Attachment A

Review of quarantine risks associated with the importation of elephant semen from Singapore, the United States of America and Member States of the European Union

The following is a draft report of a review of the quarantine risks associated with the importation of elephant semen from Singapore, the United States of America (USA) and Member States of the European Union (EU). The review is based on relevant scientific literature and the risk assessment underpinning the quarantine policy for the importation of elephants from Thailand that was finalised in 2004.

Introduction

Biosecurity Australia is responsible for developing and reviewing quarantine policy for the import of animals and plants and their products. It does this through science-based risk analysis processes.

Australia has a long-standing conservative approach to quarantine risk. The level of risk Australia is prepared to accept is known as Australia's appropriate level of protection (ALOP) and is expressed as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Those risks that are very low or less meet Australia's ALOP and no risk management measures are required. For those quarantine risks that exceed Australia's ALOP, ie those risks that are greater than very low, risk management measures are proposed to reduce the level of risk in order to achieve the ALOP.

Foot and mouth disease (FMD)

Foot and mouth disease (FMD) is a highly contagious viral vesicular disease of cloven-hoofed animals. Singapore and the USA have long been free from FMD with the last recorded outbreaks being in 1935 and 1921 respectively (WAHID OIE). Most EU Member States are free; at the time of writing (December 2007), the United Kingdom (UK) was in the final stages of eradicating an outbreak and Cyprus was close to eradicating the first outbreak in that country since 1964.

Asian elephants (*Elephas maximus*) are susceptible to FMD although infection is rare. It has long been Australia's policy that FMD susceptible species and semen from them are only allowed direct importation from countries or zones free from FMD.

It is proposed that certification be required, certifying the zoo in which the donor elephant resides is located in a country recognised by the World Organisation for Animal Health (OIE) as free from FMD.

Tuberculosis

Tuberculosis (TB) for the purposes of this review means infection with any member of the *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum, M. microti*). TB has not been reported in wild elephants. The majority of cases in captive elephants are due to *M. tuberculosis.*

Between August 1996 and June 2000, samples from 539 elephants were submitted to the National Veterinary Services Laboratory in the USA for mycobacterial culture. Seventeen elephants from 8 herds were diagnosed with TB, 12 by isolation of *M. tuberculosis* post-mortem and five by post-mortem. The prevalence of TB in elephants in North America was estimated to be 3.3% (Mikota et al 2000).

Between 1994 and June 2005, there were 34 confirmed cases of TB in the USA, 31 in Asian and three in African elephants (*Loxodonta africana*). *M. tuberculosis* was the aetiologic agent in 33 cases and *M. bovis* in one (Summary: Elephant Tuberculosis Research Workshop 2005). Hammat 2007 reported that a minimum of 36 culture-confirmed TB cases had occurred in the USA since 1994, ie TB had been diagnosed in about 12% of Asian elephants in the USA in that time.

Tuberculosis has also been found in captive elephants in a European zoo (Lewerin et al 2005).

The major pathology in elephants infected with *M. tuberculosis* occurs primarily in the lungs and thoracic lymph nodes. Extra-thoracic lesions are seen in more advanced cases (Mikota et al 2000). Transmission of *M. bovis* by artificial insemination in cattle is recognised. Venereal transmission of *M. tuberculosis* has also been recorded in humans.

Until recently, the best available test for TB in elephants has been mycobacterial culture of trunk wash samples. This method has poor sensitivity (Lyashchenko et al, 2006), particularly in the early stages of infection and actual prevalence is undoubtedly higher than that indicated by positive culture results.

ChemBio Diagnostic Systems, Inc., have developed a multi-antigen print immunoassay (MAPIA) (Lyashchenko et al 2000) and, more recently, a test based on lateral-flow technology for the detection of specific antibodies, ie the Elephant TB STAT-PAK Assay [also known as the Rapid Test (RT)].

Both tests show promise of high sensitivity and specificity, and have detected TB in elephants years before positive culture of trunk wash samples (Summary Elephant Tuberculosis Research Workshop 2005). This supports the view that the prevalence of TB infection in zoos is higher than estimates based on culture results.

Lyashchenko et al 2006 conclude that the RT has potential as an efficient screening tool, and the MAPIA is useful as a confirmatory test and to monitor effectiveness of treatment. Dumonceaux et al 2006 report that preliminary data have demonstrated 100% sensitivity and 97% specificity for the RT using culture as the reference standard. The RT has been licensed in the USA and is available internationally.

The introduction and transmission of TB through the import of elephant semen cannot be ruled out. It is proposed that all elephants in the zoo of origin must have been tested for TB with negative results, using the elephant TB STAT-PAK Assay, in the 12 months prior to semen collection.

Surra

Surra is trypanosomosis caused by the protozoan parasite *Trypanosoma evansi*. Surra has a wide host spectrum. The disease is most severe in horses, donkeys, mules, camels, dogs and cats but may occur in a range of other species; commonly in cattle and buffalo and occasionally as a chronic, mild or subclinical disease in sheep, goats, pigs and elephants. Singapore and the USA are free from surra. No EU Member State has reported surra to the OIE in the last decade. The disease occurs in the Middle East, north Africa, south and south east Asia and Mongolia, and in Argentina and Brazil.

Surra is normally transmitted by biting flies which suggests negligible risk of semen transmission. Dourine, a venereally transmitted disease of horses caused by a trypanosome designated *T. equiperdum*, has had a wider distribution. A single case was reported in Germany in 2002 (OIE Handistatus II). Claes et al 2005 have reviewed the published data on dourine and hypothesize that some *T. equiperdum* strains are actually *T. brucei brucei* or members of a subspecies of *T. brucei* and that all other *T. equiperdum* strains are *T. evansi.* This poses a theoretical possibility that *T. evansi* may be transmitted venereally. The hypothesis is yet to gain wide acceptance.

Regardless of the future classification of *T. equiperdum*, the risk of the entry, establishment and spread of surra through the import of elephant semen from Singapore, the US or EU is assessed as very low or negligible and risk management is not required.

Endotheliotropic elephant herpesvirus

Endotheliotropic elephant herpesvirus (EEHV), unknown before 1995, has emerged as a major cause of deaths in mainly young Asian elephants in captivity in the US and Europe. More than 40 cases have been documented to date. Four suspected cases have occurred in Asia since 1995 and one case has been confirmed in a wild-caught three-year old elephant found dead in a Cambodian elephant sanctuary Asia (Reid et al. 2006).

The disease has a sudden onset and runs a rapid course leading to death generally within five days. Viraemia leads to death of endothelial cells and capillary leakage and haemorrhage. Myocardial haemorrhage results in shock and death. A few animals have recovered following treatment with the anti-viral drug famciclovir.

There has been intense study into herpesvirus infections of elephants in the last decade. Genetic analysis of blood and necropsy tissues from many EEHV cases has been, and is being, carried out in at least two laboratories in the US.

Six EEHVs have been identified in elephants—EEHV1A, EEHV1B, EEHV2, EEHV3A, EEHV3B and EEHV4. These are classified as Sub-family *Betaherpesvirinae*, Genus *Proboscivirus*. EEHV4 has not, to date, been associated with disease. There is significant genetic variation within species and subspecies of these viruses (Hayward G. 2007).

An additional four elephantid herpesviruses, isolated from vaginal and conjunctival swabs in Asian elephants, have been described recently (Wellehan et al 2007). These have been classified as gammaherpesviruses. They have not been associated with systemic disease.

Dr Hayward (Hayward 2008) reports that wherever there have been multiple cases of EEHV associated with one cow, one bull or one facility in North America, there has always been multiple different virus species found. Even EEHV1A viruses are often genetically distinct from one another and therefore not epidemiologically related. This indicates multiple sources of the viruses among carrier adults that may infect naïve juveniles rather than transmission from parents. There is no evidence that semen or breeding by artificial insemination is a source of infection.

Dr Laura Richman (Richman 2007) also advises that there is no evidence of venereal transmission and reports that EEHV has not been found in semen samples routinely tested using PCR assay before insemination.

The risk of the entry, establishment and spread of EEHV through the import of elephant semen is assessed as very low. However, other herpesviruses including bovine herpesvirus 1 (BHV1) and herpes simplex type 2, are found in semen and have been transmitted by artificial insemination (van Oirschot 1993, Moore et al. 1989). It is proposed to require certification that there has been no case of EEHV in the zoo of origin in the two years prior to semen collection to ensure the risk is sufficiently low to meet Australia's ALOP.

Haemorrhagic septicaemia

Haemorrhagic septicaemia (HS) is an acute bacterial disease, mainly of cattle and buffaloes caused by two strains of *Pasteurella multocida*, known as B:2 and E:2 using agar gel immunodiffusion, or 6:B and 6:E by agglutination. Infection is of the upper respiratory tract.

The B:2 serotype causes the disease in Asia, the Middle East and north Africa and the E:2 serotype in Africa. The disease has been reported in elephant in Sri Lanka (deAlwis and Thambithurai 1965) and India. HS is diagnosed in elephants in Thailand, and elephants are vaccinated in that country. Transmission is by direct contact or through contaminated feedstuffs or water. There is no evidence of venereal transmission.

Singapore and most EU Member States report freedom from the disease. It has been sporadically diagnosed in the USA. There were outbreaks in introduced bison in the Yellowstone National Park in 1911, 1919 and 1922. The disease was also diagnosed

in bison in Montana in 1965, in dairy calves in Pennsylvania in 1969 and beef calves in California in 1993 (Rimler and Wilson 1994).

The risk of the entry, establishment and spread of haemorrhagic septicaemia through the import of elephant semen is assessed as negligible and risk management is not required.

Elephant pox

'Elephant pox' is caused by cowpox virus. It was first described in elephants in Europe in 1963 and many outbreaks have been recorded since, mainly in zoos in Central Europe. Wild rodents are the primary carriers of cowpox virus. There is a high seroprevalence in red foxes. Humans, zoo animals and cats are the final host species (Wisser et al. 2001).

There is no evidence of venereal transmission. The risk of the entry, establishment and spread of elephant pox through the import of elephant semen is assessed as very low to negligible and risk management is not required.

Parasites

The risk of introduction of exotic nematodes, trematodes, cestodes or external parasites through the import of elephant semen is negligible.

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