Summary and conclusions

Measles virus will continue to exist after certification of global eradication as virus stocks and infectious materials held in laboratories. Live virus may also exist in undetected foci of transmission and in persistently and chronically infected individuals. This analysis attempts to identify and evaluate the main risks for re-introduction of measles transmission post certification of eradication in a world in which universal routine measles immunization is no longer a feature.

Risk of continuing, undetected wild-type measles transmission in humans

There are, as yet, no definitive criteria for certification of global measles eradication or agreed requirements for validation of these criteria. Without these criteria, and the detailed requirements for demonstrating they have been met, it is not possible to accurately estimate the risk presented by undetected continuing transmission.

Mild or asymptomatic measles infections are probably very common among measles-immune persons exposed to measles cases, but transmission from asymptomatic cases is likely to be very rare. If it occurs it is unlikely to be efficient enough to sustain transmission, especially in the highly vaccinated populations expected in the years immediately following global certification of eradication. However, the potential role of asymptomatic infections in maintaining transmission requires further investigation.

If the criteria for global certification of eradication are firm enough, and require rigorous validation, then the risk of undetected measles transmission after certification is very low. If the certification criteria are lax, or validation requirements are inadequate, the risk will be higher.

Risk of transmission of vaccine-derived virus

The currently licensed live-attenuated measles vaccines are safe and efficient and have been used successfully to protect many millions of individuals and prevent measles transmission. All current vaccine viruses are closely related and belong to genotype A. There is no published conclusive evidence for currently licensed live attenuated vaccine viruses reverting to wild-type transmissibility or virulence. On the contrary, the vast majority of evidence points to an impressive level of genetic stability. However, since they are live viruses that replicate within vaccine recipients, the remote possibility must exist that they could revert to wild-type characteristics. There is also no evidence for the establishment of vaccine-escape mutants. Even if vaccine viruses were to revert to wild-type transmissibility, there is no reason to suspect that transmission could not be controlled using current vaccines.

Risk from persistent infections

There is no published evidence that cases of persistent measles infection are associated with the shedding of infectious virus or play any part in measles transmission. As the number of acute measles virus cases declines in the years leading to global eradication, we can expect a decline in the
number of potential SSPE and MIBE cases. Acute measles infection in HIV-infected individuals tends to be more severe, last longer and result in a shorter lived immunity to re-infection, but there is no published evidence to suggest that co-infection increases the potential for establishment of persistent measles infections, either with wild-type virus or with vaccine-derived virus.

Risk from non-human primates
Although non-human primates can be experimentally and naturally infected with measles virus, and animal-animal transmission occurs, population sizes are too small to maintain epizootic transmission or pose a threat to human populations.

Risk of laboratory-associated measles infection
Although there is no direct evidence for laboratory-acquired measles infections it is possible that they have occurred among immune laboratory staff and resulted in asymptomatic or very mild infections. There is no published evidence to suggest that these asymptomatic or mild infections result in further transmission of virus. Measles virus loses infectivity within a few hours at ambient temperatures, and infectious materials stored at temperatures above -30°C can be expected to lose all infectivity over the course of one to two years. Materials stored at or below -70°C, or freeze dried, maintain infectivity for many years.

Despite the lack of evidence for laboratory-acquired measles infections or escape of virus into the community, these must be considered possibilities in a post-eradication world. An appropriate systematic laboratory containment strategy for measles, learning from the example set by the Polio Eradication Initiative, should be developed.

Risk of intentional release of measles virus
Measles is a highly infectious virus that has had devastating effects on susceptible populations in the past. Although it is unlikely that the high mortalities seen in these isolated communities would be repeated, the threat of measles release would probably be very effective once a sizable population of susceptible individuals had accumulated. This threat could be countered by the establishment of a measles vaccine stockpile, preferably using a new, easy to mass-administer, non-replicative measles vaccine. The size and nature of any stockpile should be defined within a systematic and comprehensive post-eradication risk management strategy.

Risks for re-introduction of measles can be summarised as follows:

<table>
<thead>
<tr>
<th>Risk</th>
<th>Magnitude</th>
<th>Tendency over time</th>
<th>Mitigating actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuing wild-type measles transmission in humans</td>
<td>Low but depends on certification criteria and validation requirements</td>
<td>Decreasing</td>
<td>Base certification criteria and validation requirements on dynamic and stochastic modelling data</td>
</tr>
<tr>
<td>Transmission of vaccine-derived virus</td>
<td>Very low</td>
<td>Depends on level of vaccine use</td>
<td>Develop alternative, non-replicating vaccines</td>
</tr>
<tr>
<td>Persistent infections</td>
<td>Very low</td>
<td>Decreasing</td>
<td>Maintain surveillance</td>
</tr>
<tr>
<td>Non-human primates</td>
<td>Very low</td>
<td>Decreasing</td>
<td>Maintain surveillance</td>
</tr>
<tr>
<td>Laboratory-associated infection</td>
<td>Very low but rising post eradication</td>
<td>Increasing</td>
<td>Develop systematic laboratory containment strategy</td>
</tr>
<tr>
<td>Intentional release</td>
<td>Very low but rising post eradication</td>
<td>Increasing</td>
<td>Develop vaccine stockpiles as part of a comprehensive risk management strategy</td>
</tr>
</tbody>
</table>
Areas requiring further research and investigation include:

Greater understanding of the transmission dynamics of measles
Developing more models, particularly dynamic and stochastic models of measles transmission, persistence and elimination will be required for developing the certification criteria and validation requirements, particularly for low-income, high density populations.

Additional detailed epidemiological and molecular analysis is required on importations and outbreaks, particularly those occurring in highly immunized populations and in populations with recognized inadequately immunized sub-populations.

With the rapid increase in the number of highly immunized populations, opportunities for studying asymptomatic and atypical infections and their potential role in transmission should be taken.

Greater understanding of the changes brought about by the attenuation process
More information on the nature of the changes caused by attenuation and the potential for vaccine virus reversion to wild-type characteristics is required.

More understanding of the nature of the complex interaction between measles virus and the host immune system, including both humoral and cell-mediated responses, would probably benefit continued use of existing vaccines and development of new vaccines.

All genotype A viruses detected in association with acute cases of measles and atypical vaccine responses should be thoroughly scrutinized. Full epidemiological information will be required, and additional sequence data from both clinical samples and corresponding viral isolates will be necessary to rule out the possibility of transmission.
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Introduction.

We do not yet have an agreed, definitive definition for measles eradication, but a reasonable definition may be:

“Interruption of measles virus transmission globally for a period greater than or equal to 36 months, in the presence of high-quality surveillance” (modified from current Global and Regional definitions of Regional elimination).

According to this definition, measles virus will continue to exist, as virus stocks and infectious materials held in laboratories. Live virus may also continue to exist in persistently and chronically infected individuals. What risk do these viruses and materials pose in a post-eradication world?

For the purposes of this analysis potential risks have been divided into two categories:

• ‘natural’ – associated with circulation of wild-type virus, virus persistence, and immunization activities; and
• ‘laboratory’ – associated with laboratory work, storage and intentional release.

These two categories are not mutually exclusive, but do permit a more systematic, structured assessment.

‘Natural’ risks considered include:

a) continuing wild-type measles transmission in undetected human reservoirs;
b) transmission of vaccine-derived virus;
c) persistent and chronic infections;
d) non-human primate reservoirs.

‘Laboratory’ risks considered include:

a) laboratory-acquired infections;
b) stored infectious materials;
c) virus escape into the community;
d) intentional release.

If universal or near-universal coverage with measles vaccine is continued after global eradication, particularly if a more effective, non-replicating vaccine is used, the risk of measles reintroduction will be minimal. It is likely that a number of national authorities will, for political as well as public health reasons, choose to continue routine immunization post eradication. Some authorities may adopt a modified immunization schedule, such as a single-dose policy, or some form of campaign strategy. It is also likely that a number of national authorities, either through decision or default, will cease routine measles immunization. For the purposes of this analysis it has been assumed that universal immunization against measles will not be continued post eradication, and that an increasing global population will be susceptible to measles infection in the years following certification.
The analysis concludes with a brief discussion of actions required to reduce the risk of accidental or deliberate release of measles in a post-eradication world and areas that could benefit from further research.

**Risk of continuing, undetected wild-type measles transmission in humans**

There are, as yet, no definitive criteria for certification of global measles eradication or agreed requirements for validation of these criteria. Without these criteria, and the detailed requirements for demonstrating they have been met, it is not possible to accurately estimate the risk presented by undetected continuing transmission. However, based on current Regional and Global recommendations on certification of Regional measles elimination, it is likely that eradication criteria will include:

1. **Absence of circulating measles virus for at least one year;**
2. **Adequate surveillance including genotype data.** Adequate surveillance may be defined by:
   - Number of reported suspected measles cases that are discarded as non-measles (targets: ≥ 2/100,000 population nationally, ≥ 1/100,000 in at least 80% of districts)
   - Percentage of reported suspected cases that have adequate investigation within 48 hours of report (target: ≥ 80% of reported suspected cases)
   - Percentage of reported suspected cases that have adequate specimens collected (target: ≥ 80% of reported suspected cases)
   - Percentage of districts with access to a WHO-accredited measles diagnostic laboratory (target: 100%)
   - Percentage of specimens with IgM results within 7 days of receipt in laboratory (target: ≥ 90%)
   - Percentage of chains of transmission with RNA sequence analysis (target: ≥ 95%)
   - Some use of measles avidity assays to distinguish recent from long-standing immunological responses
   - Some demonstration of alternative surveillance mechanisms, routine or supplementary, based on case detection, investigation and reporting;
3. **Achievement of high population immunity.** Population immunity may be demonstrated by:
   - ≥95% coverage with routine MCV2 in all districts, or
   - ≥80% coverage with routine MCV1 plus ≥95% coverage with SIA follow-up in all districts, or
   - Some use of extensive serosurvey data.

From experience gained through Regional polio elimination and certification, specific criteria may be used to fulfil the three general criteria above, but it is unlikely that any single specific indicator will be required to pass or fail validation. The strictness and extent of requirements for providing evidence that certification criteria have been met will largely determine the magnitude of risk posed by undetected continuing measles transmission. But even with relatively lax criteria and validation requirements, how likely is it that ongoing measles transmission will be undetected for a minimum of one year before certification?
What is the smallest population required to maintain measles transmission?
Measles epidemics have generally been characterised by explosive cycles with highly complex pathogen- and population-level interactions that influence transmission dynamics (1). Accurately predicting the critical community size (CCS) required for maintaining measles virus circulation is difficult due to the large number of variables involved. Direct observation and a range of both deterministic and stochastic models suggest that a population of 250,000 to 400,000 with 5,000 to 10,000 births per year is required to maintain transmission (2,3). High levels of immunization, low population density, a low birth-rate and good public health care facilities increase the CCS. Low vaccine uptake, high population density, high birth-rates, high levels of immunodeficiency and poor public health care facilities decrease the CCS (1,4,5).

Although it may be difficult to accurately estimate the CCS in low-income, low vaccine coverage populations, it is easy to identify these populations. If disease surveillance and immunization activities are targeted on them, and on any new, at-risk populations that may emerge following displacement caused by conflict or climate change (6), the potential to overlook circulation of virus in the year leading up to global certification will be greatly reduced.

What role do asymptomatic infections play in virus transmission?
Measles control strategies assume that virus transmission occurs through chains of clinically recognizable measles cases, and the surveillance system largely relies on the identification of these cases for detecting and responding to outbreaks. But asymptomatic infections certainly occur and may play an important role in measles transmission. Serological evidence for acute measles infection among people exposed to measles virus but failing to develop classical symptoms has been well documented (7,8,9,10,11,12,13,14,15) and it has long been recognized that measles virus can infect previously immune persons, producing classic symptoms of measles in some, but mild or no symptoms in most (16,17,18,19,20). The estimated rates of mild or asymptomatic measles infections after exposure to measles cases are varied, however, in part because of different diagnostic techniques and different case definitions used, or because of the different types of exposure. In several studies the rates of mild or asymptomatic infection were determined during outbreaks in which persons were likely to have had multiple exposures to measles cases (16,21,12,8). A study of mild or asymptomatic measles infections among 44 persons likely to have been exposed to classic measles during a 3-day bus trip concluded that in populations with high levels of immunity to measles, non-classic measles infections can occur in at least 20% of previously immune persons with close exposure to a person with classic measles (10). It is possible that mild or asymptomatic measles infections are common among measles-immune persons exposed to measles cases and may be the most common manifestation of measles during outbreaks in highly immune populations (10). Although clinically unimportant, asymptomatic measles virus infections could be epidemiologically important if infected persons are capable of transmitting virus. Although at least one study has reported isolation of measles virus from an asymptomatic individual in close contact with an acute case (11), another study failed to find evidence of virus shedding from 11 seropositive acute case contacts (14). If transmission from asymptomatic cases does occur, it is likely to be very rare, and is unlikely to be efficient enough to sustain transmission (11,15), especially in the highly vaccinated populations expected in the years immediately following global certification of eradication.
Conclusion
If the certification criteria are firm enough, and require rigorous validation, then the risk of undetected measles transmission after Global Certification is very low. If the certification criteria are lax, or validation requirements are inadequate, the risk will higher.

Risk assessment: The risk is intuitively low, but until the criteria for global certification of measles eradication and the requirements for validation are established it is not possible to estimate the risk posed by continuing wild-type measles transmission in undetected reservoirs.

Risk of transmission of vaccine-derived virus
The development of live attenuated measles virus vaccines began soon after isolation of the virus by Enders and Peebles in 1954 (22). The first licensed attenuated measles vaccine was Edmonston B, used between 1963 and 1975 but frequently associated with fever and rash (23). The further attenuated Schwarz and Moraten strains were derived from the original Edmonston strain through additional passages in chick embryo fibroblasts (Figure 1). Despite differences in their passage history, these two vaccine strains have identical genomic sequences (24). The Moraten vaccine is widely used in the United States of America; the Schwarz vaccine is used in many countries throughout the world, and the Edmonston-Zagreb vaccine, similarly derived from the Edmonston B strain, is the most widely used strain in developing countries. Other attenuated measles vaccines have been produced from locally derived wild-type strains, particularly in the Russian Federation (Leningrad-16), the People’s Republic of China (Shanghai-191) and Japan (CAM-70, AIK-C) (23). All of the current vaccine viruses are well documented and well characterised with regard to provenance, immunogenicity, thermal stability and genomic structure (25,26,27,28,29,30,31,32,33). Although current vaccine viruses and their wild-type progenitors share more than 95% sequence homology, they can easily be distinguished genetically from currently circulating wild-type viruses.
Dr Ray Sanders. Measles re-introduction risk analysis

Figure 1. Relationships of major current measles vaccine viruses (from Moss & Scott, 2009 (23)).

Measles virus is considered to be one of the most contagious of human pathogens, with a very high level of transmissibility. Like wild-type virus, measles vaccine virus replicates effectively within vaccine recipients, inducing both humoral and cellular immune responses similar to natural measles virus infection, although these responses are of lower magnitude and shorter duration.

Approximately 5% of children develop fever and rash after receiving measles vaccine, and viral RNA can be detected in the urine and respiratory secretions for some days post-immunization (34). Vaccine virus can be isolated from the blood of recent vaccine recipients, and has been detected in samples of lung, liver, bone marrow or brain tissues in the very rare cases of severe acute disease following measles vaccination (35). Virus RNA and antigen can be detected in the urine of vaccine recipients for up to 14-16 days post-immunization (36,37), but there is no published evidence for the transmission of vaccine virus. Obviously the changes caused by the attenuation process effectively block transmissibility. Is it possible for vaccine virus to regain the transmissibility characteristics of wild-type virus?

The reasons for non-transmission of vaccine viruses are not fully understood, and are likely to be complex. It has been proposed that loss of ability to interact with epithelial cell receptors is a key factor (38,39,40). It is also possible that modification of the virus matrix (M) protein, known to be important in virus budding from infected cells (41), contributes to loss of transmissibility. The ability of vaccine viruses to interfere with the innate immune response may also be a key factor. Whatever the reason, it appears that the block on transmission of vaccine viruses is highly effective.

Measles virus is serologically monotypic and is genetically characterized into eight clades (A–H), divided into 23 recognized genotypes (42,43,44). All of the current vaccines, whether derived from Edmonston or not, share a remarkable nucleotide sequence similarity and all are members of genotype A (45,24). During the 1950s and 1960s, only measles viruses belonging to genotype A were
isolated and may have had a world-wide distribution before vaccination started (46,47,48). This is not the situation today, when the identification of non-vaccine related genotype A viruses is very unusual. Over the past fifteen years a massive amount of work has been put into characterizing measles viruses associated with outbreaks. Although there are still gaps, viruses from most major outbreaks and from importations in areas that have eliminated indigenous measles, are currently being sequenced and genetically characterized through the WHO Laboratory Network’s activities. We now have a reasonably comprehensive understanding of which viruses are circulating where (42,43,44,49,50,51,52,53).

Against a background of several thousand isolates characterized, very few genotype A viruses have been identified during the past 20 years. With the possible exception of viruses isolated in the UK in 1993 (54), none has been associated with outbreaks. When detected they have been sporadic cases with uncertain epidemiology, closely associated with very recent receipt of vaccine, or queried as laboratory contaminants (43,55,56,57,58,59,60,61,62).

Table 1 summarizes the published documentation on the detection of genotype A measles viruses since 1990.

<table>
<thead>
<tr>
<th>Year of detection</th>
<th>Country</th>
<th>State/Province/Region</th>
<th>Number of isolates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>Japan</td>
<td>Handai?</td>
<td>1</td>
<td>(62)</td>
</tr>
<tr>
<td>1991</td>
<td>Argentina</td>
<td>Buenos Aires</td>
<td>1</td>
<td>(63)</td>
</tr>
<tr>
<td>1993</td>
<td>UK</td>
<td>Coventry, England</td>
<td>5</td>
<td>(54)</td>
</tr>
<tr>
<td>1995</td>
<td>South Africa</td>
<td>Johannesburg</td>
<td>1</td>
<td>(64)</td>
</tr>
<tr>
<td>1996</td>
<td>Russian Federation</td>
<td>Novosibirsk, Siberia</td>
<td>3</td>
<td>(56)</td>
</tr>
<tr>
<td>1996</td>
<td>USA</td>
<td>Delaware</td>
<td>1</td>
<td>(60)</td>
</tr>
<tr>
<td>1996</td>
<td>China</td>
<td>Hunan</td>
<td>1</td>
<td>(55)</td>
</tr>
<tr>
<td>1996</td>
<td>UK</td>
<td>?</td>
<td>2</td>
<td>(58)</td>
</tr>
<tr>
<td>1996</td>
<td>South Africa</td>
<td>Johannesburg</td>
<td>1</td>
<td>(57)</td>
</tr>
<tr>
<td>1998</td>
<td>UK</td>
<td>Importation from Russia</td>
<td>1</td>
<td>(58)</td>
</tr>
<tr>
<td>1999</td>
<td>Argentina</td>
<td>Buenos Aires</td>
<td>2</td>
<td>(63)</td>
</tr>
<tr>
<td>1999</td>
<td>China</td>
<td>Henan</td>
<td>1</td>
<td>(55)</td>
</tr>
<tr>
<td>2000</td>
<td>UK</td>
<td>?</td>
<td>1</td>
<td>(58)</td>
</tr>
<tr>
<td>2001</td>
<td>Spain</td>
<td>Ibiza</td>
<td>1</td>
<td>(59)</td>
</tr>
<tr>
<td>2002</td>
<td>Spain</td>
<td>Madrid/Badajos</td>
<td>2</td>
<td>(59)</td>
</tr>
<tr>
<td>2003</td>
<td>Spain</td>
<td>Almeria</td>
<td>3</td>
<td>(59)</td>
</tr>
<tr>
<td>2003</td>
<td>China</td>
<td>Xinjiang</td>
<td>1</td>
<td>(55)</td>
</tr>
<tr>
<td>2005</td>
<td>Taiwan</td>
<td>Taichung/Taipei</td>
<td>2</td>
<td>(61)</td>
</tr>
<tr>
<td>2007</td>
<td>Taiwan</td>
<td>Tainan/Taipei</td>
<td>2</td>
<td>(61)</td>
</tr>
</tbody>
</table>

Table 1. Published documentation on isolation and characterization of genotype A measles viruses from 1990 to May 2010.

Table 1 includes isolates that may represent wild-type lineages that have survived since the pre-vaccination era. It also includes viruses isolated from very recent vaccine recipients presenting with classic measles symptoms. But it may also include vaccine-derived isolates that have been transmitted from vaccine recipients to unvaccinated contacts. Although some of these genotype A viruses have nucleotide substitutions that distinguish them from vaccine viruses, there is no published documentation identifying a distinct set of genetic markers that consistently differentiates wild-type viruses from attenuated viruses (46). Measles vaccine viruses re-isolated from
immunosuppressed patients with giant cell pneumonia have nucleotide sequences almost identical to those of the vaccine virus, suggesting that vaccine viruses are very stable even after prolonged replication in a human host (46).

Numerous published studies of several thousands of isolates from acute measles cases investigated over the past 20 years have failed to detect genotype A viruses (52,53,65,66,67,68,51,50,69,70) (71,72,73,74,75,76,77,78,79,80) (81,82,83). Because of the increasing intensity of measles immunization programmes, genotype A viruses, in the form of vaccine viruses, should be the most abundant measles genotype on Earth. Given that they are so infrequently isolated from measles cases, the molecular epidemiological data appears to support the contention that vaccine viruses do not readily revert to wild-type transmissibility.

What is the risk of measles vaccine-escape mutants?
There is no conclusive published evidence for the emergence of measles vaccine escape mutants (84). Measles is a typical RNA virus in that intrinsic errors of the RNA polymerase and lack of proofreading mechanisms results in a mutation rate of $9 \times 10^{-3}$ per base per replication and a genomic mutation rate of 1.4 per replication (85). This is well within the typical range of $10^{-3}$ to $10^{-6}$ mutations per site per replication (86). As a consequence of this high mutation rate, RNA virus populations, even those initiated by a single infectious unit, are not clonal but consist of a large number of genetic microvariants referred to as quasispecies. Despite the high mutation rate, and unlike other RNA viruses such as influenza and HIV, measles virus remains remarkably stable. How can live attenuated vaccines developed from wild type measles viruses more than half a century ago still be effective against circulating viruses?

The answer is probably associated with use of the signalling lymphocytic activation molecule (SLAM; also known as CD150) receptor by the measles haemagglutinin (H) protein, which is responsible for cell attachment and is a major target for neutralizing antibodies (87). The envelope of measles virus has two types of glycoprotein spikes, designated haemagglutinin (H) and fusion (F) proteins. The H protein binds to specific molecules (receptors) on target cells, while the F protein mediates membrane fusion between the virus envelope and the host cell plasma membrane through cooperation with the H protein. In 2000, SLAM was identified as a cell receptor for measles virus (88). SLAM is expressed on cells of the immune system, such as activated lymphocytes and dendritic cells (89). Studies on the crystalline structure of the H protein have shown that although most of this glycoprotein is covered by sugar chains, the large surface area that hosts the SLAM binding site is free from sugar chains (90). Mutations in this region are not permitted because they interfere with receptor binding. This extreme sequence restriction allows for very efficient production of neutralizing antibodies that block binding of the virus to its receptor. So the original vaccine strains, developed in the 1960s, are still effective against current wild-type viruses (91). Analysis of available sequence data from approximately 500 isolates suggests that despite the error-prone viral polymerase, the amino acid sequence of H is strongly conserved, with 60% of the residues being identical or very similar (92). It appears that any mutation that changes the nature of these conserved residues results in non-viable virus.

Conclusion
There is no current published data to support evidence for currently licensed live attenuated vaccine viruses reverting to wild-type transmissibility. On the contrary, the vast majority of evidence points
to an impressive level of genetic stability. However, since they are live viruses that replicate within vaccine recipients, the possibility must exist that they could revert to wild-type transmissibility. There is strong experimental evidence for the monotypic nature and genetic stability of measles virus being based on use of the SLAM receptor. There is also no evidence for the establishment of vaccine-escape mutants. Even if vaccine viruses were to revert to wild-type transmissibility, there is no reason to suspect that transmission could not be controlled using current vaccines.

Risk assessment: Available information suggests that the risk of current live-attenuated vaccine viruses reverting to wild-type transmissibility is very low, but it remains a possibility.

Risk from persistent infections

How long does measles infection usually persist?
In classic measles cases there is a 10–14 day incubation period between infection and the onset of clinical signs and symptoms, and infected persons are usually contagious from 2–3 days before and up to four days after onset of the rash. Host immune responses to measles virus are essential for viral clearance, clinical recovery and the establishment of long-term immunity. Early innate immune responses occur during the prodromal phase and include activation of natural killer (NK) cells and increased production of interferons (IFN)-α and β (23,93,94). However, the mechanisms and timing of normal measles virus clearance are poorly understood. Measles virus has been isolated from peripheral blood mononuclear cells (PBMC) up to a week, and from urine up to 10 days, after appearance of the rash (95,96). Delayed virus clearance has been documented in cases of malnutrition (97,98,99) and patients with cellular immunity deficiencies (100,101,102). Detection of measles virus RNA has been reported for up to 4 months in a case of congenital measles (103), for 1 to 4 months after uncomplicated infection in 90% of HIV-1-infected children and more than 50% of HIV non-infected children (104,105,106,107). These data are consistent with studies of rhesus macaques showing that virus clearance occurs over 120–150 days (108), suggesting that normal clearance is a prolonged process. Despite the reported persistence of viral RNA, there have been no reports of infectious virus shedding more than 3 to 4 weeks after appearance of symptoms (98,99).

Persistent infection with measles virus has definitively been associated with subacute sclerosing panencephalitis (SSPE), a progressive fatal neurological disease with high levels of neuronal infection by measles virus in the central nervous system (94). In immunocompromised patients, persistent measles virus has been linked to another neurological infection, measles inclusion body encephalitis (MIBE) (109). Multiple sclerosis, chronically active autoimmune hepatitis, Paget’s disease, otosclerosis, Crohn’s disease and autism, among many other diseases, have also been suggested at various times as long-term sequelae of measles virus infection. No confirmed evidence has been presented, however, to substantiate these associations, let alone prove a causative relationship.

What is the risk from SSPE cases?
SSPE is a slow, progressive disease that is invariably fatal. The average period from initial measles infection to SSPE symptom onset (latency) usually ranges between 4 and 10 years, but has been reported from 2 months to 23 years (110). Children are far more likely to develop this complication than adults. Reported SSPE incidence varies from approximately 0.2 to 40 cases per million population per year. Direct comparison of data from different countries is problematic because
methods and quality of diagnosis have been inconsistent. Analyses of data from the UK and USA have calculated the true incidence of SSPE to be approximately 4–11 cases of SSPE per 100 000 cases of measles. A higher risk is associated with earlier infection: the risk following measles infection under 1 year of age is 18/100 000 compared with 1.1/100 000 after 5 years of age in the UK (110). Obviously, as the number of measles infections declines, so will the number of potential SSPE cases.

The disease initially manifests as subtle cognitive losses, progressing to more overt cognitive dysfunction, followed by motor loss, seizures and eventual organ failure in virtually all affected individuals. Neurons in both the gray and white matter are infected, and the disease is histologically characterized by the presence of cellular inclusion bodies (111). A serologic hallmark of SSPE, as compared to the other central nervous system complications, is the elevation of measles specific antibodies in the blood and cerebrospinal fluid (94). Most importantly, evidence from brain biopsies of SSPE patients indicates that infected neurons do not release budding virus (112). Based on sequencing studies of virus from these specimens and from cells persistently infected with measles virus isolates from SSPE patients, it has been proposed that the failure of infected neurons to produce complete extracellular virus may be due to defects in protein expression caused by extensive point mutations in the H, fusion (F) and matrix (M) genes (113,94,114,115,116). There is no evidence for transmission of measles virus from SSPE cases.

**What is the risk from MIBE cases?**

Measles inclusion body encephalitis (MIBE) is a rare central nervous system complication following acute MV infection, has been described in children and adults receiving immunosuppressive drugs and therefore is thought to chiefly affect immunocompromised hosts. MIBE has also been reported to result from receipt of measles vaccine (117). The neurologic disease usually appears 3 to 6 months after the acute measles rash (111), with a median time of 4 months (118). Measles antigen is present in the brain, and virus has been isolated directly from the brains of affected individuals (111,119). MIBE differs from SSPE in the absence of elevated serum and cerebrospinal fluid neutralizing antibodies (94). The disease course is relatively short, lasting from days to weeks, causing seizures, motor deficits, and stupor, often leading to coma and death.

Although only a very small percentage of acute measles infections will go on to develop persistent complications, a few studies have detected measles virus RNA in various organs, on autopsy, of elderly individuals who died of non-viral causes (120,121). These findings suggest that measles virus persists in the brains (and other organs) of healthy individuals, and may manifest itself in central nervous system disease under conditions of immunocompromise or immunosuppression. This has been underlined by the case of a 13 year-old boy that developed MIBE after receiving a stem cell transplant (119). Neither the patient nor the stem cell donor had apparent recent measles exposure or vaccination, and neither had recent travel to measles-endemic regions. The patient was born in Chicago during the measles epidemic of 1989-1991 (birth year 1989). An undiagnosed case of measles in the period 1989-1991 would suggest a latency period to MIBE of 12 years, which is not typical. Cases of MIBE without clear measles exposure or infection have been reported. In a review of MIBE, 18% of patients had no documented measles exposure or infection (118); however, many of these cases occurred in years when measles was more prevalent. There are no published reports of infectious measles virus shedding from MIBE cases.
Does HIV co-infection present a risk for persistent measles infection and transmission?

As discussed above, measles virus RNA could be detected in samples from 90% of HIV-infected children one month after recovery from acute measles (104), but in this study no attempt was made to culture virus from any samples. In regions of high HIV-1 prevalence, co-infection with HIV-1 more than doubles the odds of death in hospitalized children with measles (122) and may slow the rate of virus clearance slightly, but there is no evidence that HIV-infection leads to an increased risk for persistent measles virus infection. Nor does HIV infection appear to present a risk for persistent infection with the measles vaccine virus. A search for persistent measles mumps and rubella vaccine viruses in children with HIV-1 infection failed to detect virus in peripheral blood mononuclear cells, polymorphonuclear leukocytes, or plasma (123).

Conclusion

There is no published evidence that cases of persistent measles infection are associated with the shedding of infectious virus or play any part in measles transmission. As the number of acute measles virus cases declines in the years leading to global eradication, we can expect a decline in the number of potential SSPE and MIBE cases.

Acute measles infection in HIV-infected individuals tends to be more severe, last longer and result in a shorter lived immunity to re-infection, but there is no published evidence to suggest that co-infection increases the potential for establishment of persistent measles infections, either with wild-type virus or with vaccine-derived virus.

Risk assessment: Available information suggests that the relatively small number of persistent measles virus cases, including those that may result from co-infection with HIV, pose a very low risk for reintroduction of measles.

Risk from non-human primates

A large proportion of our current knowledge of measles and measles infection mechanisms have come from experimental infection of non-human primates. In 1911, Goldberger and Anderson demonstrated that macaques inoculated with filtered secretions from measles patients developed measles, proving the causative agent was a virus (124). A wide range of non-human primate species are susceptible to experimental infection with measles virus. These include Macaca mulatta, M. fascicularis, M. radiata, M. cyclopis, Papio cristatus, Cercopithecus aethiops, Saimiri sciureus, Colobus quereza, Pan troglodytes, Callithrix jacchus, Saguinus oedipus, S. fuscicollis, and Aotus trivirgatus and Ateles species (125,126,127). As would be expected from an effective animal model, many species respond to infection in a manner very similar to humans (128,129,130). Inadvertent transmission of either measles (from humans) or the closely related canine distemper virus (from dogs) to captive non-human primates has caused numerous outbreaks with significant morbidity and mortality (131,127,132,133,134). Non-human primates in the wild appear to be free from measles, only contracting infection when they come into contact with infected humans (125). Human-to-primate disease transmission can potentially cause significant morbidity and mortality among wild primate populations. Serological evidence of measles infection in free-ranging populations of non-human primates has been well documented (135,136,137). Evidence exists of measles infection in non-human primate populations with frequent contact with human populations, as well as in wild
populations with minimal human contact (127). A cross-sectional study of wild macaques (*Macaca tonkeana*) in Sulawesi, Indonesia, found serological evidence of measles evidence in 5 of 15 animals surveyed (136).

Because human populations represent the largest reservoir of the measles virus, it is most likely that measles epizootics in non-human primate populations are initiated by human to non-human primate transmission and subsequently spread by animal to animal transmission. Due to their relatively small numbers, it is unlikely that natural populations of non-human primates are significant or sustainable reservoirs of measles virus (127).

**Conclusion**

Although non-human primates can be experimentally and naturally infected with measles virus, and animal to animal transmission occurs, population sizes are too small to maintain epizootic transmission.

**Risk assessment:** Available information suggests that infections in non-human primates pose a very low risk for reintroduction of measles.

**Risk of laboratory-associated measles infection**

Risks posed by laboratory-maintained measles viruses, through accidental or intentional release, are largely dependent on whether universal immunization against measles is continued or if it is stopped on, or soon after, global certification. If the decision is made to continue universal immunization, possibly with non-replicating vaccines, the risk posed by laboratory-maintained virus will be very low, since there will be almost universal immunity. If, however, universal immunization stops after global certification, the risks posed by laboratory-maintained measles-infectious materials will progressively increase, as the number of measles-susceptibles in the population increases. The risks include not only accidental release of live measles virus from laboratories and attenuated virus vaccine production facilities, but threat of deliberate release.

**What is the evidence for laboratory-acquired measles infection?**

A series of surveys for laboratory-acquired infections conducted in the UK (138,139,140,141,142,143,144), the USA (145,146,147,148,149) and Japan (150) failed to include measles among the listed infections. A recent review of principles for prevention of laboratory-associated infections also failed to make mention of measles (151). An extensive literature search failed to find documented evidence of laboratory-acquired measles infection. This leaves three possibilities: laboratory-acquired measles infections have not occurred; the infections that have occurred have been below the threshold of sensitivity of the surveillance systems; or, measles has been considered a trivial disease and infections have not been reported (152,153).

Prior to the 1970s it is to be expected that almost all staff working in clinical microbiology and research laboratories would have been exposed to measles infection during childhood. From the 1970s onwards it is to be expected that all new staff coming to work in these laboratories would have received at least one dose of measles vaccine. It is unlikely therefore, that exposed laboratory staff would develop acute measles symptoms from laboratory-acquired infections. But given the very high transmissibility of measles virus, it is possible that exposure to infectious virus, and
resulting asymptomatic infections, or very mild, atypical infections have occurred. If they have occurred, it is probable that these infections have gone undetected, or simply overlooked as unimportant.

How stable is measles virus in the environment and in laboratory materials?
Measles is not a physically robust virus. It is viable for less than 2 hours at ambient temperatures on surfaces and objects, while the aerosolized virus typically remains infective for only 30 minutes to 2 hours, depending on environmental conditions (154,155). It is very sensitive to heat and is inactivated after less than 40 minutes at 56°C, even in medium containing a protein stabilizer such as 5% calf serum (156). Virus in maintenance medium loses at least 2 logs of titre when stored at +6°C for 14-20 weeks and loses all infectivity after 1 year at this temperature. Addition of a protein stabilizer improves virus longevity, with a loss of approximately 2 logs of titre after 1 year at +6°C. Interestingly, storage at -30°C offers little advantage over storage at +6°C, with a 1-2 log loss of titre over 1 year. Storage at -72°C or below results in very little loss of virus infectivity, and infectious materials maintained at this temperature should retain infectivity for many years (156). The virus survives freeze-drying relatively well and, when freeze-dried with a protein stabilizer, can survive storage for decades at -70°C (156,155). In common with many other enveloped viruses it is inactivated by solvents, such as ether and chloroform, by acids (pH<5), alkalis (pH>10), and by UV and visible light. It is also susceptible to many disinfectants, including 1% sodium hypochlorite, 70% alcohol and formalin.

Which laboratory materials present a risk?
Measles virus infectious materials include autopsy or clinical samples (e.g. pharyngeal secretions, urine, blood) from measles-infected persons or recent live-attenuated vaccine recipients, and laboratory derived materials (e.g. virus isolates and reference stocks, materials derived from inoculated cell cultures, laboratory animals). Measles virus potential infectious materials, those that are suspected to contain infectious measles viruses, include pharyngeal secretions and blood samples collected for any purpose at a time and in a place where measles viruses were circulating, and stored under conditions that would preserve virus infectivity. They also include products of these materials in measles virus permissive cells or animals (157).

What types of risk do laboratories present?
Risks post measles eradication will exist at two levels:
- occupational risk of exposure among laboratory staff,
- community risk of laboratory-associated measles exposure.

The three most common routes of exposure to infectious agents in the laboratory are ingestion, inhalation, and injection (153). Measles virus can remain infectious on surfaces, such as work benches and door handles, for up to two hours. If transferred from the hand to the mouth, nose or conjunctiva, they can initiate infection of epithelial cells (158). Although there are no recorded incidents of laboratory-acquired measles virus infections, several surveys document the frequent occurrence of ingesting more readily recognized pathogens, such as Shigella and Salmonella (139,140,141,142,143,147,153). The most common route for natural transmission of measles is believed to be by inhalation of aerosolized virus; infectious droplets being produced by talking, coughing and sneezing by infected individuals (158). Small particles (<5-μ droplet nuclei) of suspended evaporated residues can move about rooms and buildings on air currents and when
inhaled deposit primarily in the lower respiratory tract (159). Laboratory activities that expose staff to aerosols generated from infectious material (e.g. centrifugation, blending, vigorous pipetting, etc.), and exposure to infected laboratory animals, present a risk for infection. The most common route for delivery of current measles vaccines is by injection. So, injection and needle-stick injuries involving measles virus infectious materials obviously present a risk for infection.

Community members may be exposed to infectious measles virus from:

- contaminated laboratory workers,
- infected laboratory workers,
- contaminated air effluents,
- transport of infectious material,
- escaped infectious animals.

Again, no published evidence exists for the escape of infectious measles virus from the laboratory into the community. Given the rapid inactivation of measles virus under normal environmental conditions, the length of time available for infectious virus to be carried out of the laboratory and into the community, either on the body or clothes of a contaminated worker, or in contaminated air effluents, is probably limited to 2 hours. This reduces the risk to a very low level. As discussed above, available evidence suggests that immunized individuals, who develop asymptomatic or mild infections, are unlikely to transmit the virus (10), reducing the community risk. We can assume that laboratories implementing good laboratory practices (GLP) or good management practices (GMP) will minimize the risks of release to the environment by properly packaging and transporting infectious materials in accordance with current international laws and regulations. Given the security concerns that surround laboratory animal houses and research facilities, the likelihood that measles-infected animals would escape into the community must be extremely small.

**Conclusion**

Although there is no direct evidence for laboratory-acquired measles infections it is possible that they have occurred among immune laboratory staff and resulted in asymptomatic or very mild infections. There is no published evidence to suggest that these possible asymptomatic or mild infections result in further transmission of virus. Measles virus loses infectivity within a couple of hours at ambient temperatures in the environment, and infectious materials stored at temperatures above -30°C can be expected to lose all infectivity over the course of one to two years. Despite the lack of evidence for laboratory-acquired measles infections or escape of virus into the community, in a post-eradication world these must be considered possibilities due to the highly infectious nature of measles.

**Risk assessment:** In a measles post-eradication world without routine universal immunization, measles laboratories (and measles live vaccine production facilities) will pose a very low but increasing risk for reintroduction of measles.

### Risk of intentional release of measles virus

Bioterrorist threats do not work against populations that have been fully immunized. However, in a post-eradication world in which universal routine immunization has ceased, a growing population...
will be susceptible to measles, and measles will, eventually, become a credible agent for bioterrorism. The devastating effect of measles on susceptible populations in the pre-vaccination era has been well documented (158). This is particularly true for the islands of the Pacific. In 1848 in Hawaii, 10,000 natives, about 10 percent of the population, died during an epidemic (160,161,162). In 1861 on Aneityum in the New Hebrides, the population was reduced by about 60 per cent in a measles epidemic (163). In 1875 in Fiji, 20,000 natives, 20 to 25 per cent of the population, died of measles (164). In 1907, again in Fiji, 6 per cent of 30,000 cases died, and in 1911 on Rotuma 16 per cent of the population died of measles (165). In 1936 measles caused 100 deaths and 14,282 cases in the Gilbert Islands (166), and in 1937 in Hawaii, there were 205 deaths for 13,680 cases of measles (167). In 1946 in the British Islands of the South Pacific, there were 1,000 deaths for 15,000 to 20,000 cases of measles (168). There are many other accounts of similar devastating measles epidemics in isolated communities around the world.

With advances in modern medical treatment it is unlikely that similar mortality rates would be inflicted one or two generations post measles eradication, but deliberate release would cause extensive disruption to medical, public health and social services, and probably incur enormous containment costs. The threat of release, with the knowledge of the potential disruption and financial expense it could cause, would make measles an effective agent for bioterrorists once a large enough population of measles-susceptibles had accumulated. Measles is not currently included in the CDC Bioterrorism Agent Categories (169,170), but this situation will need to be reviewed in the years following eradication.

**Conclusion**

Measles is a highly infectious virus that has had devastating effects on susceptible populations in the past. Although it is unlikely that the high mortalities seen in these isolated communities would be repeated, the threat of intentional release would probably be very effective once a sizable population of susceptible individuals had accumulated.

**Risk assessment:** The risk of deliberate release of measles will be very low at the time of global eradication, but will rise rapidly with accumulation of unvaccinated measles susceptibles.

**Actions required to reduce the risk of accidental or deliberate release of measles**

One approach to reducing the risk of measles re-introduction would be adoption of a strategy to minimize availability of measles virus, through removal of live viruses from laboratories and securely containing all infectious material that remains, and establishing an insurance policy in the form of a vaccine stockpile.

**Reducing the risk of accidental release: a laboratory containment strategy**

A systematic laboratory containment strategy for measles, learning from the example set by the Polio Eradication Initiative (171), starting now and continuing into the post-eradication era, would minimise the risk of accidental re-introduction of measles virus. The strategy established for polio outlines three distinct phases. Phase 1 would last from the present, when measles continues to circulate, to the time when measles transmission ceases. Phase 2 would cover the certification
period, and Phase 3 would take place in the post eradication, post global certification period. These three Phases for polio have been clearly described in a series of published Global Action Plans (172,173,157).

The laboratory-associated risks posed by measles are considerably lower than those posed by polio, and strategies for reducing the risk even further should not simply duplicate the activities developed for polio, but be proportionate and appropriate for measles. The general approach taken by the Polio Eradication Initiative, and lessons learned from implementing the polio containment strategy, should provide a sound starting point for measles. Strategies for reducing the risk in the pre-eradication phase should be based on the following principles:

• minimizing the number of laboratories retaining measles virus infectious and potential infectious materials;
• minimizing the risks of operations in laboratory and measles live vaccine production facilities;
• minimizing the susceptibility of workers to measles virus infection and shedding;
• minimizing susceptibility of community to measles virus spread.

The highest risks are presented by those laboratory operations involving measles virus replication, including the growth of vaccine strains for live vaccine production. The lowest risks are non-replicative, biosafety-appropriate operations performed with potentially infectious clinical materials. In the years leading up to global eradication all work with wild measles viruses should require biosafety level-2 (174), with additional requirements for restricting laboratory access, and maintenance of accurate records of measles virus materials. Establishing national measles inventories, and calls to safely dispose of all unwanted measles infectious and potential infectious materials, as has been accomplished for polio, would also be required.

The second phase of risk reduction would consist essentially of validating the containment activities at national, regional and global levels as a requirement for Global Certification. Stopping universal measles immunization post certification (third phase) will alter the relative weights of the principles on which minimizing the risk from the laboratory is based (157):

• minimizing susceptibility of communities to measles virus spread will no longer apply in those countries that elect to stop measles immunization;
• minimizing the susceptibility of workers to measles virus infection and shedding, in the absence of a non-infectious vaccine, will rely solely on prevention of infection;
• minimizing the number of laboratories retaining measles virus materials and minimizing the risks of operations in those laboratories becomes much more important.

We are currently considering the prospect of global cessation of measles transmission approximately a decade from now, allowing reasonable time to develop an appropriate measles laboratory containment strategy and for laboratory research on measles viruses to continue under current, biosafety level-2, conditions. It also allows time for continued development of alternative measles vaccines and specific antivirals.
Developing a vaccine stockpile

Live attenuated measles vaccines have been highly successful in protecting populations against measles and stopping measles transmission. As discussed above, these vaccines are very safe, and pose only a small potential risk for establishing transmission of vaccine-derived viruses in a post eradication world. To remove this risk a new vaccine that has no capacity for replication or transmission is required (175). The ideal measles vaccine would be inexpensive, safe, heat-stable, immunogenic in neonates or very young infants, and administered as a single dose without the need to use a needle or syringe (93), be 100% effective and 100% incapable of transmission. While such a vaccine would have clear benefits for the eradication of measles, it would be as a vaccine for stockpiling post eradication that it would come into its own. Several vaccine candidates with some of these characteristics are undergoing development and testing. Features of these new, potential measles vaccines have been extensively reviewed (175,176).

How large a measles vaccine stockpile would be required is very difficult to predict without modelling. Requirements would obviously be dynamic, depending on some fairly complex variables, including the number of susceptibles accumulating in the community, the effectiveness of the vaccine, transmission dynamics of the virus and the effectiveness with which any event requiring an immunization response was detected, reported and responded to. Decisions on such big, expensive items as establishing a measles vaccine stockpile should not be taken in isolation, but considered systematically and included in a consensus risk management strategy, as has been achieved for polio (177,178,179,180,181,182). Development of a post measles eradication risk management strategy should begin as soon as possible.

Areas requiring further research

The risks of re-introduction of measles post global eradication may be reduced by applying knowledge acquired through key areas of research conducted in the years leading up to eradication. These key areas include the following:

Greater understanding of the transmission dynamics of measles

In drawing up the certification criteria and validation requirements it will be necessary to engage experts familiar with the development of dynamic and stochastic models of measles transmission, persistence and elimination. This will be particularly important for determining the certification and validation requirements for low-income, high density populations. Based on the experience gained in polio eradication, this will be most relevant for selected populations in Africa, the Indian sub-continent and large refugee/migrant population camps.

Important information can also probably be gained from detailed epidemiological and molecular analysis of outbreaks, particularly those occurring in highly immunized populations, high-density populations, and in generally highly-immunized populations with inadequately immunized sub-populations.

With the rapid increase in the number of highly immunized populations, opportunities for studying asymptomatic and atypical infections and their potential role in transmission should be taken.

Greater understanding of the changes brought about by the attenuation process
If currently licensed attenuated measles vaccines are to be used in a post-eradication world, more information on the nature of the changes caused by attenuation and the potential for reversion to wild-type characteristics will be required. An alternative would be to speed up development, testing and introduction of new measles vaccines that are not dependent on live attenuated virus.

More understanding of the nature of the complex interaction between measles virus and the host immune system, including both humoral and cell-mediated responses, would probably benefit continued use of existing vaccines and development of new vaccines.

In the years leading up to global eradication, all genotype A viruses detected in association with acute cases of measles should be thoroughly scrutinized. Full epidemiological information will be required, and additional sequence data from both clinical samples and corresponding viral isolates will be necessary to rule out the possibility of transmission of vaccine-derived virus. Thorough genetic analyses, including full genomic sequencing, should be performed on selected vaccine viruses that are associated with common vaccine reactions as well as those detected in the very rare severe reactions to vaccination.
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