13.8 Guidelines – Managing risk of Herpes B virus zoonosis in Australian and New Zealand zoos holding macaques

Contents

1. Executive summary

Herpes B virus infection is an important zoonosis carried by macaques. Infection in humans is associated with a high fatality rate.

Zoos in Australia and New Zealand successfully manage various zoonotic disease risks. The ZAA Primate Veterinary Advisory Group (VAG) and Veterinary Special Advisory Group (Vet SAG) have prepared this document to provide information on herpes B virus, for use by organisations that elect to house macaques.

Herpes B virus risk management policies and protocols should be part of a zoo's broader biosecurity risk management plan, based on the (Australian) National Zoo Biosecurity Manual.

Institutions holding (or considering holding) macaques should ensure:

- All macaques are assumed to be herpes B positive, and managed accordingly, regardless of test results.
- They maintain current and up-to-date information about herpes B virus, as part of a wider staff zoonotic risk management policy.
- They develop and maintain appropriate, documented risk management and emergency response policies and protocols for herpes B virus.
- A local medical health practitioner, preferably at a hospital, has been approached to develop post-exposure treatment protocols.
- All zoo staff or contractors coming into contact with macaques or their biological products are fully briefed on risk management; regular training should be undertaken.
- Members of the public do not have any contact with macaques or their biological products.
- Veterinarians suppling services to the zoo are fully conversant with herpes B risks and associated risk management.

2. Purpose

This document provides current information about herpes B virus, and gives guidelines for preventing human exposure, and appropriate response to human exposure events. The information within this document references several leading authorities. Please refer to the document history section to determine the currency of the information in this document.

These guidelines are to be read in conjunction with the National Zoo Biosecurity Manual (www.zooaquarium.org.au/wp-content/uploads/2011/10/National-Zoo-Biosecurity-Manual-March-2011.pdf).

3. Definitions

Association means the Zoo and Aquarium Association Inc. (ABN 71 836 556 156).

Board means the board of management of the Association.

Executive Director means the Executive Director of the Association.

Member means a member of the Association, as defined in the Association's Membership Policy, and may include an employee, officer or agent of a Member of the Association.

Organisation means an unincorporated entity, or an entity incorporated under Commonwealth, State or Territory legislation.

Zoonosis means a disease that is transmissible between animals and humans (plural zoonoses).

4. Herpes B virus

Herpes B virus (also known as *Herpesvirus simiae,* Macacine herpesvirus 1, Cercopithecine herpesvirus 1) is a member of the alpha-herpesvirus group in the *Herpesviridae* family. It is closely related to the human viruses: herpes simplex viruses 1 (HSV1) and 2 (HSV2). It was first isolated from the brain and spinal cord of a researcher who died of a rapidly progressive meningoencephalitis after a bite from a macaque.

HOST RANGE

Herpes B virus occurs as a common, life-long and generally asymptomatic infection of macaques (*Macaca* spp.). *Macaca* genus monkeys are native to North Africa and Asia. Many thousands of macaques are maintained around the world in zoos, laboratories, circuses and private homes, making the distribution of the virus virtually worldwide. **All *Macaca* spp. should be assumed to be hosts for herpes B virus.**

No other monkey species are known to act as carriers for the herpes B virus. However, infection with associated disease and mortality has been reported in patas monkey (*Erythrocebus patas*), black and white colobus (*Colobus abyssinicus*), capuchin monkey (*Cebus apella*), common marmoset (*Callithrix jacchus*), DeBrazzas guenons (*Cercopithecus neglectus*) and humans. Infection in primate species (including humans) other than macaques is usually fatal. Signs typically include classic herpes vesicles on the nares, oral cavity and conjunctiva, respiratory disease and high mortality rate.

TRANSMISSION IN MACAQUES

Herpes B virus is transmitted through sexual and biting behaviour, and by fomites (inanimate objects such as cage furniture, utensils etc.). Sexual transmission between macaques is thought to be the primary mechanism of transmission in established captive colonies.

Primary infection in captive macaques usually occurs in juvenile animals as they lose their maternal antibodies, or in new arrivals. Clinical signs of disease may or may not be seen. A latent infection persists for the life of the animal. Re-activation of latent infection, with viral shedding ± clinical signs, can occur; the factors leading to this are poorly understood, but may include stress, fever, injury and immunosuppression However, infected macaques exposed to such factors do not necessarily shed virus. When re-activation does occur, virus can potentially be shed in saliva, blood, faeces, and urine.

Many colonies of macaques have endemic herpes B virus infection with no apparent impact on the general health of the animals. Prevalence of virus shedding at any given time is typically very low (2-3%), even in colonies where all animals carry the virus.

DISEASE IN MACAQUES

The disease in macaques is usually mild and self-limiting. Clinical disease is characterised by the appearance of small vesicles on the lips or in the oral cavity. The vesicles typically rupture, scab and heal within 7-14 days. Conjunctivitis or nasal discharge may also be seen. Fatal infection occurs rarely in macaques. Herpes B virus particles have been recovered from saliva, blood, faeces, urine, serum, eye, brain, genital tracts and kidney tissue cultures from infected macaques.

5. Herpes B virus in Australia

Macaques are likely to have first been imported into Australia in the early 1900's. Cynomolgus macaques were first held in Taronga Zoo's collection in 1914. Many other macaque species have been imported into Australia, including rhesus (*M. mulatta*), bonnet (*M. radiata*), Japanese (*M. fuscata*), stump-tailed (*M. arctoides*), pig-tailed (*M. nemestrina*), Sulawesi crested (*M. nigra nigra*), Tonkean (*M. tonkeana*), Formosan rock (*M. cyclopis*), and lion-tailed (*M. silenus*) macaques. These animals are held in zoos, fauna parks, research facilities, circuses and in private hands.

As herpes B virus is endemic in macaques it is likely that it has been present in Australia since macaques were first imported. Due to the lack of suitable diagnostic tests in Australia, minimal testing of macaques has been carried out. Herpes B virus was confirmed in the 1930s in macaques imported into Adelaide Zoo and other institutions at that time. There is also an anecdotal report of positive cases among 40 macaques at a Sydney research facility in the early 1980s. In 1999, 9 out of 11 cynomolgus macaques at Taronga Zoo, and 1 out of 11 crested macaques from Perth Zoo, tested positive for herpes B virus antibodies. The Taronga Zoo macaques were all euthanased, and only one individual was positive for herpes B on post mortem testing of tissues. This illustrates one of the challenges of interpreting herpes B virus blood tests – a positive antibody result does not necessarily correlate with active infection. Many other macaques from around Australia have since been tested, frequently with positive result.

6. Detecting herpes B virus infection in macaques

There are currently no Australian laboratories offering herpes B virus testing. To date, all testing of samples from Australian macaques has been done in the United States of America. The B Virus Research and Resource Laboratory at Georgia State University offers diagnostic and screening tests for herpes B virus (www.gsu.edu/~wwwvir/). Shipping biological specimens across international borders can be a complex process, especially for CITES-listed species.

Testing macaques for herpes B virus infection is not straight forward. Available diagnostic tests include antibody detection, antigen detection, viral culture, and PCR. These tests can be used to detect infection in individuals or in colonies.

The potential for false negative results is the most problematic aspect of interpreting herpes B virus tests. For example, latently-infected individuals will not necessarily maintain an antibody response, and hence can test negative with antibody detection tests. False negative results are most problematic when testing one-off samples. **Negative tests do not**

indicate an individual, or colony, is not infected with herpes B virus, and should not be relied upon for risk management purposes.

Testing new animals prior to their introduction to a known antibody-negative group may be warranted to reduce the risk of infection in potentially naïve animals. However, routine screening of macaque colonies is not typically recommended, primarily due to the difficulties in interpreting negative results.

Risk management protocols should be based on the assumption that any macaque may be herpes B virus positive, regardless of test results.

7. Herpes B virus infection in humans

While the risk of human exposure to herpes B virus may be low, the consequence of herpes B virus infection in humans is very high, with an estimated **70-80% case fatality rate** without treatment. Most human cases have involved injury by a macaque (bites, scratches); other reported methods of transmission include urine splashed in eyes, needle stick injury, laboratory exposure (handling infected macaque tissues, especially brain and kidney cell cultures), aerosolisation of excretions/secretions, and human to human contact. **Many cases have occurred as a result of exposures that were considered trivial at the time.** In some cases, patients report no known exposure event.

Immediate post exposure treatment can significantly reduce the risk of humans developing infection and associated disease.

At least 50 human fatalities have been described, all involving those working with laboratory macaques or exposure in the field. At the time of writing there have been no known cases of human infection in zoos or wildlife parks worldwide.

The incidence in humans is low despite the common use of macaques for display, circuses, pets and research. The reasons for such an apparently low rate of infection may include the infrequent shedding of herpes B virus by macaques, antibodies to human herpes simplex virus providing some 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 inoculation by a scratch or bite, or exposure of mucosa (eye, mouth etc.) or broken skin to secretions (saliva etc.) or excretions (urine and faeces) from macaques shedding the virus.

DISEASE IN HUMANS

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.

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. Latent infections can develop with subsequent reactivation. In recent years the case fatality rate has declined, likely due to the early initiation of antiviral therapy, better supportive care, and/or earlier diagnosis of infections.

8. Guidelines for minimising the risk of human exposure to herpes B virus

Herpes B virus risk management protocols should be underpinned by sound understanding of the general principles of biosecurity, and be developed in the context of a broader biosecurity risk management plan. Each institution holding macaques should have an institution-specific written post-exposure protocol. **In developing policy and protocols for managing macaques and herpes B virus, institutions should refer to the National Zoo Biosecurity Manual**.

Although the risk of exposure to herpes B virus is low, the consequences of human exposure can be devastating. The safety of both the public and zoo employees is the responsibility of the institution holding the animals. Macaques should be managed on the assumption that they are infected with herpes B virus, regardless of any test results.

We recommend that macaques are not utilised for direct contact experiences for the general public, and that incidental contact of the general public with macaque body fluids (e.g. urine, spitting, throwing faeces) is prevented.

We recommend that zoo employees minimise direct and indirect contact with macaques, and do not enter enclosures containing macaques.

The following guidelines are recommended for institutions holding or planning on holding macaques in Australia:

INSTITUTIONAL POLICY AND WORKPLACE CULTURE

All zoos holding macaques should have a policy for managing the risks associated with herpes B virus in macaques, supported by up to date, written protocols for risk management and emergency response, which are reviewed on a regular, predetermined basis.

Institutions should promote a workplace culture that allows the risk associated with herpes B virus to be taken seriously, just as for managing large carnivores. However, while the risks associated with large carnivores are generally well understood in the community, the risk associated with herpes B virus (as with many other zoonotic diseases) is typically poorly understood.

INSTITUTIONAL PROTOCOLS TO MINIMISE EXPOSURE RISK

All personnel, including contractors, students and volunteers, that may be exposed to macaques, to their excretions/secretions, or may enter the macaque exhibit, should be fully briefed on the risk management guidelines for the prevention of herpes B virus and should read and understand the written protocols.

Specific work procedures applicable to the institution should be written using the guidelines outlined in this document. All personnel should receive appropriate training on the risk associated with herpes B virus, appropriate methods of restraint, and the use of protective clothing. Regular staff refreshers should be conducted and a training and awareness log should be maintained to document that personnel have completed such requirements. Personnel may be required to sign off that they acknowledge comprehension of institutional guidelines. Access to macaque areas should be restricted to people that have completed

this process. Periodic reviews of safety procedures with regard to herpes B virus should occur.

Institutions should identify and collaborate with a local medical practitioner for establishing policy and protocols, and coordinating the response to an exposure event (see Section 6). In consultation with their designated medical practitioner, institutions may consider collection and storage of pre-employment and subsequent periodic serum samples from all staff who will be working with macaques to serve as a baseline for retrospective studies in the event of a suspected exposure. These samples should be held in an ultralow freezer (-80°C).

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Personnel coming into contact with macaques or their biological products should wear appropriate clothing that minimises the risk of contamination of skin and mucous membranes (e.g. eyes, mouth) with macaque secretions or excretions, and prevents scratches. This should include (at a minimum) long-sleeved and long-legged garments, and sturdy slip-resistance footwear.

Additional PPE may be considered based on a risk assessment of individual facilities and work practices. When physically restraining animals, particularly if not chemically restrained (see 5.8.), arm-length reinforced leather gauntlets should be worn. When handling chemically restrained animals, latex gloves and face protection (e.g. face shield, surgical mask, respirator, goggles) should supplement standard PPE.

Personal protective clothing and equipment should be supplied and laundered by the employer.

PERSONAL HYGIENE

Strict personal hygiene is essential for herpes B virus risk management. Hand washing is the most effective means of preventing transmission of zoonotic diseases generally. Disposable gloves should be used in higher risk situations, but they do not negate the importance of hand washing after working around macaques, or after servicing areas potentially contaminated by macaques.

High standards of hygiene in terms of food preparation, clothing and footwear management should be followed in accordance with the National Zoo Biosecurity Manual.

CLEANING AND DISINFECTION

The use of high pressure hosing in macaque areas should be avoided to minimise the production of aerosols from faeces, saliva and other secretions/excretions. When cleaning exhibits, as much solid material as possible (faeces, food, bedding) should be picked up, using such as a broom and shovel, to minimise the need for hosing.

Herpes viruses have limited abilities to withstand environmental conditions outside a host animal. Under optimal conditions, the virus can remain viable for up to 7 days at 37°C, and for several weeks at 4°C. Viability on dry surfaces has not been evaluated, but is likely similar to that of other mammalian herpesviruses: typically 3–6 hours.

Herpes B virus is readily destroyed by common detergents and disinfectants; these should be used when cleaning enclosures and associated equipment. Common disinfectants (e.g. F10, Virkon, Trigene, Chlorhexidine, bleach) at concentrations and contact times effective

against other enveloped viruses, can reasonably be assumed to effective, though controlled trials have apparently not been conducted.

ENCLOSURE DESIGN

Display and holding facilities for macaques should be designed in a manner that:

a) Provides protection from scratches, bites and exposure to secretions/excretions for workers during their daily routine and during procedures that require animal restraint. We recommend that zoo employees do not enter enclosures containing macaques.

b) Ensures the public do not have contact with or exposure to macaques, their excretions and secretions, equipment used to care for macaques. We recommend that macaques are not utilised for direct contact experiences for the general public.

It is strongly recommended that macaque facilities:

- Provide security with respect to preventing animal escapes and unlawful entry into facilities. Access should be limited to authorized personnel that are properly trained in procedures to avoid exposure risks. Security systems (alarm, video surveillance etc.) may be considered.
- Be designed and arranged so that staff and public cannot be grabbed, scratched, spat on, urinated on, or defaecated on. The strategic use of plexiglass or similar can minimise exposure to excretions/secretions while maintaining visualisation of animals.
- Be designed so that there is no need for employees to have direct contact with the animals. Facilities should incorporate means to physically restrain animals without direct handling by workers. Tunnels and raceways with remotely-operated slides should allow for individual macaques to be separated. One or more squeeze cages should be incorporated into this system to allow easy physical restraint of animals for examination or injections. Raceways and squeeze cages should be at or below waist height so that animals are not above personnel when being moved or restrained, thereby minimising risk of humans receiving facial exposure to biological contaminants.
- Are designed to adequately provide for both the physical and psychological needs of the animal. Stress is believed to be a major factor in the recrudescence of infection and subsequent shedding of virus. It is essential to provide an environment in which the animals feel comfortable.
- Are designed to allow easy, unobstructed access and cleaning and have adequate drainage with waste passing directly into a sewer system or treatment plant. Good ventilation facilitates drying, minimising the persistence of aerosol. Use materials and surfaces that are easily cleaned and disinfected, durable, slip resistant and have no sharp edges or protuberances that may cause scratches or wounds to workers.
- Are constructed with multiple compartments that allow for easy visualisation and movement of animals at all times, provide multiple options for isolation and separation of animals, and give animals an opportunity to escape from each other.
- Should incorporate ample natural or artificial lighting to improve visibility of animals and structures.

- Include a suitable work area for food preparation, cleaning utensils, storage of utensils and equipment, and for veterinary procedures. Adequate staff amenities (toilet, shower, change room, lockers) should be provided.

MINIMISING STRESS

Minimising stress in a colony of macaques is critical. Stressors of any kind have the potential to effect re-activation of herpes B virus infection and subsequent shedding. Measures to minimise stress include provision of an appropriate environment, behavioural enrichment, optimal nutrition, preventative medicine, breeding and genetic management (maintain the size and social structure of the colony to ensure harmony within the colony, and genetic management to reduce inbreeding).

ANIMAL RESTRAINT

Direct handling of macaques should be minimised. Capturing, restraining or otherwise handling fully conscious macaques is not recommended. Macaques should be physically restrained in a squeeze-cage and hand injected with a chemical restraint agent prior to handling. If a squeeze cage is not available, if an animal will not enter the squeeze system, or if an animal escapes the enclosure, chemical restraint via dart, or physical restraint in an appropriate net, may be necessary. Only experienced personnel wearing appropriate PPE should attempt physical capture of macaques.

Behavioural conditioning of macaques is a practical and very useful technique to complement physical and chemical restraint options.

VETERINARY PROCEDURES

All veterinary procedures on macaques should be carried out by suitably experienced and competent veterinarians with a full understanding of herpes B risk and risk management. Veterinary procedures should only be performed on animals that are sufficiently chemically restrained. Protective clothing (see 5.3.) should be worn when handling macaques. Special care must be taken to prevent needle stick and other sharps injuries. Double gloving is recommended, especially for surgery. Masks and eye protection should be used for endotracheal intubation and other oral procedures to protect against exposure to aerosolised secretions. Veterinary staff handling surgical packs, drapes, swabs, and other equipment used for macaque surgery should wear disposable gloves.

Macaques that require hospitalisation should be housed in a facility that complies with the guidelines for enclosure design (see 5.6.).

All macaque deaths should be thoroughly investigated. Necropsies should be carried out by an experienced veterinarian or pathologist. Refer also to the National Zoo Biosecurity Manual. Personal protective equipment (including gloves, masks, eye protection, protective clothing and footwear) should always be worn. The necropsy should preferably be carried out in a biohazard safety cabinet. Zoos which are unable to meet sufficient biosecurity standards for necropsy of macaques should consider submitting the body to an appropriate diagnostic laboratory. Laboratory personnel handling secretions, excretions, bodily fluids, and tissues from macaques should wear protective clothing and work in a biohazard safety cabinet.

All biological waste (including tissues, secretions and excretions) should be disposed of using an approved contaminated waste system.

9. Post-exposure protocols – first aid

Appropriate and timely post-exposure response can significantly reduce the risk of humans developing herpes B infection. All macaques should be considered to be a risk of transmitting herpes B to humans. Post-exposure protocols should be established to ensure that any potential exposure is dealt with in an appropriate manner, including first aid, reporting and contact with a medical practitioner. Each institution holding macaques should have an institution-specific written post-exposure protocol. All employees that may be exposed to herpes B virus should be familiar with the protocol.

A local medical practitioner, preferably at a hospital, should be identified and included in the development of post-exposure first aid and medical management protocols. The zoo should work with the medical practitioner to ensure they, and other doctors who may be called upon to attend an exposed patient, are aware of herpes B virus and have their own procedures in place to deal with herpes B virus exposures. Injured or exposed persons should receive prompt evaluation by the identified medical practitioner.

FIRST AID

The adequacy and timeliness of wound decontamination procedures are the most important factors in preventing the risk of infection after herpes B virus exposure. The first few minutes following the exposure event is the most critical period for preventing herpes B virus infection.

A dedicated herpes B virus first aid kit should be maintained.

Immediately after the exposure event:

SKIN WOUNDS

i. **Cleanse** the exposed area by thoroughly washing and scrubbing the area or wound with povidone-iodine surgical scrub, chlorhexidine surgical scrub, soap, or concentrated detergent, for 15 minutes

Then:

ii. **Irrigate** the washed area with running water for 15-20 minutes.

MUCOUS MEMBRANES (eyes, mouth etc.)

i. **Irrigate** the area for 15-20 minutes with saline or running water.

(soap, detergent and disinfectants are too harsh for application to the eye or other mucous membranes)

As soon as possible following first aid procedures the patient should receive medical attention, ideally by the zoo's identified medical practitioner who has been involved in developing institution specific protocols.

Information sheets containing basic information about herpes B virus, post-exposure treatment protocols, and contacts for further information should be readily accessible and accompany the patient to the medical facility. The identified medical practitioner(s) may be unavailable at the time and the patient could be seen by a doctor unfamiliar with herpes B virus.

For more information and resources refer to:

- Centers for Disease Control and Prevention (CDC), Govt USA. Herpes B virus website: http://www.cdc.gov/herpesbvirus/index.html
- B Virus Research and Resource Laboratory, Georgia State University: www.gsu.edu/~wwwvir/
- Cohen *et al.* 2002. Recommendations for prevention of and therapy for exposure to B virus (*Cercopithecine herpesvirus* 1). Clinical Infectious Diseases. 235: 1191–1203.

SPECIMEN COLLECTION FOR VIRUS DETECTION

Collection of B virus culture specimens from the wound before cleansing **is not recommended**. This serves to delay wound cleansing and can force viral particles deeper into the tissue.

Swabs from the involved macaque's buccal mucosa, conjunctiva or urogenital areas (see 6.5.) are more likely to reflect the actual risk of wound contamination than a swab of the wound. If a wound is associated with caging, swabs may be obtained from the macaque most recently housed in that cage.

INCIDENT REPORTING

Without delaying first aid, the details of the events associated with exposure should be documented, including the time of exposure, the nature of the exposure, the location of any injuries, the identification of the macaque(s) involved, the first aid measures administered and who administered them. This information assists the examining physician to determine the relative risk of B virus exposure, and allows for modification of existing protocols to prevent similar exposure events in the future.

VETERINARY EVALUATION OF THE MACAQUE(S) FOLLOWING AN EXPOSURE EVENT

The clinical and virologic status of a macaque at the time of a human exposure event may provide information useful to the medical practitioner. The decision to assess a macaque for herpes B virus-associated lesions and/or collect samples for virus detection should be made by the zoo veterinarian(s) in consultation with the medical practitioner. A risk assessment should be carried out as soon as possible post-exposure based on the realistic and predictive values of testing the macaque(s) and further operator risk. Swabs collected for virus detection will be most useful if collected as close to the time of exposure as possible; swabs collected at a later time may not accurately reflect the macaque's status at the time of the exposure. The macaque should be anaesthetised for any such procedure, to facilitate safe and thorough examination and sample collection.

Herpes B virus testing is not currently available in Australia. The decision to collect samples for testing needs to consider the practicalities of shipping such biological specimens across international borders, especially for CITES-listed species.

Samples from the macaque that may be considered for viral screening include:

i. Serum samples collected at the time of human exposure, and 14-21 days later

ii. Swabs from buccal cavity (inside the cheek), right eye, left eye and genitalia. Swabs should be placed immediately into viral transport medium. Use a separate swab for each site.

If the specific macaque associated with the injury is unknown, the value of testing all animals in the group is generally considered to be low.

10. Further information

B Virus Research and Resource Laboratory, Georgia State University: www.gsu.edu/~wwwvir/

Centers for Disease Control and Prevention (CDC), Govt USA. Herpes B virus website: http://www.cdc.gov/herpesbvirus/index.html

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12. Document history and approval

Original document created	2000
Author	Larry Vogelnest
Document reviewed and expanded	May 2014
Author	David McLelland and Primate Veterinary Advisory Group
Reviewed Veterinary SAG	May 2014
Reviewed Dr Bernie Hudson (Infectious Diseases Physician Royal North Shore Hospital / Uni Sydney)	May 2014
Reviewed ZAA Board	**August 2014**
For review	as required only (or within 5 years of the date of approval or last review).

These guidelines were endorsed by the Board of the Association on 13/08/2014.