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Zoo adaptations for primates with viral zoonotic diseases

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1. Summary

Out of millions of viruses inhabiting Earth, there are over 250 known zoonotic viruses, meaning able to be transmitted between animals and humans. Through different routes of transmission, these zoonotic viruses can cause or not clinical symptoms, which may also be fatal to humans. Zoos are among the tools which contribute to education, conservation and animal health monitoring, through animal-pathogen detection, which helps us understand that the health of humans and wild animals is intertwined. The goal of the thesis is to bring to the attention how close a relationship between humans and animals is, and which are the measures needed to prevent the transmission of diseases. Some suggestions of types of barriers that can probably be used in zoos to prevent infections will be discussed. As examples of transmission from primates to humans, is going to be described Simian T cell lymphotropic virus (STLV) and Herpes B.

2. Introduction

Zoonoses are diseases transmitted between vertebrate animals, which can be mammals, birds, reptiles, and amphibians acting as reservoirs for infectious agents like viruses, bacteria, parasites, fungi, and humans. In this thesis, the focus will be on viral agents which can cause little or no disease in their nonhuman vertebrate hosts. These viruses may infect a limited or wide range of vertebrates, thanks to the variety of transmissible routes. The transmission can occur through:

- direct contact: coming in direct contact with, blood, saliva, mucous, feces and other body fluids of an infected animal (bites, scratches),
- indirect contact: encountering surfaces or objects that have been contaminated with germs (soil, water dish),
- vector-borne: insect bites (tick, mosquito, or flea),
- foodborne: eating contaminated food (raw milk, undercooked meat),
- waterborne: drinking or encountering water that has been contaminated with feces from an infected animal (1).

The transmission can be:

- horizontal: virus is transmitted among individuals of the same generation,
- vertical: virus is transmitted from mother to offspring.
- intraspecies: the transmission of an infectious pathogen (virus) between hosts belonging to the same species,
- cross-species: the transmission of an infectious pathogen (virus) between hosts belonging to different species.

Zoos are important due to their contributions to education, conservation, and animal health monitoring.

- Educating people about animal populations, the relationship between wildlife animals and humans and how their health is related to each other.
- Investing and creating funds for conservation of wildlife.
- Testing for antibodies in a controlled environment (2).

Public health educators, institutions, and zoos have an alliance which helps to explore connections between human, animal and ecological health and to create innovative solutions. This alliance is defined as the collaboration across disciplines to improve the health of humans, animals, and the environment (3).

Emerging Infectious Diseases (EID) are recognized as a serious threat to both human health and biodiversity by estimating that 75% of EID are zoonotic and, of these, 70% originate in wildlife populations (3).

Wild animals are imported by zoos because of:

- ex-situ conservation,
- illegal pet/meat trade (emergence of antibiotic-resistant pathogens) (4),
- private owners (exotic animals),
- rescue centers (temporary housing),
- other zoos.

But even zoos are less prone to importing animals with zoonotic diseases. This is because infection diseases are the most common cause of mortality in zoo animals (2), which can probably cause economic and management issues. Also because of all the preventive measures that need to be applied to not have other animals, staff and the public infected or in contact with anything contaminated. While being housed and imported, animals may be kept in high density and in unnatural groupings of species creating the possibility to have cross species transmission (5).

3. Simian T cell lymphotropic virus (STLV)

3.1 *Retroviruses*

Retroviruses are enveloped ribonucleic acid (RNA) viruses of the *Retroviridae* family, that replicate in a unique manner by transcribing the RNA genome into linear deoxyribonucleic acid (DNA) using the viral reverse transcriptase (RT) enzyme.

Retroviruses can be:

- exogenous in nature, replicating independently of the host genome and transmitted as infectious virions, or
- endogenous as proviral DNA that integrates into the host germline and is transmitted vertically.

Retroviruses are divided into 2 subfamilies:

- *Orthoretroviridae*: six genera (*Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Deltaretrovirus, Epsilonretrovirus, Lentivirus*)
- *Spumaretrovirinae*: one genus (*Spumavirus*)

Retroviruses typically cause lifelong persistent infection and a long clinical incubation period before disease onset (6).

3.2 STLV Epizootiology and etiology

Simian T cell lymphotropic virus, STLV, is part of the subfamily *Orthoretroviridae* and part of the genus *Deltaretrovirus*.

There are 3 subtypes of STLV, STLV-1, STLV-2 and STLV-3 (6, 7, 16).

More than 33 species of Old-World primates are susceptible to STLV, in the wild and in captivity. (5,6, 9) In the latter, the seroprevalence of STLV was in the range from 0% to 95% (6). However, the seroprevalence in free-ranging populations is thought to be high (7). Prevalence increases with age, and it is higher in female than male primates (6,7).

Different subtypes were observed in different animals:

STLV-1 → in a wide range of Asian and African primates.

STLV-2 → only in captive bonobos

STLV-3 → only in African primates, red-capped and agile mangabeys, greater spot-nosed monkeys, baboons.

Co-infection can also be found, and the most common one involves STLV-1 and STLV-3, which is commonly found in agile mangabeys and baboons.

STLV was not observed in any of the New World monkeys in the wild, only in Old World monkeys and Apes, but experimental infections of squirrel monkeys and common marmosets were able to reproduce infection (6, 8).

A survey shows that wild-living bonobos are endemically infected with STLV-2, but have got occasional STLV-3, probably by cross-species transmission from monkey species on which they prey (13).

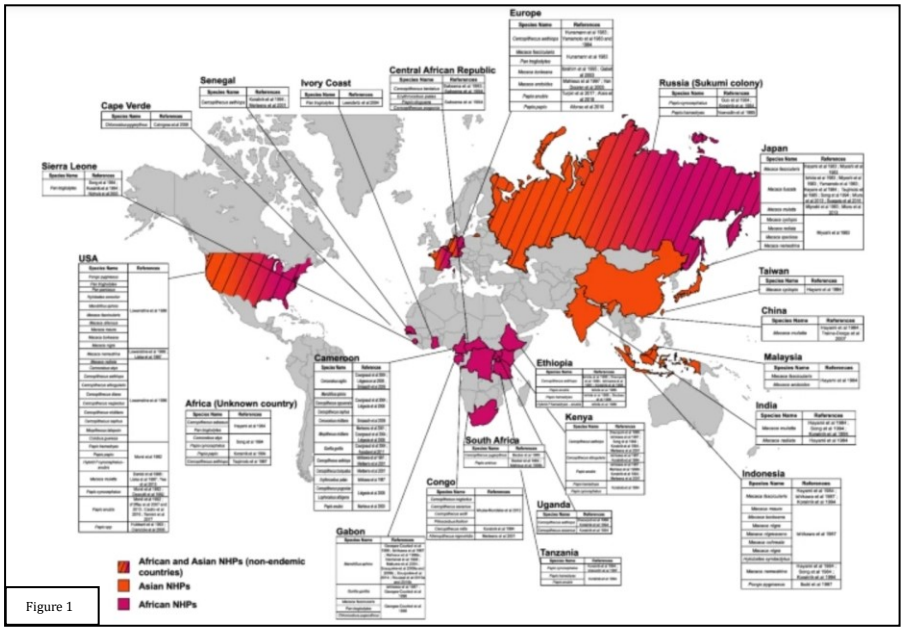


Figure 1. Orange represent Asian STLV-1 infected NHP and purple African STLV-1 infected NHPs (8)

3.3 Transmission

Horizontal transmission can happen between the same species and across species, due to mixed-species exhibitions, fighting, breeding, transfusion with infected blood cells, and blood contamination of wounds. Vertical transmission from dam to offspring through the infected cells in the milk has been reported. Natural transmission, from one animal to the other without conducting experimental research, was unknown until several studies showed that it requires transfer of lymphocytes from infected animals, in semen or cervical secretions to mucosal surfaces during breeding, or in breast milk during nursing of infants on infected dams (6, 7, 9, 10).

3.4 Clinical signs

STLV-1 usually causes an asymptomatic infection, but it has been associated with lymphoproliferative disease in baboons, gorillas, macaques, and African green monkeys. STLV-1 infection in NHP has been linked to non-Hodgkin's lymphoma and leukemia. In more than 50% of the cases was documented overt leukemia, which is occasionally associated with the presence of circulating multilobulated neoplastic lymphocytes. Involvement of the lymph nodes, spleen, liver, skin, and lung are common. In the latest, the earliest changes may be present in a perivascular or peribronchiolar distribution (6, 7, 9).

STLV-1 in baboons is clinically characterized by:

- depression,
- anorexia,
- lymph node enlargement,
- hepatosplenomegaly
- lethargy
- low body weights
- anemia
- pneumonia

Clinical signs after infection with STLV-2 and STLV-3 have not been documented (7).

3.5 Pathology

The virus infects both CD4+ and CD8+ cells, significantly more CD4+ cells. The virus replicates within the host via a clonal expansion of infected cells.
STLV-1 infected African greens also consisted of CD8+ cells suggesting that STLV-1 may preferentially transform CD8+ cells in this species (7,15).

3.6 Diagnosis

Samples, altered cytokine profiles secondary to STLV-1 infection, are initially screened by enzyme immunoassay (EIA). Positive results are further tested by confirmatory Western blot at a reference laboratory. Additionally, PCR should be used to test seronegative or sero-indeterminant animals. There may be long intervals to seroconversion indicating the need for continual monitoring (7, 15). There are ELISA reagents available to detect antibodies to STLV (12).
Fecal samples can be also used to detect STLV infection in apes.

3.7 Prevention and treatment

The incubation period could last for several years and there are no treatments available (15). Prevention of transmission of the virus is achieved thanks to the initial screening of the zoo collection and risk/benefit analysis of introducing positive animals to naïve animals.
For this, serial serologic screening of the animals for antibodies after 1 year helps identify the newly seroconverted animals.
If an individual is confirmed positive, it should be considered infected for life, with no retesting necessary. If an individual is seronegative, but housed with seropositive animals, annual retesting is necessary. Positive individuals should only be introduced to positively known groups. Because of the cross transmission, Asian and African monkeys should not be in direct contact (6, 16).

3.8 Human infection

It was known that some non-human primate species carry HTLV- related viruses, so the attention for further studies turned to monkeys and apes (14).
Nowadays, it is well known that Old World Non-Human Primates and Apes are naturally infected with a variety of STLV-1 viruses and that HTLV-1 appeared cross-species transmission approximately 27,300 years ago in Africa (11, 16).
In humans some of the clinical signs are inflammatory disorders and neurologic disease.

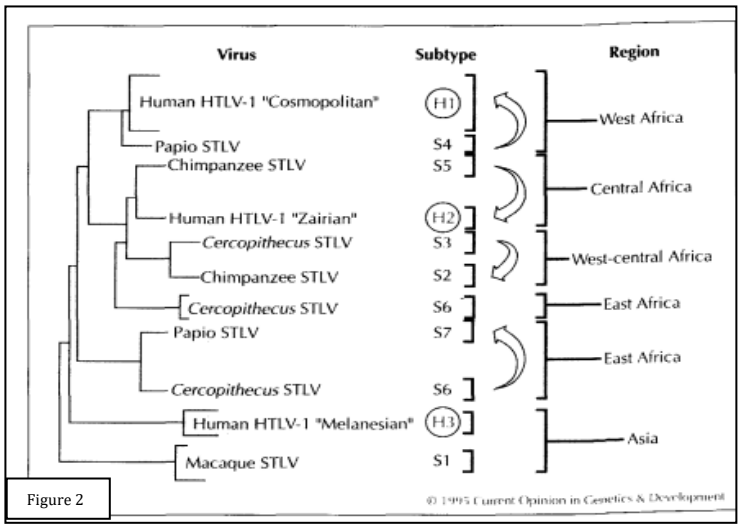


Figure 2. Possible episodes of horizontal transmission between humans and non-human primate species (8)

4. Herpes B

4.1 *Herpesviridae*

The Herpesviridae virus has a four-layered structure: a core containing the large, double-stranded DNA genome is enclosed by an icosapentahedral capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the tegument. It is encased in a glycoprotein-bearing lipid bilayer envelope. It is encoding for several enzymes involved in protein processing, DNA synthesis, and nucleic acid metabolism. DNA synthesis and capsid formation in the nucleus.

Since herpesviruses are relatively fragile, they require a lipid envelope to achieve attachment to, and penetration of, the host cell, requesting destruction of the host cell to complete the viral replicative process.

Viral persistence in a latent form within the host, which is peculiar to the success of herpesviruses as a pathogen, allows the virus to persist in causing lifelong infection in the presence of a fully developed immune response (17, 18).

The family Herpesviridae is divided into three distinct subfamilies:

- ***Alphaherpesvirinae***
  - herpes simplex virus types 1 and 2, and varicella-zoster virus,
  - have a short replicative cycle,
  - induce cytopathology in monolayer cell cultures,
  - have a broad host range.
- ***Betaherpesvirinae***
  - cytomegalovirus, and human herpesviruses 6 and 7,
  - with a long replicative cycle
  - restricted host range
- ***Gammaherpesvirinae***
  - Epstein-Barr virus and human herpesvirus 8,
  - with a very restricted host range (17,18).

TABLE 166-1	Biologic Properties of Herpesviridae
Common properties	Spherical enveloped virions, 150–200 nm in diameter Large, linear, dsDNA genome of 125–290 kbp contained within a T = 16 icosahedral capsid, which is surrounded by a proteinaceous matrix named the tegument and then by a lipid envelope containing membrane-associated proteins Synthesis of DNA and assembly of capsid within the nucleus, acquire envelope by budding through host cell membranes Specify a large array of enzymes involved in nucleic acid metabolism and synthesis Production of progeny virus results in destruction of the host cell Establish latency in their natural host
Alphaherpesvirinae	Variable host range Short reproductive cycle Rapid spread in cell culture Efficient destruction of infected cells Establish latency primarily but not exclusively in sensory ganglia
Betaherpesvirinae	Restricted host range (a nonexclusive property of this subfamily) Long reproductive cycle Infection progresses slowly in culture, frequently forming enlarged (cytomegalic) cells Latency in secretory glands, lymphoreticular cells, kidneys and other tissues
Gammaherpesvirinae	Experimental host range limited to family or order of natural host *In vitro* replication in lymphoblastoid cells *In vivo* replication and latency in either T or B cells

Table 1

Table 1. Biologic properties of Herpesviridae (18)

There are several herpesviruses that have yet to be assigned to a subfamily. The classification of herpesviruses and some names have been recently updated by the International Committee of the Taxonomy of Viruses (ICTV).

Example: Names for the nonhuman primate herpesvirus species are based on the host genus with the name ending in *–ine* (i.e. *Macacine herpesvirus 1* replaces *Cercopithicine herpesvirus 1*) (17).

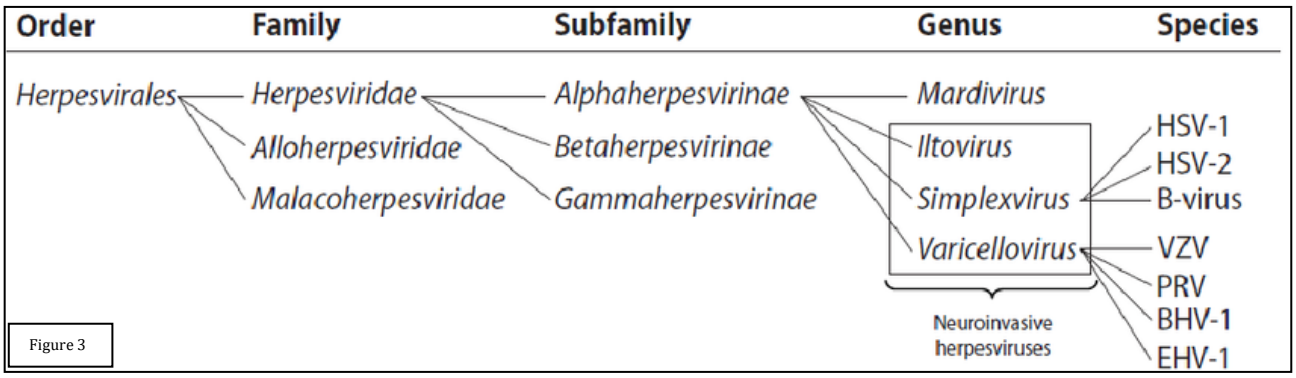


Figure 3

Figure 3. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2); monkey B virus (B-virus); varicella-zoster virus (VZV); pseudorabies virus (PRV); bovine herpesvirus type 1 (BHV-1); equine herpesvirus type 1 (EHV-1) (19).

4.2 Herpes B Epizootiology and Etiology (*Macacine Herpesvirus-1*)

Herpes B virus, or Macacine Hepesvirus-1, is a member of the subfamily *Alphaherpesvirinae* and genus *Simplexvirus*, and it was first isolated in 1932 (17, 24).
This is mainly endemic in Old World macaques, since macaques are the natural host of the herpes B virus (24).
Herpes B virus commonly causes a latent and usually asymptomatic infection in Asian and north African macaques including:

- rhesus macaques,
- bonnet macaques (*M. radiata*),
- Japanese macaques,
- stump-tailed macaques,
- Formosan rock macaques (*M. cyclopis*),
- pig-tailed macaques,
- lion-tailed (*M. silenus*),
- and cynomolgus macaques (17).

The incidence of infection in immature rhesus macaques is low, but it increases rapidly with sexual maturity, 80–90% in some colonies by 3–4 years of age (17).
Macaques under 1 year of age can be infected through close contact with infected mothers. The chance of infection increases drastically for macaques in the prepubescent and pubertal period (2–4 years of age), since they are socially and reproductively active. Wild adult macaques over 2.5 years of age are nearly 100% positive for herpes B virus antibodies (24).

However, there is also a high rate of seropositivity (70%–100% by adulthood) in conventionally reared, captive macaques.
The virus causes lifelong persistent infection, and it is latent in the trigeminal and lumbosacral ganglia (20).

Infection of non-macaque species, including the Patas monkey, black and white colobus (*Colobus abyssinicus*), DeBrazza's monkey *(Cercopithecus neglectus),* capuchin monkey (*Cebus apella*), and common marmoset, can cause the death of the infected animals (17).

A persistent and asymptomatic B virus infection in a colony of capuchin monkeys housed near but not in direct contact with rhesus macaques has been reported and indicates that safe practices should be employed when working with all NHP species (17).
Overall, all Macaca spp. should be considered the main hosts for herpes B virus.
No other monkey species are known to act as carriers for the herpes B virus (21).

4.3 Transmission

The Herpes B virus is transmitted through:

- sexual behavior
- biting
- fomites (inanimate objects such as cage furniture, utensils)
- open skin lesions
- oral mucosa
- ocular mucosa
- genital mucosa
- overcrowding
- unsanitary conditions (17, 24).

There is no evidence for vertical transmission of B virus.
There has only been one documented case of human-to-human transmission, although the potential for secondary transmission is probably low (23).

Macacine herpesvirus 1 (herpes B virus) establishes a latent infection in which the virus resides within sensory ganglia near the site of primary infection, most commonly the neurons of the trigeminal ganglion and the lumbosacral plexus. Primary infection in captive macaques usually occurs in juvenile animals as they lose their maternal antibodies, or in new arrivals, while clinical signs of disease may or may not be seen. But a latent infection persists for the life of the animal. Re-activation of latent infection, with viral shedding in oral and genital secretions (saliva, blood, feces, urine), with or without clinical signs, can occur and the factors leading to this are not completely understood, but stress related to changing social dynamics, transportation or relocation, fever, ultraviolet light, tissue or nerve damage, and immunosuppression may play a role (17, 21).

Virus isolation and molecular detection of Herpes B virus DNA indicate that the frequency of shedding in a population is low (1–5%). There is evidence to suggest that shedding frequency may go up during breeding season (23).

Seroprevalence rates in conventionally housed macaque colonies indicate that upwards of 100% of animals in a colony may be carriers (22).

4.4 Clinical signs

Disease in macaques is usually mild and self-limiting.

- Vesicular lesions on oral and genital mucosae, which progress to ulceration and resolve within 10–14 days, when the vesicles typically rupture, scab and heal
- Conjunctivitis or nasal discharge
- Fatal infection occurs rarely in macaques (17, 24)

Herpes B virus particles have been recovered from saliva, blood, faeces, urine, serum, eye, brain, spinal cord, genital tracts and kidney tissue cultures from infected macaques (21, 24).
Viral dissemination of the lung, liver, spleen, bone marrow, and adrenal cortex has been documented, but it is rare and usually fatal (17).
Characteristic vesicle formation occurs on the mucous membranes and mucocutaneous border of the nose and oral cavity, on the skin of the nose and the eyelids, and conjunctiva secondary to reactivation of latent infection in the trigeminal ganglion. When genital involvement occurs due to reactivation of a lumbosacral plexus infection, lesions are present on the skin of the prepuce and the vulva (24).

4.5 Pathology

The virus replicates initially in epithelial cells, producing a characteristic vesicle on an erythematous base. It then ascends the sensory nerves to the dorsal root ganglia, where, after an initial period of replication, it establishes latency. During reactivated infection, the virus spreads distally from the ganglion to initiate new cutaneous and/or mucosal lesions (18).
Primary infection results in an initial round of replication at the site of inoculation. Histologically, this is characterized by the ballooning degeneration of keratinocytes with progression to

vesiculation. Multinucleated, syncytial cells and eosinophilic to basophilic, intranuclear viral inclusions may be prominent.
Inflammatory cells may be found within vesicles, epidermis, and subjacent dermis. Endothelial cell necrosis with intranuclear viral inclusions may be seen.
In disseminated disease, there is widespread, hemorrhagic necrosis within the liver, lung, brain, adrenal gland, and lymphoid organs.
The Herpes B virus is responsible for multifocal necrotizing hepatitis in macaques.
Following initial viral replication, the virus (virion or capsid) is transported by retrograding axonal flow to the sensory ganglion where a latent infection is established for the life of the animal (17).

4.6 Diagnosis

Available diagnostic tests can be used to detect infections in individuals or in colonies and the virus isolation and culture should be carried out in Biosafety Level 3 (BSL-3) facilities.
Examples of the tests used for detection:

- The antibodies may be detected by enzyme-linked immunosorbent assay (ELISA) and western blot methods.
- PCR detection has low sensitivity and efficacy due to the low frequency of viral shedding.
- Immunohistochemistry using a commercially available rabbit polyclonal antibody against the closely related human herpesvirus-1 can be used to demonstrate viral antigen in equivocal lesions. However, this antibody detects viral glycoproteins shared by all simplex viruses and therefore is not able to fully confirm herpes B viral infection.
- serological assays and viral isolation have limitations on fully confirming Herpes B virus

That is why SPF (specific pathogen free) status can be ascertained only by knowing the virologic history of the animal under consideration and the history of all its contacts. For these reasons, treating all macaque species as if they are potentially infected with herpes B virus and taking appropriate precautions is advisable (17, 18, 21, 24).

For virus isolation, swabs of clinical specimens or other body fluids can be inoculated into susceptible cell lines and observed for the development of characteristic cytopathic effects. This technique is useful for the diagnosis of *Alphaherpesviridae* infections because of their relatively short replicative cycles.
Newer and more rapid diagnostic techniques involve the detection of viral gene products. This can be done by applying fluorescence antibody directed against immediate-early or late gene products

to tissue cultures after 24 to 72 hours of incubation. A positive result is the appearance of intranuclear fluorescence. Alternatively, fluorescence antibodies may be applied directly to cell monolayers or scraps of clinical lesions, with intranuclear fluorescence again indicating a positive result (18).

4.7 Prevention

Animals are initially screened by ELISA and western blot. Negative animals should be kept in single cage housing or in small groups and be periodically tested by a modified ELISA for at least one year. Animals that are repeatedly negative can then be moved to larger groups.
In breeding groups, animals should be periodically tested for seroconversion. Repeated testing during the quarantine period is required because animals may be chronically infected and immunologically unreactive or in the early stages of disease prior to seroconversion. Serologic testing on an annual or semi-annual basis should be continued as a component of colony management as seropositive macaques have been detected as late as 7 years post establishment of a SPF.
Breaks in the SPF barrier status may occur from introduction of new animals, contact with contaminated fomites, or reactivation of latent infection in seronegative animals. Ideally, SPF colonies should be self-sustaining and not require the introduction of new animals (17).

Animals actively infected and shedding viruses should not be treated as this entails considerable risk to the attending personnel (17).

4.8 Human infection

There are less than 50 documented human cases described. Most human cases have involved:

- injury by a macaque (bites, scratches),
- urine splashed in eyes,
- needle stick injury,
- laboratory exposure (handling infected macaque tissues, especially brain and kidney cell cultures),
- aerosolization of excretions/secretions,
- and human to human contact.

In some cases, patients report no known exposure event (21).

The symptoms in humans are gradually developing but at a fast rate:

- Vesicles on the skin develop at the site of inoculation 1–5 days post exposure in many cases, followed by swelling of local lymph nodes.
- Itchy skin and pain at the inoculation site may be intense.
- Fever, skin tingling, muscle weakness and conjunctivitis may develop.
- In some cases, flu-like symptoms and/or fever of at least 48hr duration is the only sign of infection.
- Common sites of viral infection include buccal, gingival, conjunctival, anal, and genital mucosa. Infection can be accompanied by erythema, vesicles, and ulcerations.
- Spinal inflammation typically develops after 3-7 days, resulting in neurological signs such as gait, confusion, difficulty swallowing, and paralysis.
- Death occurs 10–14 days post exposure in 70–80% of herpes B virus infected humans, principally due to severe encephalitis.
- As treatment: Early recognition of clinical signs is critical as the administration of nucleoside analogs (acyclovir, valacyclovir, ganciclovir, or famciclovir) may be beneficial during the initial stages of infection (17).
- In recent years the fatality rate has declined, likely due to the early initiation of antiviral therapy, better supportive care, and/or earlier diagnosis of infections (17, 21).

Zoonotic infection of humans with B virus is almost invariably fatal (>70%) in the absence of antiviral chemotherapies, and severe non-fatal infections can result in encephalomyelitis or severe neurological impairment (23).

- Antiviral therapy

Oral valacyclovir (1 g) administered three times daily is recommended as the preferred drug for the post-exposure prophylaxis of herpes B virus in adults and nonpregnant women, because valacyclovir results in much higher serum levels than does acyclovir.
If there are any signs or symptoms of B virus disease, or laboratory confirmed positive results, intravenous antiviral treatment must be carried out instead of the oral administration.
Because herpes B virus is more susceptible to ganciclovir than it is to acyclovir, intravenous ganciclovir (5 mg/kg) is recommended for patients with definitive signs and symptoms of peripheral nervous system or CNS involvement. In addition, a patient with brainstem encephalitis recovered completely after ganciclovir treatment (24).

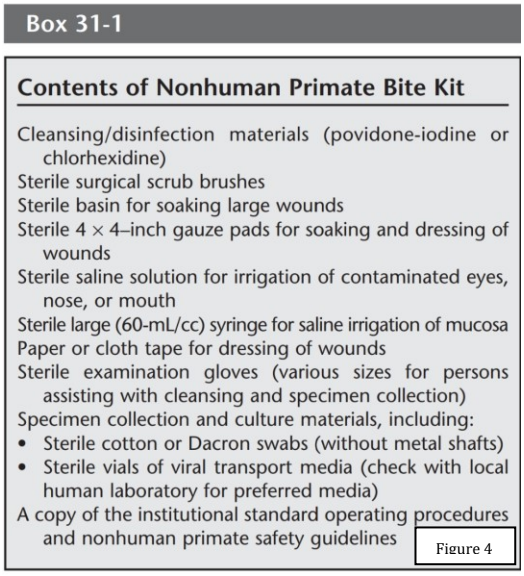
The reasons for this low rate of infection may include the infrequent shedding of herpes B virus by macaques, the presence of antibodies against human herpes simplex virus which provide some level of cross protection against herpes B virus, and undetected asymptomatic infection. The virus is also very fragile once shed into the environment; human infection requires direct exposure from macaques shedding the virus (21).

5. Preventive measures for staff

People working with infected animals have a higher risk of exposure, especially the staff that perform the invasive procedures and experience longer exposure. A survey was done by CDC (Centers for Disease Control and Prevention) to evaluate the needlestick or mucocutaneous exposures reported by animal workers, which could be from naturally or experimentally infected NHPs, and it was reported by 35% of workers.

However, apart from the potential adverse health effects on people working with animals, there is the risk of secondary transmission, from infected workers to other people.
Offering the staff the necessary training in how to prevent the transmission of the virus, as well as providing them with the proper guidelines (OHSPs- occupational health and safety plans) and equipment, is extremely important.

As animal reactions are unpredictable, since they can bite or scratch the animal carers, first-aid kits should be easily accessible from anyone.
In case of exposure, all the names, mailing addresses, emergency contact numbers of health professionals should be clearly posted near the animal working area (6, 29).


Box 31-1

Contents of Nonhuman Primate Bite Kit

Cleansing/disinfection materials (povidone-iodine or chlorhexidine)
Sterile surgical scrub brushes
Sterile basin for soaking large wounds
Sterile 4 × 4–inch gauze pads for soaking and dressing of wounds
Sterile saline solution for irrigation of contaminated eyes, nose, or mouth
Sterile large (60-mL/cc) syringe for saline irrigation of mucosa
Paper or cloth tape for dressing of wounds
Sterile examination gloves (various sizes for persons assisting with cleansing and specimen collection)
Specimen collection and culture materials, including:
- Sterile cotton or Dacron swabs (without metal shafts)
- Sterile vials of viral transport media (check with local human laboratory for preferred media)

A copy of the institutional standard operating procedures and nonhuman primate safety guidelines

Figure 4

Figure 4. is a list of the supplies needed in a first-aid kit (6).

Animal carers, being daily in contact with the animals infected by a zoonotic disease, should be in possession of personal protective equipment (PPE). It includes:

- Clothes – coveralls, lab coat- which should be laundered separately from the clothes used for uninfected animals,
- Gloves – disposable or reusable, must be impermeable to liquids,
- Face mask – for respiratory protection,
- Boots or shoe cover – easy to dispose or disinfect,
- Googles/ face shield – for eye and mucous membranes protection in case of water splash or exposure (25, 26).

All the disposable equipment used for the infected animals should be disposed of into a closed, lidded container before leaving the animals' area. All the clothes, cloths, gloves used for those animals should be washed separately from other clothes. All the materials used for cleaning the enclosures holding the infected animals should be kept in a different area from the uninfected materials. The use of the soap/detergent for the materials and the equipment after use is necessary (6, 29).

Frequent handwashing and performed before leaving the primate area, before eating, drinking or smoking. All the latter is forbidden in the NPH area.

The staff occupying with primates that pose a zoonotic risk, should be vaccinated from the preventive medicine department. An ill human should not prepare food for NPH or handle them (38).

A bite or skin exposure to body fluids should be scrubbed immediately with soap or detergent for 15 minutes and rinsed after. If there has been exposure to the eyes, then rinsing with sterile saline for 10 minutes and applying disinfectant with 0.5% tincture of iodine for another 10 minutes is necessary (6).

6. Zoo adaptations for infected primates

6.1 Staff training

Zoos should provide training for staff members who are working with animals infected with zoonotic diseases. Giving them the instructions and guidelines on how to prevent the transmission, on how to proceed in case of wounds caused by infected animals, on how to proceed in case of escape of an infected animal, on how to transmit the information they have to people who are going to be near the animal areas (new colleagues, visitors, technicians) (29).

The goal of the safety training is to reduce occupational exposures, accidents and injuries. That is why it should be:

- Constant - should be covering every task and implementing new findings or techniques
- delivered routinely – not only after an incident happened, because this way the workers will concept it as punishment and not a necessary safety measure
- efficient – should cover topics and tasks that are related to the work that they do, since there might be a difference between what people are expected to do and what they actually do, this affecting the efficacy of the training

To help reduce the number of exposures, the training program should:

- provide information to improve knowledge
- demonstrate safe work techniques
- provide instruction on emergency response
- yearly updates on safety policies and procedures
- motivate staff to work safely

Every institution holding NHPs, especially macaques, should have an institution-specific written post-exposure protocol and all the employees should be familiar with it.
Every staff member involved in primate experiments should be provided with an occupational health care system to document and advise on potential exposures, treatment, and follow-up for the exposed team, by the government.
As an example, the staff involved in experiments using monkeys should be informed of the biohazards associated with rhesus monkeys and should be required to report bites, scratches, or mucocutaneous contact to supervisors and occupational health workers (30).

6.2 Public

Zoos often are seen as a form of entertainment, an activity that people attend in their leisure time. In 2011 a survey on zoos and aquariums was conducted and it was indicated that around 700 million people visit those annually, and thanks to this participation, around 350 million dollars was spent on wildlife conservation internationally. A report of 2012 from AZA indicated that 2700 conservation programs spent around 160 million dollars on field conservation for 650 individual species. It is the high attendance of visitors that gives the income to help and support the zoos into conversation programs (3).

Visitors are more likely to have positive perceptions about zoos and animals and to participate in on-site conservation after their visit and engagement in activities that zoo offers (3, 32).

We can measure the visitors' response to a zoo exhibition, like interest or satisfaction, by measuring:

- time spent in front of an exhibit
- attention toward an exhibit
- Crowd size (3)

Modern zoo aims to:

- complement the design of enclosures
- support the visitor in making sense of what they see
- draw connections between the animals and conversation issues (33)

A naturalistic enclosure design is better for visitors and animal perspective.

Visitors: it offers the opportunity to see the animal in close range and engaging in natural behavior (33)

Animals: it stimulates species natural behavior and cognitive engagement through enrichment and design of the enclosure (3)

Enrichment is important to promote the wellbeing of the animal and to reduce stereotypical behavior. Keepers of NPH are challenged daily with having to find and create enrichment activities which challenge them and engage them in problem solving activities since they are a very intelligent species. (33)

Human-animal interaction can have benefits for both

- greater commitment to conservation

- improved health and increased wellbeing in humans
- improved immune and reproductive status in animals (33)

Examples of visitor engagement:

- connecting with prior knowledge
- presentations/media with facts or campaign messages
    - signs
    - photo boards
    - videos
- enrichment items
    - watching them solve puzzles, using monitors, hunting, foraging
    - touching and creating enrichment
- feeding stations
    - watching them eat from up close
    - chop food, create bowls
- interact with staff/volunteers
    - asking questions
    - explaining PPE
    - explaining where wild animals come from
    - explaining the disease, virus, symptoms
- animal training
    - with interpretation from staff, by explaining step by step the procedures and the goals
    - Without interpretation from staff, by watching the animal achieve the final goal
    - Touching training tools (3, 33)

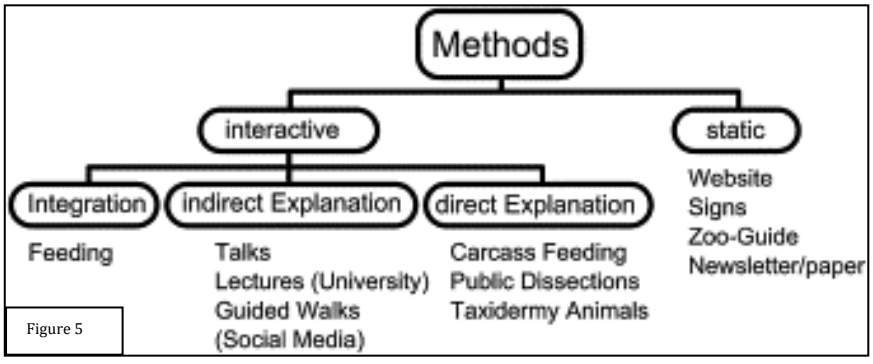


Figure 5. Methods used to communicate with visitors (35).

6.3 The design of enclosures

It should provide maximum safety for the staff, while allowing easy management of the animals. This is to prevent physical danger.

Attention should be given to the:

- Doors
- Hatches
- Personnel access areas inside the enclosure
- Fences
- Visibility of the animals (use of cameras, mirrors)
- Warning systems (radio telephones, emergency buttons)
- Easy cleanable platforms (for disinfection)

Enclosures should be away from the kitchen where the food or medication is prepared, so they will not be contaminated (28).

Holding facilities for macaques should be designed to:

- Provide protection from scratches, bites and exposure to secretions/excretions for workers while cleaning, restraining or modifying the enclosure.
- Ensure the public does not have contact with or exposure to macaques' excretions and secretions, equipment used to care for them.
- Staff and public cannot be grabbed, scratched, spat on, urinated on, or defaecated on.
- Use of plexiglass or similar can minimize exposure to excretions/secretions while maintaining visualization of animals. Always having easy visualization and movement of animals.
- Access only for authorized and properly trained staff to avoid exposure risks. Security systems (alarms, video surveillance etc.) in case of animal escaping and unlawful entry into facilities.
- Tunnels and raceways with remotely operated slides for individual separation. Squeeze cages to allow easy physical restraint of animals for examination or injections.
- Easy, unobstructed access and cleaning and have adequate drainage with waste passing directly into a sewer system or treatment plant.
- Good ventilation facilitates drying, minimizing the persistence of aerosol.
- Use materials and surfaces that are easily cleaned and disinfected, durable, slip resistant and have no sharp edges.

- Only experienced personnel wearing appropriate PPE should attempt physical capture of macaques (36).

6.4 Barriers

Barriers in zoos are important to keep the public and animals safe, aiming to avoid physical and psychological issues (27). Considering that we are talking about zoonotic diseases, there shouldn't be any kind of direct or indirect contact with animals or their body fluids. This means avoiding:

- Animals (no petting)
- Bites
- Scratches
- Blood (from wounds or menstrual cycle)
- Saliva (spitting)
- Feces (throwing them at the public)
- Contaminated objects (throwing them at the public)
- Contaminated food/water

The enclosures in zoos should be built based on the animals' requirements and skills like dimensions and ability to jump.
For example, the enclosures for the arboreal species, which are able to jump and climb (Rhesus Macaque), the number of the monkeys housed in one enclosure (20-30), feeding habits (on the ground or in the trees), their abilities (good swimmers) have as suggested front barrier 4.5m wide and 4.5m deep V-shaped dry moat and rear barrier 5m high wall (27).

Some other suggested barriers for primate species:

- Creating a monkey island, surrounded by different types of moats → it depends on the possibilities of zoos providing the space and all the other requirements like proper drainage, maintenance, expenses.
- U-shaped moats → less chances of transmission of infectious diseases.
- V-shaped moats → reduced contact with animals.
- Fences → hot-wired fences to prevent escaping.
- Glass → help keeping public safe from spitting or throwing contaminated objects (27).

We have barriers that are used alone or in combination to offer higher security.

- Unclimbable barrier - glass
    - has a smooth surface (glass, smooth concrete vertical walls)
    - make sure animals are unable to gain limb holds
- Climbable barrier - mesh
    - Mesh or netting fences in combination with an internal overhang and/or electric wires.
- Electric wires
    - Not used as primary barrier
    - Used as an additional psychological deterrent
    - Used to discourage animals from climbing, minimize damage to barriers or vegetation
- Wet moat/dry moat
    - Gently sloping from animal area (36)

Common name *Latin name*	Unclimbable Barrier: minimum height (m)	Climbable Barrier: minimum height (m)	Climbable Barrier with electrified wire: minimum height (m)	Minimum Wet Moat Specifications	Foundation	Building Materials	Rationale	Containment Area Recommendations	Contingency Plan
Cercopithecidae									
Macaque *Macaca* spp. Mandrill *Mandrillus sphinx*	3	Not suitable	3.5 3m mesh fence topped with 1m internal overhang at 45° with at least 5 electrified wires about 100mm apart (top of overhang is clad in 500mm mesh from fence top), **and** 500mm horizontal in-rigger on barrier, about 1.9m from ground, with at least 4 electrified wires about 100mm apart	3.5m wide, 1.2m deep moat with: 2m unclimbable barrier **or** 1.5m unclimbable barrier with an electric wire at top of barrier	300mm and 1m anti-dig material	-	AZ used climbable with electric wires and wet moat for over 14 years	2	2

Table 2

Table 2. According to appendix 2 on zoo enclosure guidelines, the barrier for macaques. AZ-Auckland Zoo (36)

For outdoor enclosures

- Daily check for signs of digging from native wildlife, domestic species or enclosure residents.
- Fence material is well secured to supporting posts in a way that the weight of primates could not detach it.
- Gates and doors are strong and effective in containing primates.

For open enclosures

- Minimum fence height of 4,6 m
- With upper 30% of the barrier made of smooth, non-climbable material.
- Maximum mesh dimension of 50.8mm x 50.8mm is recommended for Old World species

Fences

- Energizers are connected to battery or generator in case of no power
- Safety signs visible for staff and bystanders indicating the presence of electric wire

Solid barriers

- Concrete block, poured concrete and artificial rock

Moats

- Dry moats plus non-climbable barriers
- Animals can get back into the enclosure in case of falling into the moat
- Surrounded by fences, walls, hedges to keep the others from approaching
- Are accessible with tractors in case of erosion or need of repair
- Caregivers have safe and easy access
- Wet moats are not recommended for macaques because they can swim, and not recommended for baboons because they cannot swim so they can drown
- Water quality is measured on a weekly basis

Glass

- Glass is laminated (glass-clad polycarbonate) with a minimum thickness of 2.53cm
- Glass is set into a steel or aluminum frame for security purposes (37)

Type of barrier	Advantages	Disadvantages
Walls (concrete, cement, wood)	- Easy to build - Natural shelter (wind, sun) - Safe	- Not esthetical, if nothing is added unusable surfaces for the animals
Water	- Natural barrier - Good landscape integration	- Risks of drowning - In winter, risks of ice formation (escape)
Wire mesh	- Increase the useful surface for capuchins (to climb) - Safe	- Not esthetical - Possible transmission of disease
Electric fences	- Discreet - Good complement for other types of barrier - Cheap	- Risks of escape (if short-circuit with vegetation) - Risks of electrocution
Glass walls and glass windows	- Esthetic - Safe	- Shorter distance between animals and visitors, if nothing is added unusable surfaces for the animals

Table 3

Table 3. Advantages and disadvantages of barriers (38)

7. Conclusion

Many animals, and including non-human primates, that are being rescued from illegal trades, private owners, circuses or abandoned near the cities, are getting treatment in rescue centers all over the world. However, many of them are not able to be reintroduced in the wild as they lack the defense mechanism and the instinct of survival, or they want and search for human approach, being a risk for the people they encounter, physically or by transmitting some zoonotic diseases.

Zoos help with the education of the public and conservation of wild animals, this way offering a new permanent home to the animals. However, housing large groups of animals that carry zoonotic diseases represents an issue for zoos, both economically and physically, by making the necessary adaptations.

There are some adaptations that can be considered to improve these animals' lives and to raise awareness about the zoonotic diseases and their management.

- Avoiding direct contact with non-human primate and considering them all as carriers of some zoonotic disease, is the best way to be safe.
- Using all personal protective equipment and continuous staff training about the new developments in the zoonotic field.
- Using the right barriers to keep the staff and public safe, by avoiding being in direct contact with the animals or their secretions.

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