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Is Smallpox Dead?
(The Story of Highly Contagious and Most Feared Disease)

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For thousands of years, smallpox raged as a scourge of mankind, causing death and disfigurement. Smallpox has existed for at least 3000 years and was one of the world's deadliest and most feared diseases. The disease that killed Queen Mary II of England, Tsar Peter II of Russia, King Louis XV of France and hundreds of millions more during the past century alone is gone – but not forgotten. Smallpox lives on in the memories of those who witnessed its awful impact. We have an obligation to ensure that our children's children never have to worry about seeing this ancient scourge kill yet again. The global eradication of smallpox, achieved after ten years of concerted campaigns under the auspices of the WHO, has been a most impressive medical achievement.

The earliest credible clinical evidence of smallpox is found in medical writings from ancient India, as early as 1500 BC. In India and Nepal, **Shitala Maata**, the Hindu Goddess of smallpox was worshipped in Temples. It was believed that this Goddess was both evil and kind and had the ability to inflict victims when angered, as well as calm the fevers of the already affected. Portraits of the Goddess show her holding a broom in her right hand to continue to move the disease and a pot of cool water in the other hand in an attempt to soothe victims. Temples were built where Hindus went to worship and attempt to protect themselves.

Fig 1: The Hindu Goddess Shitala Maata was worshipped to prevent or cure smallpox (Source: enwikipedia.org)

SMALLPOX IN NEPAL

From time immemorial, there has been a Temple in Nepal for Sitala, Goddess of smallpox, although the present temple at Swayambhu in Kathmandu was built less than two centuries ago after the abdication of King Rana Bahadur Shah.

The smallpox affected the Royal Family in Nepal and the King Girvana died of smallpox on 20 November, 1816 at the young age of 21. The disease also affected the Royal Court of King Rana Bahadur Shah. A prominent figure in the court suffered from the disease and his disfigurement and subsequent suicide led to the desecration and destruction of many temples including the previous Sitala temple at Swayambhu.

In conformity with the decision of the WHO, His Majesty's Government of Nepal launched the smallpox eradication program in 1967. The geographical distribution of outbreaks in Nepal was closely related to the epidemiological pattern in India and to the routes of population movement. In 1973, smallpox spread to Western Nepal from adjoining Uttar Pradesh, and suffered from its worst epidemic for many years. In 1974, the disease spread to Eastern Terai region from the adjoining state of Bihar. Twenty of the 75 districts of Nepal are in the Terai region, bordering India were more commonly affected than the districts of the other terrains in 1973 and 1974. A cash reward of Rs 100 was offered to the public in March 1975 for reporting an outbreak of smallpox. It was later raised to Rs 1000 by which time no smallpox cases were being reported. The last case of smallpox in Nepal occurred on 6 April, 1975. Eradication of the smallpox in Nepal was declared on 13 April, 1977 (1st Baishak, 2034).

Although Nepal has a long border with the Tibet region, there has been no report of importation of smallpox from Tibet. Travel across border is much more restricted than across the Indian border which is freely open without any requirement for documents.

DR. EDWARD JENNER

Dr. Edward Jenner (1749-1823) was an English physician, who discovered a safe and efficient vaccination against smallpox, which ultimately led to eradication of small pox (***Variola***). Jenner is often called the **Father of Immunology**. Folklore claimed that milk maids were immune to smallpox. Dr Jenner also observed that milk maids exposed to occupational cowpox infection, were immune to smallpox. He proved experimentally that resistance to smallpox can be induced by injecting cowpox material (***Vaccinia***) from disease pustules into man. The first experiment to test this theory involved milkmaid Sarah Nelmes and James Phipps, the nine year old son of Jenner's gardener. Dr. Jenner took material from a cowpox sore on Nelmes' hand and inoculated it into Phipps' arm on May 14, 1796. Months later, Jenner exposed Phipps a number of times to variola virus, but Phipps never developed smallpox. Jenner published his findings in 1798 in a pamphlet "*An inquiry into the cause and effect of variole vaccine*". Louis Pasteur gave the general term **vaccine** (Vacca = cow) in honor of Jenner's cowpox vaccine to various materials used to induce active immunity.

Fig 2: Dr. Edward Jenner (1749-1823) (Source: bbc.co.uk)

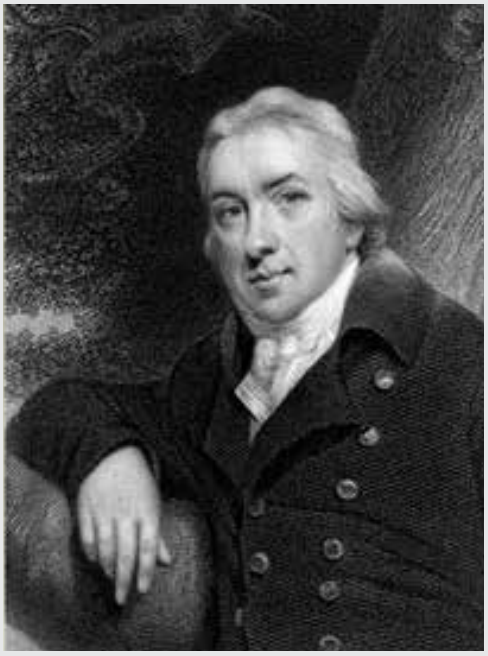
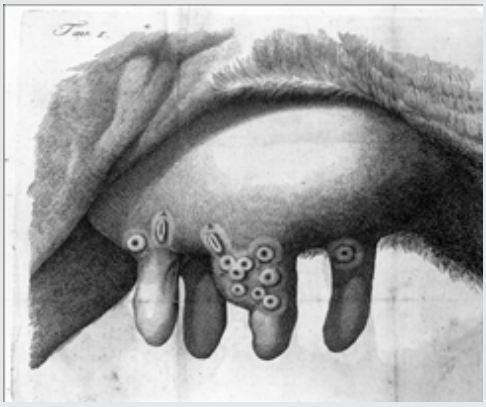


Fig 3: Cowpox lesions on udder (Source: livescience.com)



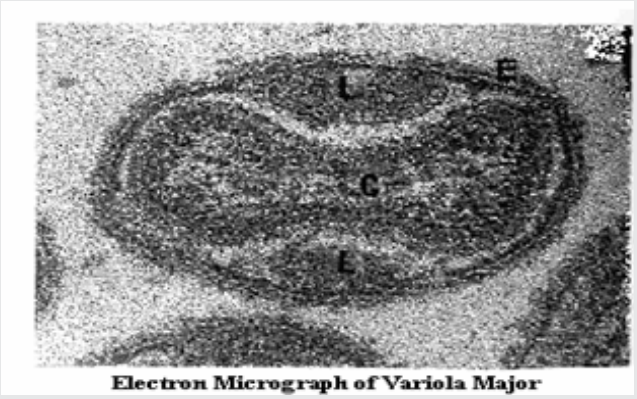
VIROLOGY

The ***Variola virus*** is the causative agent of smallpox, closely related to cowpox virus, belongs to family ***Poxviridae*** (Dermotropic DNA viruses), subfamily ***Chordopoxvirinae***, and genus ***Orthopoxvirus***, which includes vaccinia (smallpox vaccine), monkeypox virus, buffalopox, mousepox, camelpox, cowpox and variola (smallpox) viruses. Poxviruses are the largest viruses (400 x 240 x 100 nm)-large enough to be seen under light microscope. They are brick shaped and enveloped. They possess a double layered membrane surrounding a biconcave nucleoid containing DNA core. The nucleocapsid does not show any symmetry (complex symmetry). The lipoprotein envelope encloses a core and two lens shaped structures of unknown function, called lateral bodies. The core contains large viral genome, a single linear molecule of double-stranded DNA. The genome possesses the capacity to code for more than 200 polypeptides.

Vaccinia virus used for smallpox vaccination is derived from Jenner's original cowpox vaccine. The virus since Jenner's time was maintained by arm to arm passage in humans.

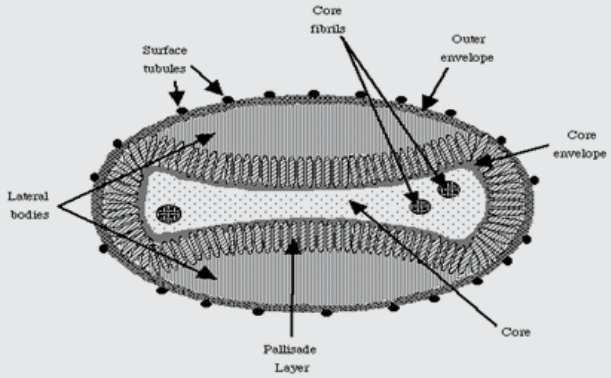
During this passage, the virus underwent some permanent changes differing in some respects from the freshly isolated cowpox virus, resulting in an artificial virus. Thus vaccinia virus is unique in that it is an artificial virus, which is not found in nature as such. The virus has been studied in detail as it is found to be safer to work with. Vaccinia virus is used as vector for introducing foreign genes and live virus (recombinant) vaccines has been developed. The genome of vaccinia virus can accommodate approximately 25,000 foreign base pairs – sufficient for introducing several genes. Many genes have been inserted in the genome, such as antigens of hepatitis B virus, rabies, HIV, and several pharmacological products (e.g. neuropeptides).

Fig 4: Electron microscopic picture of Variola major



Electron Micrograph of Variola Major

Fig 5: The structure of Variola virus



Smallpox epidemics in the past appeared in two distinct clinical varieties-the florid, highly fatal disease (**Classical smallpox**) typically seen in Asia, and the mild, nonfatal disease (**Alastrim**) typically seen in Latin America. The virus causing classical smallpox was called ***Variola major*** and that causing alastrim ***Variola minor***. Variola major and Variola minor were antigenically identical but differed in certain biological characteristics. They were stable variants as the diseases produced by each always bred true; alastrim did not lead to smallpox and vice versa.

PATHOGENESIS

Smallpox was an exclusively human infection, with no animal reservoir. There were no carriers as the virus was eliminated completely from the patient on recovery. Smallpox was highly contagious and spreads through person-to-person contact and saliva droplets in an infected person's breath or through formites such as bedding. The source of infection

was a patient in the early phase of the disease, though infectivity extended from the appearance of buccal mucosal lesions to the disappearance of all the skin lesions. Infection usually occurred only in close contacts. Virus entered the body by inhalation. After initial multiplication in the local lymphoid tissues, the virus reached the reticuloendothelial cells, where further multiplication took place, leading to severe viremia. The virus multiplies in the mucus membranes of mouth and pharynx and invades capillary epithelium of the dermal layer in skin heralding the clinical disease. Oropharyngeal and skin lesions contain virions abundantly, particularly in the early phase of illness.

CLINICAL MANIFESTATIONS

The incubation period was around 12 days (7 – 17 days). The prodromal phase, which lasts for two to three days, was characterized by severe headache, backache, and fever, all beginning abruptly. The temperature often rises to more than 40°C and then subsides over a period of two to three days.

During viremia, viruses settle at skin and mucosa; two to five days of fever precedes the appearance of skin rashes. The rash first appears on the tongue, mouth, and oropharynx (enanthema), and then on skin (exanthema). A **maculopapular rash** begins on the face and extremities and spreads to the trunk. The lesions were initially maculopapular and evolve rapidly from small, reddish **macules** to **papules** with a diameter of 2 to 3 mm over a period of one or two days; after an additional one or two days, the papules become **vesicles** with a diameter of 2 to 5 mm, and finally **pustules** that are 4 to 6 mm in diameter develop about four to seven days after the onset of the rash and remain for five to eight days, followed by umbilication and crusting. The **crusts** begin separating by the second week of the eruption. **Scabs** form over the lesions leaving survivors with **pitted scars**. These lesions were common on the face because the large sebaceous glands tend to become infected.

Smallpox lesions have a peripheral or centrifugal distribution and are generally all at the same stage of development. Death from smallpox is due to toxemia, associated with immune complexes, and to hypotension. There was a 10 – 30% mortality in classical smallpox and 5 – 10% of naturally occurring smallpox cases appear as either of two highly virulent atypical forms–hemorrhagic and malignant.

Fig 6: Classical smallpox patient (Source: owlcation.com)

Fig 7: A baby with smallpox (From the CDC)

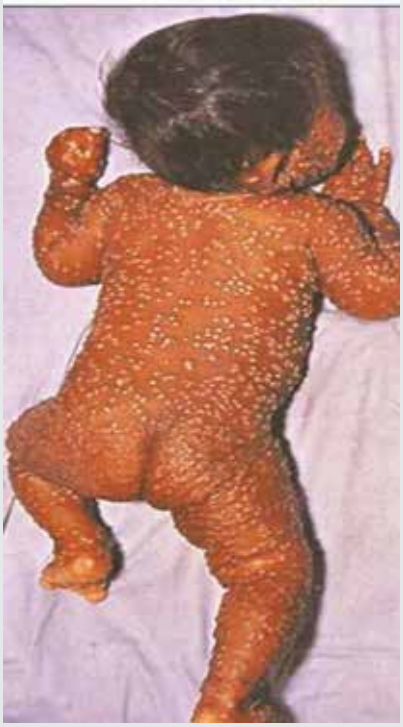
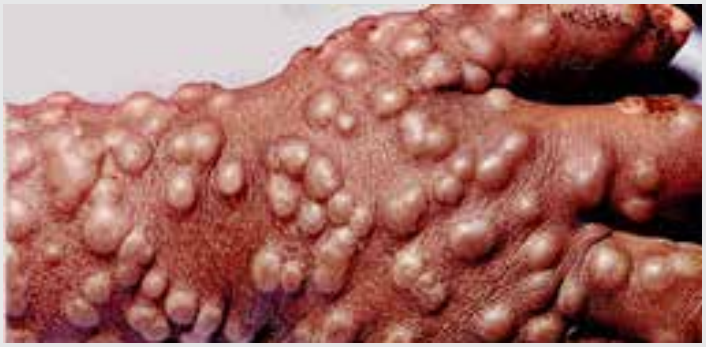


Fig 8: The lesions (vesicles) in classical smallpox on skin (Source: cdc.gov)



LAB DIAGNOSIS

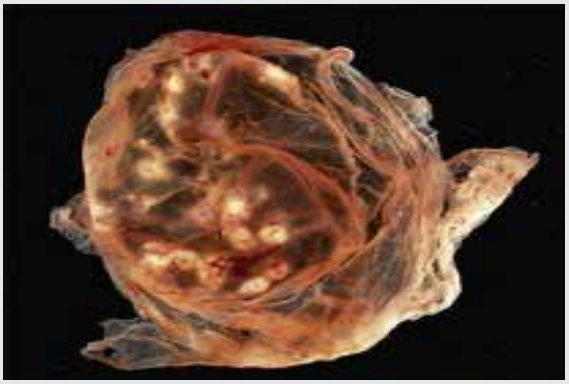
Scrapings of skin lesions, popular, vesicular or pustular fluid, crusts, blood samples, and tonsillar swabings can be used for the detection of virus. Several methods are available to confirm the diagnosis of smallpox.

Microscopy: Paschen (1906) devised a staining technique of virus particles and demonstrated the elementary bodies (**Paschen bodies**) in smears prepared from vesicular lesions of smallpox. On account of the distinctive morphology of the virion, rapid diagnosis was possible by electron microscopy.

Cultivation: 1) **Variola** and **vaccinia virus** can be cultivated by inoculating onto **chorio-allantoic membrane (CAM)** of 11 -13 day old chick embryos. Viruses produce visible lesions on CAM, known as **pocks**. Under optimal condition, each infectious virus particle can form one pock. Therefore **Pock counting** can be used for **assay** of pock-forming viruses. **Variola pocks** are small, shiny, white, convex, non-necrotic, non-hemorrhagic lesions. **Vaccinia pocks** are larger, irregular, flat, greyish, necrotic lesions, some of which are hemorrhagic.

Fig 9: Variola pocks (small, shiny, white, convex, non-necrotic, non-hemorrhagic) on CAM (Source:enwikipedia.org)

Fig 10: Vaccinia pocks (larger, irregular, flat, greyish, necrotic lesions, some of which are hemorrhagic) on CAM 9Source: veteriankey.com)



2) The viruses can be grown in cell lines of monkey kidney, HeLa and chick embryo cells. Cytopathic effects are produced by vaccinia in one to two days and more slowly by variola. Eosinophilic inclusion bodies, known as **Guarieneri bodies**, can be demonstrated in stained preparations. Vaccinia virus produces plaques in chick embryo tissue cultures but not variola virus.

Fig 11: Eosinophilic inclusion bodies (Source: cram.com)

3) Monkeys, calves, sheep and rabbits can be infected by scarification leading to vesicular lesions

Detection of virus antigen: Viral antigen can be detected by immunodiffusion in gel by Ouchterlony procedure.

Serology: Antibodies appear after the first week of infection and can be detected by heamagglutination inhibition (HI), neutralization, immunofluorescenece, RIA and ELISA.

DIFFERENTIAL DIAGNOSIS

Many eruptive diseases can be misdiagnosed as smallpox. Severe chickenpox is most frequently misdiagnosed as smallpox. Smallpox must be differentially diagnosed as it may be confused with pustular acne, meningococcemia, secondary syphilis, drug rashes and other diseases associated with a skin eruption.

IMMUNITY

An attack of smallpox confers complete life-long protection against reinfection. Ancient healers knew that smallpox could be prevented by transferring pustular material from a patient with the disease to an uninfected individual. The term **variolation** was used for this crude form of immunisation. This was done either by scratching the material into the arm or inhaling it through the nose. The practice of variolation was prevalent in ancient India since time immemorial. The practice of variolation spread from India to the West and in the 18th century became very popular in Europe, till it was replaced by vaccination introduced by Edward Jenner in 1796.

PREVENTION

Smallpox vaccine was a live attenuated vaccinia virus prepared from vesicular lesions produced on the skin of calves or sheep, or it can be grown in chick embryos. The final product contained 40% glycerol to stabilize the virus and 0.4% phenol to prevent bacterial contamination.

The vaccine was administered into the basal layers of epidermis by **multiple-puncture** with a **bifurcated needle** by scarification (scratching live virus into skin). In some mass vaccination situations, a compressed-air gun device was used. But during the global eradication program, the bifurcated needle was universally used for multiple-puncture vaccination, as recommended by WHO. Each bifurcated needle is sterile and individually wrapped. The needle is designed to hold a minute drop of vaccine. The preferred site for vaccination is the deltoid area on the upper arm. Three to fifteen perpendicular insertions within an area of 5 mm in diameter are made. Three insertions are recommended for primary vaccination and 15 for revaccination. Strokes should be vigorous enough to evoke a trace of blood at the site after 15 to 30 sec. the vaccination site should be inspected six to eight days after vaccination, to ensure that a take has occurred.

Fig 12: Bifurcated needle for smallpox vaccination: A needle is shown both empty and containing vaccine in (Source: reserachgate.net)

Fig 13: Smallpox vaccine being administered by the bifurcated needle (Source: en.wikipedia.org)

Fig 14: compressed-air gun device for smallpox vaccination

Following a successful primary vaccination, there will be no visible reaction for the first three to four days. A **papule** develops at the site in 3 – 4 days, which progresses to **vesicle** with surrounding erythema by five to six days. The formation of a vesicle is indicative of a 'take' (Success). The centre of the vesicle umbilicates and become **pustular** by seven to nine days. The pustule crusts and a dark brown or black **scab** forms by approximately day 12, which detaches in 2 – 3 weeks, leaving a **depressed scar**, which remains life-long. Smallpox vaccination may carry a small risk of complication such as developing a mild smallpox disease and rarely encephalitis.

Fig 15: Smallpox vaccination adverse reaction (Source: dxline.info)

In spite of widespread and, in many places, compulsory vaccination, smallpox was not eliminated till a concerted program of its global eradication was initiated by the WHO in 1967, with the cooperation of the member countries. The disease was then present in 44 countries, with a global incidence of around 10 million cases annually. After ten years of intense effort, the disease was wiped out.

The **last natural case of *Variola major*** detected was Rahima Banu in late 1975, a three year old girl from Bangladesh; the last person in Asia to have active smallpox. She was isolated at home with house guards posted 24 hours a day until she was no longer infectious. A house-to-house vaccination campaign within 1.5 mile radius of her home began immediately, and every house, public meeting area, school, and healer within five miles was visited by a member of the smallpox eradication program team to ensure the illness did not spread. A reward was also offered to anyone for reporting a smallpox case.

The **last case of *Variola minor*** occurred in Merca, Somalia, in October 1977. The patient was Ali Maow Maalin, a hospital cook. Maalin was isolated and made a full recovery.

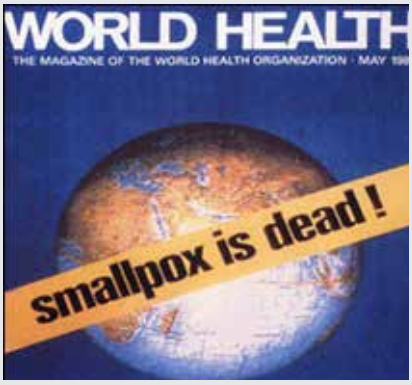
Janet Parker was the last person to die of smallpox on September 11, 1978. She was a medical photographer at **Birmingham University Medical School** in England and worked one floor above the **Medical Microbiology Department** where smallpox research was going on. An investigation performed afterwards suggested that she had been injected either via an airborne route through the Medical school building's duct system or by direct contact while visiting the Microbiology corridor one floor above.

It was promptly identified and controlled but the incident showed the hazard of keeping variola virus stocks in laboratories. Following a directive by the WHO, all such laboratory stocks of the virus have been destroyed. The last stocks of smallpox virus were held under high security in the Centres for Disease Control and Prevention, Atlanta, Georgia (USA) and the Centre for Research on Virology and Biotechnology, Koltsova (Russia). These stock cultures were also to have been destroyed by June 30, 1999, but fears of the possible use of smallpox in bioterrorism led to an indefinite extension of the deadline.

Variola virus is a category A **bioterrorism agent** along with anthrax, botulism, plague, tularemia and hemorrhagic fever. Because of eradication of smallpox (Variola virus) and discontinuation of vaccination, at present the world's human population possesses no immunity against smallpox and hence highly susceptible to this viral infection. All those born from 1978 are susceptible to infection. Due to recent concerns about bioterrorism (Particularly after terrorist attacks of September 2001 in USA); smallpox vaccination is recommended in certain groups such as military and health workers.

When two years after the last case of smallpox, no further case could be detected anywhere in spite of active surveillance, the whole world was certified free of smallpox in October, 1979. **Global eradication of smallpox was formally declared by the 33rd World Health Assembly of the WHO on 8 May, 1980**, almost two centuries after Jenner published his hope that vaccination could annihilate smallpox.

Fig 16: The poster depicting the eradication of smallpox



The factors which contributed to the elimination of smallpox were

- Availability of very effective, attenuated vaccine - freeze-dried vaccine
- Antigenically stable and only a single antigenic type existed
- The absence of asymptomatic cases, subclinical cases or persistent carriers
- The absence of an animal reservoir
- The emotional effect of this highly fatal, disfiguring diseases helping gain public cooperation in the eradication efforts
- Long term (Life-long immunity) conferred by infection
- Aggressive surveillance-containment measures
- Technique of vaccination by multiple puncture with bifurcated needle, which was simple, effective and economical

The process of eradicating smallpox was long and complicated, requiring the coordinated efforts of people around the globe. Not every disease can be eradicated; it just so happened, that smallpox has many characteristics that lend ease to eradication. The incubation period, the time between initial infection and visible symptoms, is relatively short, which prevents the disease from spreading undetected. The symptoms are also very distinctive, allowing for easy identification of smallpox patients. The WHO put in place a **ring vaccination** method whereby vaccines were not given only to infected people, but also to anyone who may have been exposed to an infected person. Ring vaccination effectively hindered the mass spread of smallpox since officials were able to isolate and treat affected areas early. In remote areas, WHO workers tracked down infected persons by showing locals pictures of people with smallpox symptoms and asking if they had seen anyone with them.

However, as a measure of protection against the remote danger of smallpox re-emerging, large stocks of smallpox vaccine are maintained by the WHO for rapid deployment, if needed. The future generations are unlikely to witness the disease but its disppearance has been too recent for it to be ignored altogether.

Anyone who has been vaccinated against smallpox (in most countries, this means anyone aged 40 or over) will have some level of protection; though not fully effective, but is likely to protect them from the worst effects of the disease.

OTHER DISEASES ON THE VERGE OF ELIMINATION

Six other diseases have been identified as possible candidates for eradication by the Carter Center International Task Force for Disease Eradication: Dracunculiasis caused by Guinea worm (*Dracunculus medinensis*), poliomyelitis, mumps, rubella, lymphatic filariasis (caused by *Wuchereria bancrofti*, *Brugia malayi, Brugia timori*), cysticercosis (caused by larval stage *Cysticercus cellulosae* of *Taenia solium*) and measles.

Guinea worm disease is likely on the verge of eradication. Only 30 cases were reported in 2017, from just two countries (Chad-15 cases, Ethiopia-15 cases)