A REVIEW OF MEASLES VIRUS

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ABSTRACT:
Measles is a highly contagious, acute febrile illness that results from infection with measles virus (MV). MV is a single-stranded, negative-sense RNA virus in the genus Morbillivirus of the family Paramyxoviridae. The wild-type MV consists of 24 genotypes, three of them (B3, D8 an H1) have dominated circulation in the world. MV is transmitted by the respiratory route and illness begins with fever, cough, conjunctivitis followed by a rash and measles enanthem (Koplik spot). Laboratory confirmation of measles is provided by serological (ELISA test for detection of IgM and IgG antibodies), molecular (detection of viral nucleic acid) and viral isolation in Vero/hSLAM cells methods. As a vaccine-preventable infection, measles has a global importance and is a target of WHO strategic goals in the European region. Despite significant progress in measles control in recent years, it is necessary to improve the national vaccination coverage, and the epidemiological and laboratory monitoring of the infection. High vaccination coverage across all of the population is crucial to reach the goals of measles elimination.

Keywords: measles virus, morphology, diagnosis, epidemiology, elimination

INTRODUCTION
Measles is a highly contagious, acute febrile illness that results from infection with measles virus (MV). The virus is transmitted by the respiratory route and illness begins with fever and typically at least one of the three “Cs”: cough, coryza, and conjunctivitis.

MV belongs to the family Paramyxoviridae, subfamily Orthoparamyxovirinae, genus Morbillivirus. The family Paramyxoviridae is divided into 4 subfamilies: Avulavirinae, Metaparamyxovirinae, Orthoparamyxovirinae, and Rubulavirinae. Subfamilies are further subdivided into 14 genera, and three viruses are members of species that are not assigned to a genus or a subfamily. The current taxonomic structure of Paramyxoviridae is based on a comparative analysis of the complete amino acid sequences of the L-protein (1, 2). They are large enveloped RNA viruses that infect mammals and birds, in some cases reptiles and fish. Many paramyxoviruses are host-specific, several such as MV, mumps virus, Hendra virus, several parainfluenza viruses, respiratory syncytial virus (RSV) are pathogenic to humans (3, 4). Viral transmission is horizontal, mainly through direct contact or by airborne droplets, no vector transmission is known.

STRUCTURE
MV is an enveloped virus, containing non-segmented negative sense RNA. The virions have spherical to pleomorphic shape, they range in size of 120 nm to 300 nm in diameter and are composed of six structural proteins and two nonstructural proteins C and V (Figure 1). MV RNA genome consists of approximately 16,000 nucleotides and is enclosed in a lipidcontaining envelope derived from the host cell. Two envelope glycoproteins are important in the pathogenesis – transmembrane haemagglutinin (H), which is responsible for binding of the virion to cells and fusion (F) glycoprotein, responsible for fusion of virus and host cell membranes, viral penetration,
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The matrix (M) protein lies the interiors of the virion envelope, which strengthens the structure of the virion.

GENOME ORGANIZATION

MV genome consists of six genes, each encoding a single structural protein (Figure 2). One of these genes, the phosphoprotein (P) gene, also encodes two non-structural proteins (V and C). Structural proteins are the nucleoprotein (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H) and large polymerase (L) proteins. Non-structural C and V proteins are both products of the gene P. C and V are transcribed from an overlapping reading frame of the P gene by alternative reading frame and mRNA editing, respectively. Each coding region is preceded and followed by untranslated regions, of which the longest (1012 nt) is the noncoding region between the M and F protein (M/F NCR) genes (6). The 3′ leader and 5′ trailer of the MV genome are non-coding regions composed of 107 and 109 nucleotides, respectively (6). These structurally homologous regulatory elements serve as binding sites for the viral RNA-dependent RNA polymerase (vRdRp) to viral RNA (vRNA), for transcription and synthesis of full-length positive chain replication intermediates, and negative-chain viral genomes. vRdRp requires a bilateral/bipartite promoter to initiate genome replication (7). vRdRp binds to the nucleocapsid template through its co-factor, phosphoprotein (P) (8).

The most variable part of the MV genome is represented by 450 nucleotide sequences encoding the carboxyl terminus of the N-protein (N-450) and nucleotide variability in different genotypes can reach up to 12% (10). Based on the variability in the nucleotide sequence of the hemagglutinin (H) and nucleoprotein (N) genes, wild-type viruses are distinguished into eight strains (A-H), which are divided into 22 genotypes and one possible genotype. Strains B, C, D, G and H show multiple genotypes (B1 - 3, C1 - 2, D1 - 10, G1 - 3, H1 - 2), while strains A, E and F contain one genotype (A, E, F).

Sequences of vaccine strains show that they are all members of genotype A, which does not circulate in wild type in the world (11,12). Specific measles genotypes are not associated with differences in the severity of the disease, nor do they alter the efficacy of the vaccine (13).

Figure 1: Paramyxovirus virion structure. (A) Negative-contrast electron micrograph of intact MV particle (genus *Morbillivirus*). Scale bar = 100 nm. (B) Schematic diagram of paramyxovirus particle in cross-section. (https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna-viruses/w/paramyxoviridae)
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VIRAL LIFE CYCLE
As transmembrane glycoproteins, H and F are exposed on the virus surface and binding of the H protein to a host receptor triggers conformational changes in F protein. This induces fusion of the viral envelope with the plasma membrane and release of ribonucleoprotein (RNP) complexes in the cytoplasm of target cells. Replication and transcription of the viral genome takes place entirely in the cytoplasm (14). Encapsidated viral RNA serves as a template of the RdRp complex for both transcription and replication (15). Transcription begins at the 3′ end of the genome and viral genes are transcribed in the 3′ to 5′ direction with a sequential “stop–start” mechanism. Newly synthesized viral mRNAs are translated to viral proteins by using the host translation machinery. The newly synthesized genomic RNA is tightly wrapped with the N protein to provide a helical template for viral transcription and replication (16, 17). Coordinated interactions between viral components (the assembling of the M protein, the RNP complex, and the glycoproteins at selected sites on the plasma membranes of infected cells) as well as between viral and cellular factors, lead to the formation of fully infectious MV particles (Figure 3) (18,19).

Figure 2. Schematic representation of the genes encoding the MV proteins. The second gene encodes three proteins - P, C, V, and the other genes one protein each. The structural genes for the N protein are marked in blue and for the P protein in red (9).

Three are the host cell receptors which are responsible for the entry of the virus particle – CD150 (signaling lymphocytic activation marker or SLAM), expressed by thymocytes, macrophages, mature dendritic cells, Langerhans cells, lymphocytes and platelets (20, 21), Nectin-4, which is expressed as epithelial cell receptor for MV and CD46 is a complement regulatory molecule expressed on all nucleated cells in humans. Wild-type MV binds to cells primarily through the cellular receptor SLAM, whereas most vaccine strains bind to CD46, as well as to SLAM (22,23).

Although the receptor remains to be identified, MV can replicate in endothelial cells lining blood vessels. It is therefore possible that replication of MV in endothelial cells of brain capillaries may allow infectious virus particles to bud directly into the brain parenchyma.

Figure 3. MV replication cycle (according to Moss et al., 2006)

PATHOGENESIS
Measles is transmitted by the respiratory route and is highly infectious. The aerosolized MV enters the susceptible host through the respiratory tract where it infects epithelial cells (24). Symptoms usually develop after an incubation period of 7–14 days and last 7–10 days. The incubation period for measles is about 10 days to the onset of fever and 14 days to the onset of rash. During this period MV replicates and spreads in the infected host. Initial viral replication begins in the epithelial cells of the upper respiratory tract, followed by infection of the regional lymphoid organs, which is followed by viremia (the presence of virus in the blood) and the dissemination of MV to many
organs, including lymph nodes, skin, kidney, gastrointestinal tract and liver, in which the virus replicates in the epithelial and endothelial cells, and in lymphocytes, monocytes and macrophages (25). As the virus replicates, the host immune response is developed, and MV infection is even clinically apparent during the incubation period. Evidence of these processes is the occurrence of lymphopenia – the number of circulating lymphocytes is reduced during the incubation period. Host immune responses to MV are essential for the clinical recovery and the establishment of long-term immunity. Activation of natural killer (NK) cells and increased production of interferons (IFN)-α and β are an early innate immune response of the host. The adaptive immune response includes production of specific MV antibodies. The most abundant and rapidly produced antibodies are against the nucleoprotein (N). Antibodies to the haemagglutinin (H) and fusion (F) proteins contribute to virus neutralization and are sufficient to provide protection (25). Measles begins with a prodromal phase, characterized by fever over 38.5°C, malaise, dry cough, coryza (runny nose), conjunctivitis, and a pathognomonic exanthema on the oral mucosa, referred to as Koplik spots, followed by a maculopapular rash spreading from the head to the trunk and to the lower extremities (26). Symptoms intensify over 2-4 days before the onset of rash and peak on the first day of rash. The rash lasts for 3-4 days after which it fades, disappearing from the face first (25,26,27). People with measles are infectious for several days before and after the onset of rash, when concentrations of MV in blood and body fluids are presumed to be the highest. Recovery from measles produces lifelong immunity (28).

MEASLES IMMUNE RESPONSE

Immune response to MV is crucial for the viral clearance and the establishment of protective immunity (29). Host immune responses at sites of virus replication are responsible for the signs and symptoms of measles, which might be absent or delayed in people with cellular immune deficiencies (30). A manifestation of the cellular immune response to infection (with lymphocyte infiltration) is the maculopapular rash that appears 10–14 days after infection (31). MV-specific IFN-g-producing T cells and IgM antibodies are detectable in blood as the rash is fading and infectious virus is cleared within a week after appearance of the rash (32). Congenital inability to produce antibodies allows recovery from measles, while defects in T-lymphocyte function can lead to fatal progressive pulmonary or neurologic disease, which is clinical evidence of the importance of cellular immunity to MV for virus clearance (33, 34). The predominant initial cellular response is characterized by appearance of MV-specific IFN-g-producing CD4+ T cells and cytotoxic CD8+ T cells which is important for the control and clearance of infectious virus (35, 32, 36). During convalescence, a Th2 response promotes the development of protective MV-specific antibodies and is characterized by high concentrations of interleukin 4, interleukin 10, and interleukin 13 (37). The initial humoral response consists of IgM antibodies that arise at the time of the rash and persist for 6–8 weeks and it is commonly used to confirm the diagnoses of measles. This is followed by the sustained synthesis of MV-specific IgG. Measles induces a robust MV-specific immune response, but leads to suppression of immunity to other pathogens and increased susceptibility to other infectious diseases. Immune suppression is evident during acute disease and for many weeks after recovery (Figure 4) (38).

DIAGNOSES AND COMPLICATIONS

Measles is an acute febrile illness associated with a characteristic erythematous, maculopapular rash. The measles case definition includes a generalized maculopapular rash, fever (≥38.3°C) and either cough, coryza, or conjunctivitis. Koplik’s spots appear on the buccal mucosa as small white papules and provide an opportunity to clinically diagnose measles a day or two before the rash. The rash appears first on the face and behind the ears, and then spreads to the trunk
and extremities, coinciding with development of the adaptive immune response. The fever and catarrhal symptoms typically peak with the rash, which persists for 3–4 days (Figure 4) (29,39). Other acute viral infections might be confused of measles, including rubella virus, parvovirus B19, human herpes virus type 6 and dengue virus. That is why adequate laboratory diagnosis is crucial (40, 41). After the introduction of specific prophylaxis of measles, atypical forms of the disease are often observed, which necessitates mandatory laboratory diagnostics. Serology is the most common method of laboratory confirmation (42, 43).

Laboratory diagnosis of measles is based on one of the following indicators (specimen required, optimal timing of sample collection) (13).

- antibody testing: positive IgM antibody or seroconversion to IgG (serum, oral fluid: > 4 days to 2-3 months);
- molecular testing: detection of measles RNA (oral fluid, urine and serum; up to 5 days);
- virus isolation in cell cultures: isolation of MV from clinical specimen (throat swab, nasopharyngeal aspirate, conjunctival swab, urine; up to 5 - 7 days);
- detection of MV antigen by direct fluorescent assay in a clinical specimen using MV-specific monoclonal antibodies.

Complications of measles can affect most organ systems and are most common in young infants, adults older than 20 years, pregnant women, and those who are immunocompromised or undernourished, particularly children with vitamin A deficiency (44). The respiratory tract is a frequent site of complication. Pneumonia is most often caused by secondary viral or bacterial pathogens, or by MV itself, causing a giant cell pneumonitis. Other respiratory complications include laryngotracheobronchitis (croup) and otitis media. Many children with measles develop diarrhoea, which can result in considerable morbidity and mortality, often due to secondary infections with bacteria or protozoa. Keratoconjunctivitis is common after measles in children with vitamin A deficiency and can cause blindness (39, 45). Measles during pregnancy increases the risk of low birthweight, intrauterine fetal death, spontaneous abortion, and maternal death (46). MV can cause serious disease of the central nervous system (CNS) as a complication. Measles inclusion body encephalitis (MIBE) and subacute sclerosis panencephalitis (SSPE) are severe CNS disorders that occur months to years after acute infection, especially when persons are infected with MV before 2 years of age (47, 48).

Figure 4. Schematic diagram of immune response (A) and clinical manifestation (B) of a typical measles infection (William J Moss, Diane E Griffin, Measles, Lancet 2012)
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PREVENTION
Before the introduction of measles vaccine in 1963, major epidemics occurred approximately every 2 to 3 years and it is estimated that 30 million cases of measles and more than 2 million deaths occurred globally each year, and that by the age of 15 years, more than 95% of individuals had been infected with MV (23). Measles is best prevented through measles vaccination. Currently licensed measles vaccines are attenuated viral vaccines that replicate within the host to induce protective immunity. Measles vaccines can be administered as combined vaccines with those for rubella (MR), mumps (MMR), or varicella (MMR-V). Two doses of measles-containing vaccine (MCV) are recommended. National schedules differ but all countries recommend that the first dose is given during the second year of life (age 12 to 24 months). The timing of the first and second doses of MCV (MCV1 and MCV2) varies across countries and regions. Measles vaccine induces both humoral and cellular immune responses similar to those induced by wildtype MV infection, although antibody concentrations are usually lower. Measles-containing vaccine (MCV) protects without the risk of the severe illness, complications and death that comes with having the disease. After vaccination, transient MV-specific IgM antibodies appear in the blood and IgA antibodies appear in mucosal secretions. IgG antibodies are produced subsequently and persist in the blood for years. Vaccination also induces MV specific CD4+ and CD8+ T lymphocytes (27, 28). There is no evidence that having natural disease is an advantage that justifies not getting vaccinated. Two doses of MCV vaccine are about 97% effective at preventing measles; one dose is about 93% effective. High population immunity is required to interrupt MV transmission due to its high infectivity (27). Use of combined measles–rubella vaccines provide an opportunity to eliminate rubella and congenital rubella syndrome (29).

EPIDEMIOLOGY
Over the past several decades’ measles mortality declined in the developed countries, due to the economic development, improved nutritional status, antibiotic therapy for secondary bacterial pneumonia., increased measles vaccine coverage. The seasonality in the regions with temperate climate is winter-spring, and in those with tropical climate the morbidity is evenly distributed throughout the year. Significant changes occurred in the epidemiology of the disease after the introduction of the specific vaccine prophylaxis of measles – prolongation of the inter-epidemic periods to 6-10 and more years, reduction of the number of patients by 90-99%, displacement of the disease to older age groups (49, 50).

The high contagiousness of MV is expressed by the basic reproductive number (R0), which is the average number of secondary cases resulting from the introduction of an infectious individual into a completely susceptible population. A function of pathogen transmission characteristics, population density, and social contact patterns, R0 of MV has been estimated to be 9–18 in different settings. Measles has one of the highest R0 for a directly transmitted pathogen, significantly higher than that for smallpox (R0 = 5–7) or influenza (R0 = 2–3) viruses. This epidemiological characteristic of measles is the major obstacle to elimination as the virus spreads rapidly in susceptible populations and requires high levels of population immunity to interrupt transmission (29).

A sharp decrease in measles cases has been observed globally during the COVID-19 pandemic. A few measles cases are being reported in the EU/EEA, including countries that had previously eliminated or interrupted endemic transmission. Registered cases within the European continent have been reduced from a few hundred per month to a sporadic cases. So far in 2021, no new deaths have been reported by EU/EEA countries (51).
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MV IN BULGARIA
Despite the active surveillance and prevention of measles, epidemic outbreaks have been reported in many European countries and around the world in the early 21st century.
One of the largest epidemics of measles in the European region, that affected Bulgaria also, occurred in the period 2009-2011. It re-emerged after a long interepidemic period (7 years), affecting over 24,000 persons (24365) of whom 24 died (mortality-0.3‰ and lethality-0.1(52, 53).
In the period 2011-2013, large epidemics of measles were registered in France, Ukraine, Georgia and Turkey. Outbreaks of measles in Bulgaria were reported in 2013 (54) and in the first half of 2017 (55,56).
In 2019-2020, an outbreak of measles was registered in the country with a total number of 1488 confirmed cases in 16 districts (57).
In Bulgaria, measles has been a mandatory notifiable disease since 1921. National case-based surveillance started in 2004 and in 2005, the European Union case definition and case classification were adopted for surveillance purposes. The National Measles Surveillance System was developed and introduced in 2009 (Figure 5) (58).
National Center for Infectious and Parasitic Diseases has played a major role in the laboratory diagnosis and surveillance of measles in Bulgaria. Since 2019, there is a National Program for Elimination of Measles and Rubella in the Republic of Bulgaria, funded by the Ministry of Health.

Figure 5. Number measles cases reported in Bulgaria for the period 2009-2020 (https://mmr.gateway.bg/en/disease/mor.php?c=15&filter[date_reported]=04.01.2009..31.12.2020&interval=year)

CONCLUSION
As a vaccine-preventable infection, measles has a global importance and is a target of WHO strategic goals in the European region. The main reason for the unsustainable success of measles control is the insufficient immunization coverage achieved in the implementation of planned immunization programs or mass vaccination campaigns. This imposes increased requirements for epidemiological and laboratory monitoring of the disease. Unfortunately, the epidemic outbreaks in Europe and in Bulgaria in particular, in recent years prove that the virus is still a public health problem. With the development
of methods and technologies in virological science, the diagnosis of measles virus has also undergone development, which has become an incommutable part of monitoring the elimination process.

Competing Interest
The author does not have any competing interest.

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