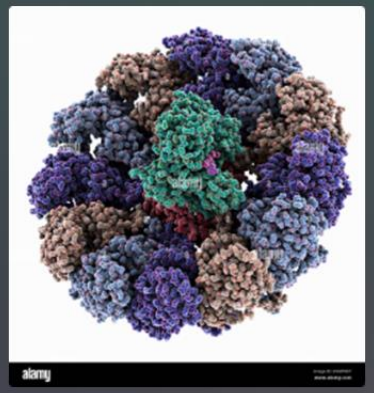
The viral envelope fuses with the host cell membrane, allowing the viral genome to enter the cytoplasm.

3

Uncoating

The viral capsid is disassembled, releasing the RNA genome for translation and replication.





Viral Replication and Assembly

1 RNA Translation

The viral RNA is translated into non-structural proteins that form the viral replication complex. Site of viral replication is Lymh node, Liver, Muscle, Spleen.

2 Genome Replication

The non-structural proteins facilitate the replication of the viral genome.

3 Structural Protein Synthesis

The viral structural proteins, including the capsid and envelope proteins, are synthesized and assembled.

4 Virion Assembly

The viral genome and structural proteins are packaged into new viral particles, which are then released from the host cell.

- There are several potential receptors for CHIKV, including Mxra8, CD147, GAGs, and TIM .
- CHIKV enters the target cells via clathrin-mediated endocytosis pathway Other entry pathways, such as micropinocytosis, are not depicted.

- Upon formation of the early endosome, clathrin molecules dissociate from the endocytic vesicle . The pH acidification of endosome (endocytic vesicles) triggers the fusion of the endosomal membrane with the viral membranes (the E1 protein), releasing the genomic RNA, followed by an immediate translation of the non-structural polyproteins (P1234 precursor) by the ribosome .

- The P1234 polyprotein is then cleaved by the nsP2, releasing the individual non-structural proteins, to form the viral replicase complex. The complex mediates the synthesis of the negative-strand RNA that serves as templates for the synthesis of new positive-strand RNA as well as for the synthesis of 26S subgenomic RNA .
- The synthesis of negative-strand RNA intermediate, genomic, and subgenomic RNA occurs in the specialized replication compartments termed spherules (not depicted).

- The subgenomic RNA is subsequently translated into the structural polyprotein precursor C-pE2-6K-E1 in the rough endoplasmic reticulum (RER).

- The C protein contains a protease domain responsible for its self-cleavage. It dissociates from the polyprotein and assembles with the genomic RNA to form the icosahedral nucleocapsid core in the cytoplasm .

- The pE2-6K-E1 precursor will be addressed to the lumen of the RER for maturation process culminating in the formation of E1-E2 heterodimers [11]. E1-E2 heterodimers will be inserted in the cell membrane forming the “virus budding microdomain” .

- The assembled icosahedral nucleocapsid core migrates to this domain, and new viral particles will be extracellularly released by budding process.

Clinical Manifestations

Acute Phase

Symptoms typically appear 3-7 days after infection, including fever, joint pain, headache, and rash.
Its is marked by interferons like IFNα , IL6, MCP1

Chronic Phase

In some cases, the joint pain and stiffness can persist for months or even years, leading to chronic arthritis. Marked by GM-CSF, IL6

Complications

Severe cases may result in complications such as neurological disorders, cardiovascular issues, and even death.

Diagnosis and Detection

Serological Tests

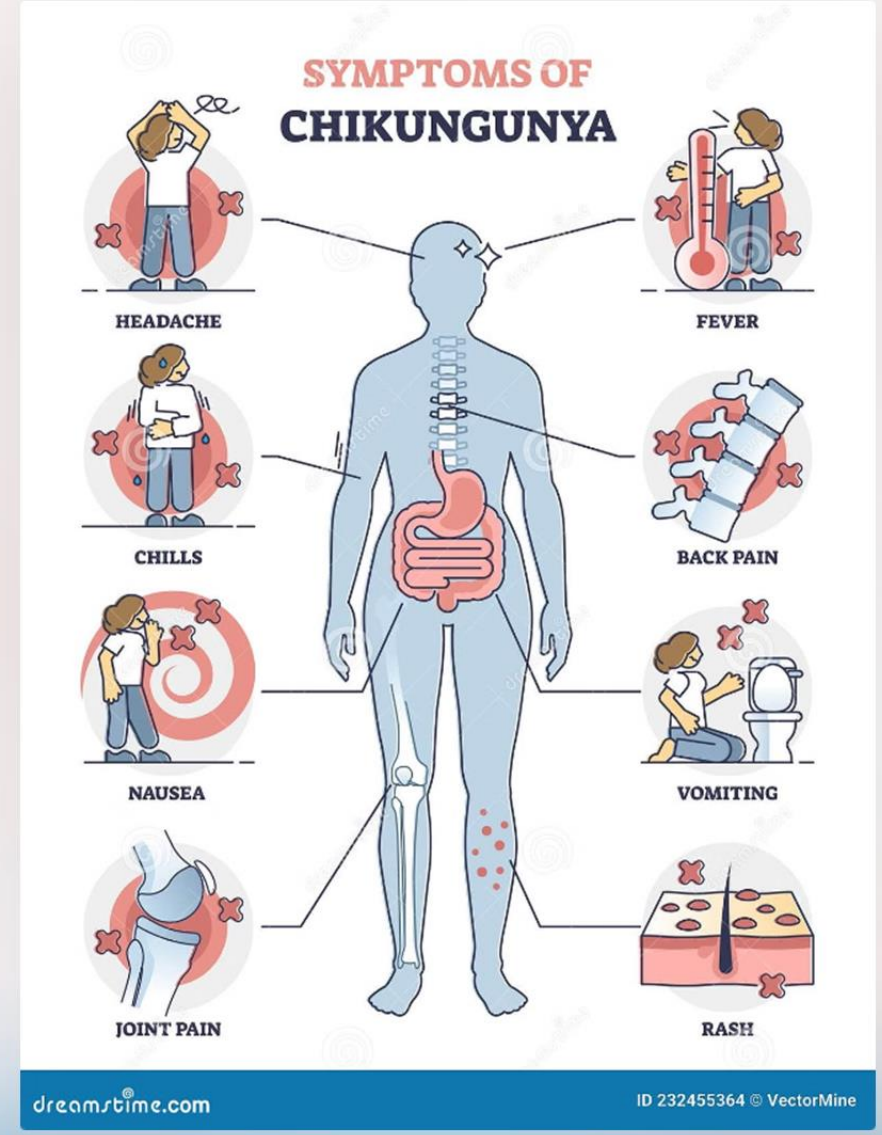
Antibody detection assays, such as ELISA and neutralization tests, can confirm chikungunya virus infection.

Molecular Diagnostics

RT-PCR and other molecular techniques can detect the presence of the viral genome in patient samples.

Virus Isolation

Virus isolation in cell culture can provide a definitive diagnosis, but is typically more complex and time-consuming.



Treatment and Management

Symptomatic Treatment

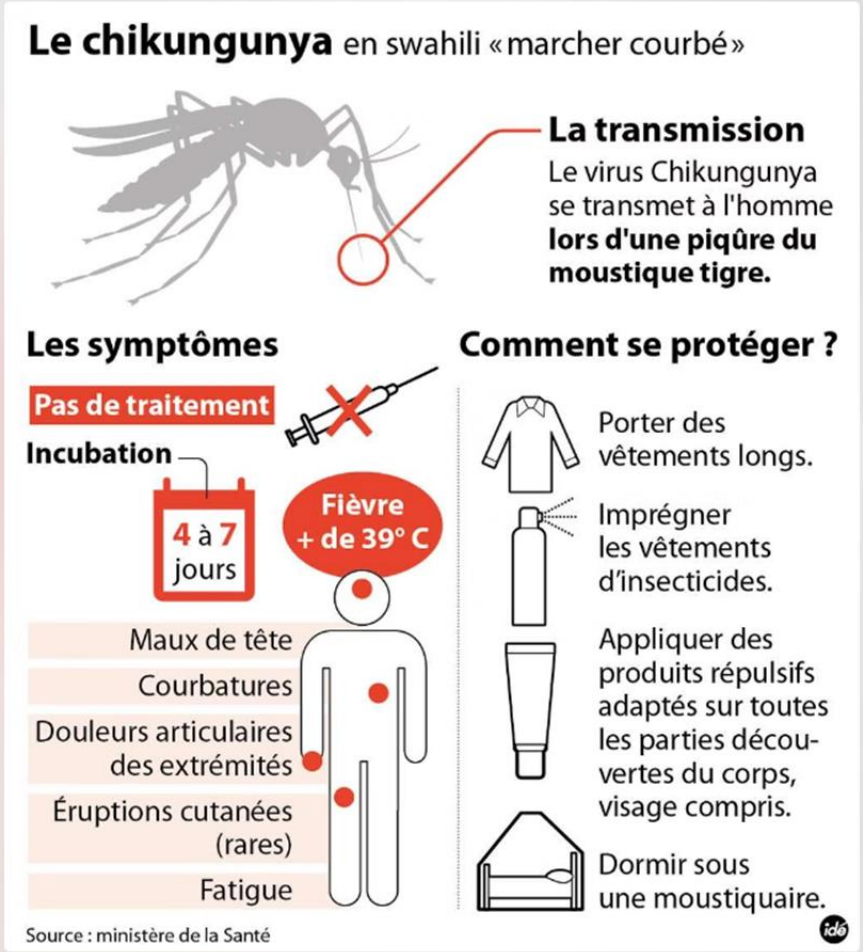
There is no specific antiviral treatment, so management focuses on relieving symptoms with pain relievers and fluids.

Vaccine Development

Efforts are ongoing to develop effective vaccines to prevent chikungunya virus infection and transmission. but one vaccine **live attenuated VLA1553**

Vector Control

Reducing mosquito populations through insecticides and environmental management is crucial for preventing transmission.



Prevention

1

Personal Protection

Using insect repellents, wearing long clothing, and avoiding mosquito-infested areas can reduce the risk of infection.

ACKNOWLEDGEMENT

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used to create this presentation.