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Department of Agriculture, Fisheries and Forestry

Importation of zoo hippopotamuses and their semen from approved countries – draft report

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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

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Summary

This policy review takes into consideration the biosecurity risks for Australia associated with the importation of live zoo hippopotamuses (hippos) and their semen from approved countries. It considers relevant peer-reviewed scientific information, advice from international scientific experts, and relevant operational practicalities. Australia does not currently import zoo hippos. Under previous conditions, the last import of zoo hippos occurred in the 1980s.

This policy review proposes that the importation of live zoo hippos and their semen to Australia from approved countries be permitted, subject to a range of biosecurity measures.

The Department of Agriculture, Fisheries and Forestry has prepared this draft with the assistance of technical and scientific experts. This policy review identifies hazards that require biosecurity measures to manage risks to a very low level in order to achieve Australia's appropriate level of protection (ALOP). The hazards that were identified as associated with the importation of zoo hippos or their semen, and the determination of whether they were retained for detailed review or not, are listed in Table 3 of this report. A list of diseases retained for assessment is provided as section 2.1 of this report.

General biosecurity measures that are not disease-specific are commonly applied when international trade in zoo hippos and their semen occurs. These measures are described in chapter 4 of this report. As part of the risk review process, the level of risk management achieved by those measures during the import of live zoo hippos or their semen was considered in the risk assessment process when determining final risk estimates. In addition, where the WOAH Terrestrial Code stipulated measures applicable to zoo hippos or their semen, application of these measures were considered to be part of the baseline requirements when arriving at a final risk estimate.

Detailed risk reviews for each of the hazards retained for review are found in chapter 3 of this report and include, as appropriate, disease-specific risk mitigation measures proposed for each of live zoo hippos and/or zoo hippo semen.

The hazards requiring disease-specific measures in relation to the importation into Australia of live zoo hippos and/or their semen are as follows:

- anthrax (*Bacillus anthracis*)
- brucellosis (*Brucella abortus* and *Brucella melitensis*)
- external parasites
- internal parasites
- *Mycobacterium tuberculosis* complex (*M. bovis, M. caprae, M. tuberculosis*)
- New World screwworm (*Cochliomyia hominivorax*) and Old World screwworm (*Chrysomya bezziana*)
- rabies virus
- Rift Valley fever

- surra (*Trypanosoma evansi*)
- *Trypanosoma vivax.*

This policy review proposes a combination of risk management measures and operational systems that will reduce the risk associated with the importation of live zoo hippos and their semen from approved countries into Australia to achieve Australia's ALOP. These biosecurity measures are provided in their entirety in chapter 4 of this report.

The department recognises that there may in future be new scientific information and technologies, or other combinations of measures that may provide an equivalent level of biosecurity protection for the diseases identified as requiring risk management. Submissions requesting consideration of equivalence for alternative measures will be considered on a case-by-case basis using available evidence.

This draft contains details of the identified hazards and the proposed biosecurity measures to allow interested parties to provide comments and submissions to the Department of Agriculture, Fisheries and Forestry within the consultation period.

Introduction

Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, agricultural industries that are relatively free from serious pests and diseases, and human health.

Risk analysis is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods (including live animals and semen) into Australia. If the biosecurity risks do not achieve Australia's appropriate level of protection (ALOP), risk management measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified.

Successive Australian Governments have maintained a conservative, but not a zero risk, approach to managing biosecurity risks. This approach is reflected in Australia's appropriate level of protection (ALOP), which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

The Department of Agriculture, Fisheries and Forestry conducts Australia's import risk analyses. We use appropriate technical and scientific experts and consult with stakeholders during the drafting process.

Risk analyses conducted by the department are consistent with Australia's international biosecurity obligations including those under the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the World Organisation for Animal Health (WOAH). Risk analyses aim to establish a balance between our international obligations and the various risks that goods may pose.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis (such as scientific review of existing policy and import conditions, or scientific advice).

More information about Australia's biosecurity framework is provided in the Biosecurity import risk analysis guidelines 2016.

The department recognises that new scientific information and technologies, or other combinations of measures, may provide an equivalent level of biosecurity protection for the disease agents identified as requiring risk management. The department will consider technical submissions that objectively demonstrate alternative biosecurity measures.

This policy review

Background

The Hippopotamidae family are even-toed ungulates in the Artiodactyla order. There are 2 extant species: *Hippopotamus amphibius* (common hippopotamus) and *Choeropsis liberiensis* (syn. *Hexaprotodon liberiensis*) (pygmy hippopotamus). The natural distribution of common hippos is

limited to sub-Saharan Africa with an invasive population established in Colombia, while the pygmy hippo is found in Guinea, Sierra Leone, Liberia, and Côte d'Ivoire. Both species are listed on the International Union for Conservation of Nature Red List of Threatened Species, with the common hippo currently considered vulnerable and the pygmy hippo endangered (Lewison & Pluháček 2017; Ransom, Robinson & Collen 2015). Both species are exhibited in zoos and are hereafter referred to as zoo hippos.

Internationally, zoo hippo populations are considered self-sustaining, with wild-caught hippos rarely present in approved countries. Direct acquisitions from the wild are subject to the requirements of the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Zoo hippos are typically housed singly or in small groups, often in mixed-species exhibits. Housing in small groups enables close monitoring of individual zoo hippos by zoo staff. Generally, zoological institutions approved by the competent authority of a country have preventative health programs with well-maintained, written health and husbandry records for each individual animal. Many zoos employ veterinarians and/or veterinary paraprofessionals with expertise in wildlife and exotic zoo animals. Deaths of collection animals will typically be investigated through a full post-mortem examination conducted by the zoo's veterinary service, followed by histopathology and ancillary testing where necessary. In Australia, as per the National Zoo Biosecurity Manual (Reiss & Woods 2011), animals culled within the zoo grounds are not fed out to other collection animals, unless the veterinary service has assessed the risk of transmissible diseases, and the practice is compliant with state and territory regulations.

Where possible, this review has examined literature on diseases of hippos in a captive setting. However, there is little literature relating to disease agents in hippos, either in captive or free-range settings. As such literature from related wildlife studies and domestic livestock has been considered in order to extrapolate knowledge. Furthermore, much of the current knowledge pertaining to disease agents of hippos is based on serological studies. Serological studies are subject to several limitations of interpretation, and the finding of seropositive individuals in a population does not necessarily equate to an important role in the maintenance and transmission of infectious diseases (Gilbert et al. 2013). Judgement is also required regarding the use of tests and vaccines in zoo hippos as they are not often validated in hippos. Assumptions and extrapolations made because of a lack of information have been clearly identified in the text.

Some of the disease agents reviewed have significant consequences, particularly for Australia's livestock industries and international trade. Some of them could have an immediate adverse impact on country freedom status and therefore trade, even if only reported from a single animal within a zoo setting. For example, a confirmed case of foot-and-mouth disease virus in a zoo may result in immediate trade restrictions and economic losses even if infection is contained within the zoo. Additionally, in some instances onshore treatment of an animal may not adequately address the biosecurity risk and therefore the animal may be required to be re-exported or destroyed. These outcomes are undesirable and the policy review considers these consequences when proposing biosecurity import conditions, in addition to the general risks to Australia.

The operational implementation of clinical procedures in zoo hippos is challenging. Both hippo species are considered dangerous. Some individuals in captive collections may be habituated to handling, allowing limited clinical procedures under physical restraint and/or minor sedation.

However, general anaesthesia for procedures may be required. These challenges limit the feasible options for risk management and these factors have been considered in the measures recommended.

In addition, established principles and options for disease management exist for the collection of semen from domestic artiodactyls. This is not the case for the collection of semen from zoo hippos. Extrapolation of applicable principles from domestic artiodactyls, whilst considering the operational practicalities of semen collection from zoo hippos, has been used to determine suitable disease management options for zoo hippo semen in this policy.

Scope

The scope of this review is to assess the biosecurity risk associated with the import into Australian zoos of live zoo hippos and their semen from approved, licensed or registered zoos or wildlife parks in approved countries.

For the purpose of this review, approved countries are Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Japan, Luxembourg, Netherlands, New Zealand, Portugal, Singapore, Spain, Sweden, the United Kingdom, and the United States. These countries were considered for inclusion on the basis of historical trade and their ability to provide accurate export certification. The department has not assessed these countries to determine whether they are able to meet the import requirements developed in this review. The addition of other countries would require a submission to and then assessment and potential approval by the department, and may require re-assessment of the specific disease hazards identified.

This review covers the 2 extant species of hippos: *Hippopotamus amphibius* (common hippopotamus) and *Choeropsis liberiensis* (syn. *Hexaprotodon liberiensis*) (pygmy hippopotamus).

Existing policy

International policy

Zoo hippos were previously imported into Australia under historical conditions, with the last imports of zoo hippos occurring in the 1980s when conditions were determined on a case-by-case basis. In addition, hippos intended for circus display were imported on a temporary basis in the mid-1990s. No current import policy exists for live zoo hippos or their semen.

The department has considered the pests and diseases previously identified in existing zoo artiodactyl and related wildlife policies. Where relevant, the information in those assessments has been considered in this risk analysis.

Domestic arrangements

The Australian Government is responsible for regulating the movement of animals and animal products into and out of Australia. State and territory governments are responsible for animal health and environmental controls within their individual jurisdictions.

Once animals and animal products have been cleared by Australian Government biosecurity officers, they may be subject to state and territory controls (including movement conditions). The importer is responsible for identifying and ensuring compliance with all relevant requirements of the states and territories where the imported animals will transit and/or reside.

Next steps

This review provides stakeholders the opportunity to comment and draw attention to any scientific, technical or other gaps in the data, misinterpretations and errors.

The department will consider submissions received on this draft policy review and may consult informally with stakeholders. The department will then prepare a final report, considering stakeholder comments.

The final policy review will be published on the department's website with a notice advising stakeholders of the release. The department will also notify the proposer, the registered stakeholders and the WTO Secretariat about the release of the final report. Publication of the final report represents the end of this review process. The conditions recommended in the final report will be the basis of any import permits issued.

1 Method

The World Organisation for Animal Health (WOAH), in its Terrestrial Animal Health Code (the WOAH Terrestrial Code), describes 'General obligations related to certification' in Chapter 5.1 (OIE 2015a).

The WOAH Terrestrial Code states in Article 5.1.2. that:

> The import requirements included in the international veterinary certificate should assure that commodities introduced into the importing country comply with the standards of WOAH. Importing countries should align their requirements with the recommendations in the relevant standards of WOAH. If there are no such recommendations or if the country chooses a level of protection requiring measures more stringent than the standards of WOAH, these should be based on an import risk analysis conducted in accordance with Chapter 2.1.

Article 5.1.2. further states that:

> The international veterinary certificate should not include measures against pathogenic agents or diseases which are not WOAH listed, unless the importing country has demonstrated through import risk analysis, carried out in accordance with Section 2, that the pathogenic agent or disease poses a significant risk to the importing country.

The components of risk analysis as described in Chapter 2.1 of the WOAH Terrestrial Code are:

- hazard identification
- risk assessment (entry assessment, exposure assessment, consequence assessment and risk estimation)
- risk management
- risk communication.

Hazard identification, risk assessment and risk management are sequential steps within a risk analysis. Risk communication is conducted as an ongoing process and includes both formal and informal consultation with stakeholders.

1.1 Risk review

Risk – defined by the WOAH Terrestrial Code as 'the likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health' – is dynamic in nature, changing with time. Consequently, risk should be kept under regular review.

Although not defined or described in the WOAH Terrestrial Code, risk review is recognised by risk analysts as an essential component of the risk analysis process (Barry 2007; FSA 2006; Purdy 2010).

Australia applies a process of risk review to the biosecurity risks associated with the importation of an animal commodity (animal product or live animal) for which biosecurity measures exist or have previously been developed.

Risk review differs from the monitoring and review component of risk management, as described in the WOAH Terrestrial Code, in that each component of the risk analysis process (hazard identification, risk assessment and risk management) is reviewed under the risk review process. If a change (either an increase or a decrease) in the biosecurity risk associated with a live animal or animal product is identified based on updated scientific information, risk management measures can be revised accordingly.

This policy review has drawn on these sources of information:

- the WOAH Terrestrial Code
- Australia's existing import policies for other zoo artiodactyls
- a review of relevant scientific literature and other information
- expert opinion coordinated through the Australasian Zoo and Aquarium Association (ZAA).

For this review, conditions for zoo hippo imports have not been re-examined since the last import of zoo hippos occurred in the 1980s and circus hippos in the 1990s, and substantial new information relating to biosecurity risks associated with zoo hippos and related species has become available since this time. Therefore, each component was reassessed entirely, drawing on relevant previous policies, scientific literature, and expert opinion.

1.2 Hazard identification

Hazard identification is described in the WOAH Terrestrial Code (Article 2.1.2) as a classification step that is undertaken to identify potential hazards that may be associated with the importation of a commodity.

In accordance with the WOAH Terrestrial Code, a disease agent was considered to be a potential hazard relevant to the importation of live zoo hippos and their semen if it was assessed to be appropriate to the species being imported.

A list of potential hazards was developed following a review of scientific literature and expert consultation. Disease agents in previous policy reviews or import risk assessments for zoo artiodactyls conducted by the department were also considered as potential hazards.

A hazard was retained for further review (hazard refinement) if it was:

- identified as being capable of infecting or infesting and being spread by hippos
- identified as emerging and/or capable of producing adverse consequences
- not present in Australia, or present in Australia and a notifiable disease or subject to official controls or eradication.

Where evidence for the inclusion or exclusion of a particular disease agent was equivocal, a judgement was made based on the strength of the available evidence to implicate zoo hippos and/or their semen in disease transmission.

1.3 Risk assessment

Where hazard refinement led to the conclusion that a risk assessment was required for the disease agent, this was conducted in accordance with Chapter 2.1 of the WOAH Terrestrial Code.

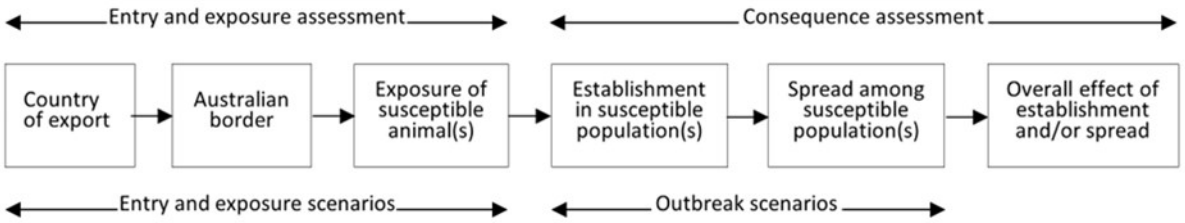
Risk assessment is the evaluation of the likelihood and the biological, economic, and social consequences of entry, establishment and/or spread of a hazard within the territory of an importing country. A review of risk factors relevant to the entry, exposure and consequence assessment was conducted for each hazard retained. If definitive information on risk factors was not found through literature review or contact with relevant experts, any uncertainties were identified and documented. Assumptions and judgements that were made in drawing conclusions for each hazard retained were documented in the relevant risk review sections (chapter 3)

For each disease agent requiring risk assessment, the risk assessment resulted in an unrestricted risk estimate for that agent. For the purposes of this draft policy review, the unrestricted risk estimate was defined as the level of risk that would be present if there were no safeguards in place other than the baseline requirements for the importation of zoo animals, and any disease-specific requirements as set out in the WOAH Terrestrial Code as applicable to live zoo hippos or their semen. These baseline requirements can be found in chapter 4 of this report. Estimation of the unrestricted risk included consideration of:

- likelihood of the disease agent entering Australia in imported live zoo hippos or their semen (entry assessment)
- likelihood of susceptible animals in Australia being exposed to the disease agent in imported zoo hippos or their semen (exposure assessment)
- most likely outbreak scenario that would follow exposure to the disease agent and the overall effect of establishment and/or spread associated with the outbreak scenario (consequence assessment).

Figure 1 shows how we estimate unrestricted risk.

Figure 1 Components of the unrestricted risk estimate



If the unrestricted risk estimate for the disease agent did not achieve Australia's ALOP, then risk management measures in addition to baseline requirements were recommended to reduce the risk to achieve Australia's ALOP.

1.3.1 Evaluating and reporting likelihood

Risk assessments were conducted using a qualitative approach and the nomenclature in Table 1.

Table 1 Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	Event would be very likely to occur
Moderate	Event is equally likely to occur or not occur
Low	Event would be unlikely to occur
Very low	Event would be very unlikely to occur
Extremely low	Event would be extremely unlikely to occur
Negligible	Event would almost certainly not occur

1.3.2 Entry assessment

The entry assessment consists of describing the pathways necessary for the importation of live zoo hippos and their semen to introduce the disease agent into Australia and estimating the likelihood of that complete process occurring.

Several factors were taken into account in determining the likelihood of the disease agent being present in imported zoo hippos and their semen. These included:

- reported detections and prevalence of the disease agent in live zoo hippos and their semen in approved countries, or, where information was not available, prevalence in other zoo artiodactyls and hippos in free-ranging states
- biology and epidemiology of the hazard (including ease of recognising clinical signs, incubation periods, transmission, latency and predilection sites, including the presence of the disease agent in semen)
- impact of general risk management conditions, such as baseline requirements or any WOAH Terrestrial Code applicable requirements.

A qualitative likelihood (Table 1) was assigned to describe the likelihood of the disease agent entering Australia in imported live zoo hippos and their semen.

1.3.3 Exposure assessment

The exposure assessment consists of describing the pathways necessary for exposure of susceptible animals in Australia to the disease agent in zoo hippos and their semen and estimating the likelihood of the exposure occurring. It considers the different groups of animals that are susceptible to infection with these disease agents as well as the possible pathways by which exposure of these animals could occur.

Several factors were taken into account in determining the exposure likelihood including:

- biology and epidemiology of the hazard (including methods of transmission, availability of vectors or intermediate hosts where relevant, persistence of the disease agent in zoo hippos and their semen, infectivity and viability of the pathogen in the environment)

- impacts of general risk management conditions such as baseline requirements or any applicable WOAH Terrestrial Code requirements
- impacts of zoo biosecurity and captive management practices including mixed-species exhibits, waste and water management practices.

A qualitative likelihood (Table 1) was assigned to describe the likelihood of susceptible animals being exposed to the disease agent from imported zoo hippos and their semen.

1.3.4 Estimation of the likelihood of entry and exposure

The likelihood of entry and exposure for the disease agent was estimated by combining the likelihood of entry and the corresponding likelihood of exposure using the matrix as shown in Figure 2.

Figure 2 Matrix for combining qualitative entry and exposure likelihoods

Likelihood of entry \ Likelihood of exposure	*Negligible*	*Extremely low*	*Very Low*	*Low*	*Moderate*	*High*
High	Negligible	Extremely low	Very low	Low	Moderate	High
Moderate	Negligible	Extremely low	Very low	Low	Low	Moderate
Low	Negligible	Extremely low	Very low	Very low	Low	Low
Very low	Negligible	Extremely low	Extremely low	Very low	Very low	Very low
Extremely low	Negligible	Negligible	Extremely low	Extremely low	Extremely low	Extremely low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

1.3.5 Consequence assessment

Once exposure of susceptible animals has occurred, a number of possible outbreak scenarios could follow. These represent a continuum ranging from no spread to widespread establishment of disease. These include:

- establishment in the directly exposed zoo animal population without spread to other populations of susceptible animals outside of the zoo
- establishment in the directly exposed zoo animal population and spread to other populations of susceptible animals within the local area
- establishment in the directly exposed zoo animal population and spread to other populations of susceptible animals within the region
- establishment in the directly exposed zoo animal population and spread to other populations of susceptible animals across multiple states or territories.

For the purposes of this review, outbreak scenarios were considered based on the epidemiology of the disease agent. For each hazard the most likely outbreak scenario following exposure of susceptible animals to the disease agent in imported zoo hippos and their semen was identified and used to determine overall likely consequences of the disease agent, using effect categories as per Table 2. For some hazards, reflecting the nature of the zoo environment into which animals are imported (including separation from the general animal population, and post arrival quarantine and

monitoring), the likely scenario was of a detection within a zoo environment, and direct effects primarily were confined to that zoo environment. Indirect effects were also considered in all cases and included in the overall consequence rating, but for some hazards were generally expected to be more limited than would occur with entry and exposure of a disease agent through a non-zoo import pathway (and therefore not restricted to occurring within a zoo environment).

Table 2 Rules for determining the likely consequences using effect categories

Effect	Descriptive definition
Extreme	Effect is likely to be highly significant at the national level. Implies that economic stability, societal values or social well-being would be seriously affected.
High	Effect is likely to be significant at the national level and highly significant within affected zones. Implies that the effect would be of national concern. However, serious effects on economic stability, societal values or social well-being would be limited to a given zone.
Moderate	Effect is likely to be recognised on a national level and significant within affected zones. The effect is likely to be highly significant to directly affected parties.
Low	Effect is likely to be recognised within affected zones and significant to directly affected parties. It is not likely that the effect will be recognised at the national level.
Very low	Effect is likely to be minor to directly affected parties. The effect is unlikely to be recognised at any other level.
Negligible	Effect is unlikely to be recognised at any level within Australia.

1.3.6 Risk estimation

Risk estimation consists of integrating the results from the entry assessment, exposure assessment and consequence assessment to produce an unrestricted risk estimate of the disease agent.

The unrestricted risk for the disease agent was estimated by combining the likelihood of entry and exposure with the likely consequences using the risk estimation matrix shown in Figure 3.

Figure 3 Risk estimation matrix – likelihood of entry and exposure and likely consequences

Likelihood of entry and exposure	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Likely consequences

1.4 Risk management

Risk management is described in the Terrestrial Code Article 2.1.5. as the process of deciding upon and implementing measures to address the risks identified in the risk assessment, while ensuring that negative effects on trade are minimised (OIE 2018a).

Components of risk management include risk evaluation – the process of comparing the risk estimated in the risk assessment with the reduction in risk expected from the proposed risk management measures – and option evaluation – the process of identifying, evaluating the efficacy and feasibility of, and selecting measures to reduce the risk associated with an importation. The efficacy is the degree to which an option reduces the likelihood or magnitude of adverse health and economic consequences.

In this policy review, if the unrestricted risk estimate for a disease agent did not achieve Australia's ALOP, then risk management measures in addition to baseline requirements and any disease-specific WOAH Terrestrial Code requirements (as applicable to live zoo hippos) were recommended to reduce the risk to achieve Australia's ALOP.

The restricted risk estimate for a disease agent is the level of risk that would be present with a particular risk management measure or combination of measures applied. If the restricted risk of the disease agent was estimated to be 'negligible' or 'very low' following application of a particular risk management measure or combination of measures, this achieved Australia's ALOP and that measure or combination of measures was considered acceptable.

Proposed risk management measures aimed to be practical, taking into account industry practices and operational feasibility, and no more trade-restrictive than necessary to achieve Australia's ALOP.

1.4.1 Baseline requirements

This policy review also considered the efficacy of long-standing general zoo policy to manage the biosecurity risks and animal welfare issues associated with the importation and handling of wild animal species. General risk management measures are implemented through application of that policy and help in achieving the ALOP in each case.

Baseline requirements for live zoo animals

The baseline risk management measures common to most current import policies for live zoo animals include:

1) The animal must be resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before export, unless otherwise approved by the department. The residency requirement may be achieved in more than one approved country or holding institution if specifically authorised by the department and the conditions for each country of residence and holding institution were met.
2) The premises of origin (zoo or wildlife park) must provide separation from other animal populations, be under veterinary supervision and have a documented health monitoring program that would be effective in monitoring for the diseases of biosecurity concern identified in this review.

   The required outcome of veterinary supervision is up to date and regular knowledge of the animals, their health status, and the general health status of the institution that allows a veterinarian to sign off on these records.

   The required outcome of separation is a sufficient distance or other barriers to maintain a distinct animal health status with regards to the diseases in this policy.

The required outcome of a health monitoring program is the regular monitoring, ongoing surveillance, and veterinary oversight to ensure that the health status of animals and an institution is known and monitored over time (e.g. post-mortem records for deceased animals, disease testing programs). This underpins official certification.

3) The animal must be held in pre-export quarantine (PEQ) for at least 30 days and isolated from all other animals not eligible for export to Australia, during which it is inspected at least daily for signs of disease and treated and tested for diseases in accordance with Australian entry requirements.
4) For the 30 days immediately before export, the animal showed no clinical signs or other evidence of infectious or contagious diseases, including the diseases retained for risk review in this policy.
5) During the PEQ period or the 90 days immediately prior, the animal was not under any quarantine restrictions (aside from PEQ).
6) The PEQ facility has acceptable documented standards of how it will meet Australian requirements.
7) Immediately following arrival in Australia, the animal must be transported to an approved arrangement site audited and approved by the department, in a manner that ensures no direct exposure to animals of a lesser biosecurity status en-route, and must undergo a period of post-arrival quarantine of at least 30 days.
8) The receiving institution must be approved under relevant Australian state or territory legislation to hold the species being imported.

Baseline requirements for zoo semen

General risk measures relevant to semen are:

1) The donor animal must be resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before collection, unless otherwise approved by the department. The residency requirement may be achieved in more than one approved country or holding institution if specifically authorised by the department and the conditions for each country of residence and holding institution were met.
2) The premises of origin (zoo or wildlife park) must provide separation from other animal populations, be under veterinary supervision and have a documented health monitoring program that would be effective in monitoring for the diseases of biosecurity concern identified in this review.

   The required outcome of veterinary supervision is up to date and regular knowledge of the animals, their health status, and the general health status of the institution that allows a veterinarian to sign off on these records.

   The required outcome of separation is a sufficient distance or other barriers to maintain a distinct animal health status with regards to the diseases in this policy.

   The required outcome of a health monitoring program is regular monitoring, ongoing surveillance, and veterinary oversight to ensure that the health status of animals and an institution is known and monitored over time (e.g. post-mortem records for deceased animals, disease testing programs). This underpins official certification.

3) The donor animal was not under quarantine restriction for the 90 days immediately prior to each semen collection, on the day(s) of each semen collection and for the 30 days immediately after each semen collection.
4) For the 30 days immediately before each semen collection, the donor animal showed no clinical signs or other evidence of infectious or contagious diseases, including the diseases retained for risk review in this policy.
5) The donor animal showed no signs of infectious or contagious disease at the time of each semen collection and for the 30 days immediately after.
6) The receiving institution must be approved under relevant Australian state or territory legislation to hold the relevant donor and recipient hippo species.

Additional baseline requirements

In addition, the WOAH Terrestrial Code specifies minimum risk mitigation standards for the movement of live animals for specific diseases. Where specific disease chapters within the WOAH Terrestrial Code stipulated measures applicable to the movement of all wild mammals, these requirements were taken to form part of the baseline requirements for import under this policy. The relevant chapters within the WOAH Terrestrial Code are New World screwworm (*Cochliomyia hominivorax*) and Old World screwworm (*Chrysomya bezziana*), and infection with rabies virus. These chapters stipulate risk mitigation measures applicable to the movement of live zoo hippos, and these requirements subsequently form part of this policy.

1.5 Risk communication

Risk communication is defined in the WOAH Terrestrial Code as 'the interactive transmission and exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions among risk assessors, risk managers, risk communicators, the general public and other interested parties' (WOAH 2023b).

Consultation with external stakeholders is a standard procedure for all import risk analyses and risk or policy reviews to enable stakeholder assessment and feedback on draft conclusions and recommendations about Australia's animal biosecurity policies.

2 Hazard identification

The list of potential hazards (disease agents of potential biosecurity concern) was compiled from:

- diseases, infections and infestations listed by the WOAH within the category of multiple species diseases, infections and infestations, that are recognised to affect any artiodactyl (WOAH 2023a)
- diseases identified in previous policy reviews and import conditions of relevant zoo animals conducted by the department
- other diseases identified as occurring in hippos or their semen, or other closely related artiodactyl species, including emerging diseases.

The method of hazard identification and refinement is described in section 1.2. The preliminary list of hazards is shown in Table 3. This table summarises the results of the hazard refinement process, including the reason for removal or retention of each identified hazard.

The department gave careful consideration to hazards for inclusion in the list. Many disease agents are ubiquitous or common commensal organisms which may be present in Australia. Others are opportunistic, not reported to be pathogenic, or are of uncertain relevance to hippos due to limited or insufficient information. These agents were considered when compiling the list of potential hazards but may not be included in the preliminary hazard list shown in Table 3.

The diseases retained after hazard identification and refinement (Table 3) are listed at the end of this chapter.

Table 3 Hazard identification and refinement

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
African swine fever (African swine fever virus)	Pigs	Uncertain[a]	Yes	Yes (Suidae)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Aino disease (Aino virus)	Domestic and wild ruminants primarily, camelids	No	Limited (already present)	No	Yes	No	No. Current evidence does not indicate that hippos are important epidemiologically. Present in Australia and not nationally notifiable.
Akabane disease (Akabane virus)	Domestic and wild ruminants primarily, camelids	Yes[b]	Limited (already present)	No	Yes	No	No. Current evidence does not indicate that hippos are important epidemiologically. Present in Australia and not nationally notifiable.
Anthrax (*Bacillus anthracis*)	Mammals, and some birds	Yes	Yes	Yes (multiple)	Yes	Yes	Yes. Common reports of infection and transmission by free-ranging common hippos. WOAH listed, present in Australia but nationally notifiable and control measures are in place.
Aujeszky's disease (suid herpesvirus-1)	Pigs, other mammals recorded as dead-end hosts	No	Yes	Yes (multiple)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically. Infection of species other than pigs typically fatal after short incubation period (2-10 days), which is controlled by non-specific measures (PEQ period and clinical inspection).

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Babesiosis (*Babesia* spp.)	Broad range of mammals and birds susceptible to specific species	Yes[c]	Yes	Yes (specific species)	Yes, specific species	Yes (specific species)	No. Current evidence does not indicate that hippos are important epidemiologically for species of concern.
Bluetongue (bluetongue virus)	Artiodactyls	No	Yes	Yes (multiple)	Yes, some serotypes	Yes (clinical disease)	No. Current evidence does not indicate that hippos are important epidemiologically.
Border disease (border disease virus)	Sheep and goats (primarily), cattle, pigs, deer, camels	No	Limited (already present)	No	Yes	No	No. Current evidence does not indicate that hippos are important epidemiologically. Present in Australia and not nationally notifiable.
Borna Disease (Borna disease virus)	Equids, sheep mainly; hippos, cattle, camelids, dogs, cats, ostriches	Yes	Yes	No	No	Yes	Yes. Reported in a zoo hippo. Not present in Australia and nationally notifiable.
Bovine anaplasmosis (*Anaplasma marginale*)	Ruminants	Yes[d]	Yes	Yes (Bovidae)	Yes	Yes (in tick free areas)	No. Current evidence does not indicate that hippos are important epidemiologically[d].
Bovine ephemeral fever (bovine ephemeral fever virus)	Cattle, yaks, water buffalo	Yes[b]	Limited (already present)	No	Yes	No	No. Current evidence does not indicate that hippos are important epidemiologically. Present in Australia and not nationally notifiable.
Bovine herpes mammillitis (bovine alphaherpesvirus 2)	Cattle, wild ruminants	Yes	Limited (already present)	No	Yes	No	No. Present in Australia and not nationally notifiable.
Bovine viral diarrhoea (bovine viral diarrhoea virus 1, bovine viral diarrhoea virus 2, HoBi-like pestivirus)	Artiodactyls	No	Yes	Yes (Bovidae)	Yes (type 1 only)	Yes (type 2 only)	No. Current evidence does not indicate that hippos are important epidemiologically.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Brucellosis (*Brucella abortus, B. melitensis, B. suis*)	Mammals	Yes	Yes	Yes (multiple)	*Brucella suis* present in Australia. *B. melitensis* and *B. abortus* absent	Yes	Yes. Several reports of positive serological results in free-ranging common hippos and wide range of mammals susceptible. WOAH listed, *B. abortus, B. melitensis* not present in Australia and nationally notifiable.
Chagas disease (*Trypanosoma cruzi*)	Wide range of mammals, particularly humans and dogs	No	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically, although likely susceptible; known competent vectors have highly restricted distribution in Australia.
Contagious bovine pleuropneumonia (*Mycoplasma mycoides* subsp. *mycoides* small colony type)	Cattle, buffalo, sheep, goats	Yes[e]	Yes	Yes (Bovidae)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically[e].
Crimean–Congo haemorrhagic fever (Crimean–Congo haemorrhagic fever virus)	Mammals, including humans	No	Yes	Yes (multiple)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Encephalitides – tick-borne (tick-borne encephalitis virus)	Rodents, birds, ruminants, equids, dogs, humans	No	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically. Mammals other than rodents are typically dead-end hosts.
Encephalomyocarditis (encephalomyocarditis virus)	Mammals, rodent reservoir	Yes	Limited (already present)	No	Yes	No	No. Present in Australia and not nationally notifiable.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Enterocytozoon bieneusi	Humans, pigs, ruminants, cats, dogs, birds, several wild mammals	Yes	Limited (already present)	No	Yes	No	No. Present in Australia and not nationally notifiable.
Epizootic haemorrhagic disease (clinical disease) (epizootic haemorrhagic disease virus)	Cervidae primarily; Bovidae, occasional other species, including rhinos, black bears, oryx.	No	Yes	Yes (multiple)	Yes (not clinical disease)	Yes (clinical disease)	No. Current evidence does not indicate that hippos are important epidemiologically.
External parasites	Wide range of species	Yes	Yes	No	Yes (some types)	No	Yes. Hippos host several exotic species that may not be managed by baseline conditions.
Foot-and-mouth disease (foot-and-mouth disease virus)	Artiodactyls primarily	Uncertain[f]	Yes	Yes (multiple)	No	Yes	Yes. Most artiodactyls are presumed susceptible to infection, although no unequivocal evidence of infection in hippos[f]. WOAH listed, not present in Australia and nationally notifiable.
Getah virus infection	Horses and pigs, other warm-blooded species, including humans occasionally	No	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Gyrovirus galga 1	Primarily chickens although reported in broader host range	Yes	Yes	No	Not reported	No	No. Current evidence does not indicate that hippos are important epidemiologically.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Haemorrhagic septicaemia (*Pasteurella multocida* serotypes 6:B & 6:E)	Ruminants, camels, deer, hares, horses, pigs, elephants, birds, potentially humans	Yes	Yes	Yes (Bovidae)	No (serotypes B2 & E2 not present)	Yes	Yes. *P. multocida* (unknown types) recorded in hippos associated with sudden death and septicaemia. WOAH listed, some serotypes not present in Australia and nationally notifiable.
Heartwater (*Ehrlichia ruminantium*)	Domestic and wild ruminants	No	Yes	Yes (multiple)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Hepatocystis hippopotami	Common hippos	Yes	No	No	Not reported	No	No. No evidence of adverse consequences.
Hippopotamus herpesviruses (*Hexaprotodon liberiensis* gammaherpesvirus 1), (*Hippopotamus amphibius* rhadinovirus 1)	Hippos	Yes	Not reported	No	Not reported	No	No. No evidence of adverse consequences.
Infectious bovine rhinotracheitis and infectious pustular vulvovaginitis (bovine alphaherpesvirus 1)	Cattle, sheep, goat, water buffalo, wild artiodactyls	Yes	Yes	Yes (Bovidae)	Yes (only less virulent subtype 1.2b)	No	Yes. Several reports of serological evidence in pygmy and common hippos, including in a zoo setting. WOAH listed, present in Australia and not nationally notifiable, but Australian strains are only of the BoHV-1.2b subtype. Overseas, the more virulent BoHV1.1 strains predominate.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Internal parasites	Wide range of species	Yes	Yes	Some (*Echinococcus granulosus, E. multilocularis)*	Some	Some (*E. multilocularis*)	Yes. Hippos host several exotic species that may not be managed by baseline conditions.
Leishmaniosis (*Leishmania* spp.)	Humans and dogs primarily. Occasional reports in other species	No	Yes	Yes (other)	Yes (a single novel species found in macropods in a discrete location)	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Leptospirosis (*Leptospira* spp.)	Wide range of species	Yes	Yes	No	Yes (but strains of serovar Canicola are exotic)	No (notifiable in humans)	No. Minimal domestic controls on existing *Leptospira* strains. Hippos have not been identified as a maintenance host for any serovar. Various reviews have determined so far that only serovar *L.* Canicola is of concern to Australia for imports. Hippos are not known to have not been identified as a host for this serovar.
Loiasis (*Loa loa*)	Humans primarily	No[g]	Yes	No	Not reported	No	No. Current evidence does not indicate that hippos are important epidemiologically.
Lumpy skin disease (lumpy skin disease virus)	Cattle, buffalo, some wild artiodactyls	No	Yes	Yes (Bovidae)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Malignant catarrhal fever (wildebeest-associated) (alcelaphine herpesvirus 1)	Artiodactyls	Yes	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically. Transmission of virus is only thought to occur from the natural host, wildebeest (*Connochaetes* spp.) with all other mammals considered dead end hosts.
Malignant catarrhal fever (Sheep associated) (ovine herpesvirus 2)	Sheep. Other artiodactyls spillover hosts	Yes[b]	Limited (already present)	No	Yes	No	No. Current evidence does not indicate that hippos are important epidemiologically[b]. Present in Australia and not nationally notifiable.
Melioidosis (*Burkholderia pseudomallei*)	Mammals	Yes	Limited (already present)	No	Yes	No	No. Present in Australia and not nationally notifiable.
Middelburg virus	Horses, humans	Yes[b]	Yes	No	Not reported	No	No. Current evidence does not indicate that hippos are important epidemiologically[b].
Mycobacterium tuberculosis complex (*M. bovis, M. caprae, M. tuberculosis*)	Wide range of mammals	Yes	Yes	Yes (multiple)	No	Yes	Yes. Evidence of infection in hippos. WOAH listed. Australia is free of *Mycobacterium tuberculosis* complex and it is nationally notifiable.
Nairobi sheep disease (Nairobi sheep disease virus)	Sheep and goats primarily	Yes[b]	Yes	Yes (Caprinae)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically[b].

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Paratuberculosis (Johne's disease) (*Mycobacterium avium* subsp. *paratuberculosis*)	Artiodactyls, rodents, lagomorphs, birds	No	Limited (already present)	Yes (multiple)	Yes	Yes	No. No reports in hippos. Present in Australia although nationally notifiable. Limited consequences expected in the event of detection in a zoo hippo.
Peste-des-petits-ruminants (peste des petits ruminants virus)	Goats and sheep, wild artiodactyls	No	Yes	Yes (Caprinae)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Q fever (*Coxiella burnetii*)	Wide range of mammals	No	Limited (already present)	Yes (multiple)	Yes	No (notifiable in humans)	No. Current evidence does not indicate that hippos are important epidemiologically. Present in Australia, notifiable in humans only.
Rabies virus	All mammals, including humans	No	Yes	Yes (multiple)	No	Yes	Yes. No evidence identified in hippos but all mammals are considered susceptible and WOAH Terrestrial Code conditions for all mammals. WOAH listed, not present in Australia and nationally notifiable.
Rift Valley fever (Rift Valley fever virus)	Artiodactyls, multiple other species, including primates, rodents, humans	Yes	Yes	Yes (multiple)	No	Yes	Yes. Several reports of positive serological results in free-ranging common hippos. WOAH listed, not present in Australia and nationally notifiable.
Rinderpest (rinderpest virus)	Artiodactyls	Yes	Yes	Yes (multiple)	No	Yes	No. Globally eradicated.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Sarcocystis hippopotami and *Sarcocystis africana*	Hippopotamus	Yes	No	No	No	No	No. Although life cycles for species found in hippopotamus have not been described, most *Sarcocystis* have 2-host life cycles, with herbivorous intermediate hosts and carnivores as definitive hosts. Hippos likely an intermediate host for both species and without access to definitive hosts infections are likely to be dead end.
SARS-CoV-2 (severe acute respiratory syndrome–related coronavirus 2)	Humans, wide range of mammals	Yes	Yes	No	Yes	No	No. Although cases have been reported in hippos, current evidence does not indicate that hippos are important epidemiologically. Currently no disease controls applied within Australia (human or animal).
Schmallenberg (Schmallenberg virus)	Domestic and wild ruminants primarily, camelids	No	Yes	No	Not reported	No	No. Current evidence does not indicate that hippos are important epidemiologically.
Screw-worm fly – New World (*Cochliomyia hominivorax*)	All mammals, including humans	No	Yes	Yes (multiple)	No	Yes	Yes. No evidence identified in hippos but all mammals are considered susceptible and WOAH Terrestrial Code conditions are for all mammals. Not present in Australia and nationally notifiable.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Screw-worm fly – Old World (*Chrysomya bezziana*)	All mammals, including humans	No	Yes	Yes (multiple)	No	Yes	Yes. No evidence identified in hippos but all mammals are considered susceptible and WOAH Terrestrial Code conditions are for all mammals. Not present in Australia and nationally notifiable.
Semliki Forest virus	Horses, monkeys, humans	Yes[b]	Uncertain	No	Not reported	No	No. Current evidence does not indicate that hippos are important epidemiologically.
Streptococcus iniae	Marine and freshwater fish, humans	Yes	Limited (already present)	No	Yes	No	No. Present in Australia and not nationally notifiable.
Surra (*Trypanosoma evansi*)	Wide range of mammals	No	Yes	Yes (multiple)	No	Yes	Yes. Although no identified reports of *T. evansi* in hippos, susceptible to closely related *T. brucei* and wide host range. WOAH listed, not present in Australia and nationally notifiable.
Transmissible spongiform encephalopathies	Wide range of mammals	No	Yes	Some types (BSE, Scrapie)	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Trichinellosis (*Trichinella* spp.)	Most mammals, some reptiles and birds	Yes	Yes	Yes (multiple)	Limited species present (*T. pseudospiralis, T. papuae*)	Yes	No. No risk pathway for transmission in a zoo setting.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Tsetse-associated trypanosomes (*Trypanosoma brucei, T. congolense, T. simiae* and *T. vivax*)	Wide range of mammals	Yes	Yes	Yes (multiple)	No	Yes	Yes. Several reports of infection in common hippo. WOAH listed, not present in Australia and nationally notifiable.
Tularaemia (*Francisella tularensis*)	Wide range of mammals, including humans	Yes[b]	Yes	Yes (multiple)	Yes (type B only)	Yes	No. Current evidence does not indicate that hippos are important epidemiologically[b].
Uganda S virus	Unknown	Yes	Not reported	No	Not reported	No	No. No evidence of adverse consequences.
Vesicular exanthema (vesicular exanthema of swine virus)	Pigs, marine mammals	No	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Vesicular stomatitis (vesicular stomatitis virus)	Artiodactyls, horses, humans	No	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically.
Wesselsbron disease (Wesselsbron virus)	Artiodactyls rodents, equids, birds, humans	Yes[b]	Yes	No	No	Yes	No. Current evidence does not indicate that hippos are important epidemiologically[b].
West Nile fever (West Nile virus)	Horses, humans, birds, occasionally other mammals	Yes[b]	Yes	Yes (multiple)	Yes (some strains)	Yes (clinical disease)	No. Current evidence does not indicate that hippos are important epidemiologically[b].
Yellow Fever (yellow fever virus)	Humans, non-human primates	Yes	Yes	No	No	No (notifiable in humans)	No. Current evidence does not indicate that hippos are important epidemiologically.

Disease (disease agent)	Susceptible species	Evidence of infection and/or transmission by hippos	Emerging or adverse consequences expected	WOAH-listed disease	Present in Australia	Nationally notifiable animal disease in Australia	Retained for risk review
Zika (Zika virus)	Humans, non-human primates	Yes[b]	Yes	No	No	No (notifiable in humans)	No. Current evidence does not indicate that hippos are important epidemiologically[b].

a Two reports of isolation of African swine fever virus in hippos, as reported in secondary literature, could not be verified. Subsequent research could not demonstrate ASFV in hippos although 2 of 297 serum samples had questionable positive serological results (Stone & Heuschele 1965). Currently the host range is believed restricted to suids. **b** Serological evidence in a single study (akabane disease (Al-Busaidy, Hamblin & Taylor 1987); bovine ephemeral fever (Hamblin et al. 1990); malignant catarrhal fever (sheep-associated) (Hamblin & Hedger 1984); Middelburg virus, Wesselsbron disease, West Nile fever, Zika (Thal 1971); Nairobi sheep disease, Semliki Forest virus (Weinbren & Hewitt 1959); tularaemia (Kuehn et al. 2013)). **c** A report of babesiosis in hippos is limited to a single report of morphological detection of 3 parasites, which was subsequently questioned by the authors (Brocklesby & Vidler 1966). **d** Evidence of *A. marginale* in hippos is limited to morphological identification of a low-level parasitaemia in a single pygmy hippo (Mbaya et al. 2008). **e** Serological evidence of contagious bovine pleuropneumonia from a single study was reported as doubtful by the authors of that study (Shifrine & Domermuth 1967). Subsequent epidemiological knowledge suggests hippos would not be a natural host for this bacteria. **f** Evidence of experimental infection of foot-and-mouth disease virus in hippos, as reported in secondary literature, could not be verified (Koenen et al. 2012). Positive serology identified in one study was suggested to be a false positive (Di Nardo et al. 2015). **g** Evidence of infection of hippos with *Loa loa*, as reported in secondary literature, could not be verified (Jacobsen et al. 2022).

2.1 Diseases retained for risk review

The diseases retained for risk review on the basis of the information provided in Table 3 were:

- anthrax (*Bacillus anthracis*)
- Borna disease
- brucellosis (*Brucella abortus, B. melitensis* and *B. suis*)
- external parasites
- foot-and-mouth disease
- haemorrhagic septicaemia (*Pasteurella multocida* serotypes 6:B & 6:E)
- infectious bovine rhinotracheitis and infectious pustular vulvovaginitis
- internal parasites
- *Mycobacterium tuberculosis* complex (*M. bovis*, *M. caprae, M. tuberculosis*)
- New World screwworm (*Cochliomyia hominivorax*) and Old World screwworm (*Chrysomya bezziana*)
- rabies virus
- Rift Valley fever virus
- surra (*Trypanosoma evansi*)
- tsetse-associated trypanosomes (*Trypanosoma brucei, T. congolense, T. simiae* and *T. vivax*).

3 Risk reviews

3.1 Anthrax

3.1.1 Background

Anthrax is a WOAH listed disease of multiple species (WOAH 2023a) with most warm-blooded species considered susceptible, including humans. It is caused by a spore-forming bacterium, *Bacillus anthracis*, with disease in some species characterised by a rapidly fatal septicaemia. Anthrax epidemics in free-ranging common hippos are frequently reported (Stears et al. 2021; Turnbull et al. 1991; Wafula, Patrick & Charles 2008).

Bacillus anthracis is globally distributed, and is present in approved countries, although some countries such as New Zealand have not reported the disease for extended periods (Herrera 2022). Anthrax is present, but uncommon, in some regions of Australia and is a nationally notifiable animal disease, with major outbreaks listed as a category 3 disease under the Emergency Animal Disease Response Agreement (EADRA) (AHA 2022b; DAFF 2022b). Government controls typically applied to outbreaks in Australia include appropriate carcass disposal and site disinfection, movement restrictions and vaccination (Agriculture Victoria 2022; Business Queensland 2020; NSW DPI 2020). Anthrax is zoonotic and is nationally notifiable in humans in Australia (Department of Health and Aged Care 2023).

3.1.2 Technical information

Epidemiology

Bacillus anthracis exists in 2 forms, a vegetative form that multiplies predominantly within the host, and a dormant environmental spore form. Following exposure to suitable environmental and nutrient conditions, sporulation may be triggered (AHA 2021a). The resultant anthrax spore is highly resistant and capable of surviving for decades under optimal conditions and decontamination is difficult (Sinclair et al. 2008). Although spores typically concentrate around carcass sites, contaminating soil, they may also be dispersed through hydrological influences, scavengers, insects and human-mediated dissemination, including via contaminated material such as wool and bone (Beyer & Turnbull 2009; FAO, OIE & WHO 2008; Hugh-Jones & de Vos 2002). In its vegetative form, the bacterium is labile and readily inactivated.

Transmission of anthrax in animals is suggested to occur primarily through ingestion of spores. However, other routes of transmission may include ingestion of contaminated meat, inoculation or contamination of skin lesions and inhalation of spores (FAO, OIE & WHO 2008; Hugh-Jones & de Vos 2002). WOAH considers the incubation period to be 20 days and instances of carrier or prolonged incubation states are rare (Beyer & Turnbull 2009; FAO, OIE & WHO 2008; OIE 2011). A carrier state has not been identified in hippos. Death of the infected host is generally considered necessary for transmission of anthrax.

In the wild, common hippos are thought to play a key role in anthrax epidemics in sub-Saharan Africa (Driciru et al. 2018), and the disease has been suggested to be the most significant disease of wild common hippos (Walzer & Stadler 2015). Mass mortalities involving hundreds to sometimes thousands of individuals in wild populations have been reported intermittently, primarily during the dry season (Cossaboom et al. 2019; Driciru et al. 2018; Gachohi et al. 2019; Mukarati et al. 2020;

Turnbull et al. 1991; Wafula, Patrick & Charles 2008). These events may reflect a high susceptibility of common hippos to infection and/or disease, or additionally may be driven by ecological and behavioural factors, including grazing habits, nutritional stressors, population densities in refugia during the dry season and carnivory (Bengis & Frean 2014; Clegg et al. 2007; Driciru et al. 2018; Dudley et al. 2015; Stears et al. 2021). Reported mortality of common hippos in epidemics range up to an estimated 21 to 55.5% of local populations (Driciru et al. 2018). Common hippo populations are reported to have higher anthrax-induced mortality compared with other species, whilst some outbreaks may be confined to common hippos alone, despite occurring in free-range settings with other species present (Dudley et al. 2015; Stears et al. 2021). Records of purported zoonotic transmission from common hippos exist (Hang'ombe et al. 2012; Van den Enden, Van Gompel & Van Esbroeck 2006).

There are no identified published reports of anthrax in wild pygmy hippos. Flacke et al. (2016) cites a reported case from a captive pygmy hippo, but the details of this case are uncertain. Historically, anthrax in zoos (in other species) has been linked to contaminated feed from local suppliers and recycling carcasses as feed (Hugh-Jones & de Vos 2002). Increased awareness and modern biosecurity, including surrounding feeding practices have led to a reduced incidence of disease in zoos (Hugh-Jones & de Vos 2002).

There is no identifiable evidence of anthrax transmission via semen. The WOAH Terrestrial Code recommends that semen should be considered be a safe commodity with respect to anthrax, with no conditions imposed (OIE 2011).

Diagnosis

Clinical signs

In wild common hippos, sudden death has been noted to sometimes be preceded by severe dyspnoea and oedema of the neck (Driciru et al. 2018). In other highly susceptible species, affected animals usually die within 1 to 3 days, often with no preceding clinical signs but frequently with haemorrhagic discharge from the carcass.

Testing

Visualisation of the agent in blood smears, culture, polymerase chain reaction (PCR) assays and lateral flow assays have been utilised for the diagnosis of anthrax in hippos (Cossaboom et al. 2019; Driciru et al. 2018; Kolton et al. 2019; Wafula, Patrick & Charles 2008). The sensitivity and specificity of these tests in hippos, as in other species, may be dependent on time elapsed since death of the host (Hornitzky & Muller 2010; Kolton et al. 2019).

Treatment

Antibiotic treatment of infected animals may be effective if given early in the course of the disease. However, antibiotic treatment may interfere with vaccination during outbreaks (AHA 2021a).

Control

Control measures for anthrax include rapid disposal of carcasses to minimise spore contamination, decontamination, vaccination, premises quarantine and movement controls (AHA 2021a).

3.1.3 Current biosecurity measures

Australia's current biosecurity measures for anthrax in some other live animals include premises freedom. The WOAH Terrestrial Code recommendations for ruminants, equids and pigs include

absence of clinical signs of anthrax and either 20 days residency in a free establishment or vaccination (OIE 2011).

3.1.4 Risk review

The following key points are relevant to the biosecurity risk of anthrax in live zoo hippos and their semen:

- Anthrax is a severe disease affecting multiple animal species, including humans.
- Wild common hippos are considered highly susceptible with outbreaks reported periodically. The incidence of anthrax in wild pygmy hippos is unknown.
- Increased awareness and modern biosecurity, including surrounding feeding practices have led to reduced incidences of disease in zoos. Outbreaks of anthrax within licensed or registered zoos, with active health monitoring programs, are likely to be identified quickly due to the severity of disease and broad range of species susceptible.
- Transmission of *B. anthracis* occurs primarily through ingestion of spores. These spores can be highly resistant in the environment.
- Affected animals are only considered infectious to others once they have died and the carcass releases spores or bacteria and no asymptomatic carrier state is recognised in hippos.
- There is no evidence that semen or artificial insemination poses a risk for transmission of *B. anthracis.*
- Hippos do not mix with other non-zoo animals (such as domestic livestock) in Australia although may be exposed to susceptible animals in a captive setting.
- Outbreaks of anthrax in animals often result in high levels of mortality and would be detected in captive hippos.
- Anthrax is present, but uncommon, in some regions of Australia and is a nationally notifiable animal and human disease. An outbreak of anthrax in a zoo animal will likely trigger government responses to some degree.

Based on the information above, the likelihood of entry and exposure for anthrax in live zoo hippos is estimated as low.

Based on the information above, the likelihood of entry and exposure for anthrax in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of anthrax is considered moderate.

For the likelihood of entry and exposure of low, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with anthrax in live zoo hippos of low which does not achieve Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall

unrestricted risk associated with anthrax in zoo hippo semen of negligible which achieves Australia's ALOP.

3.1.5 Conclusion

As the introduction of anthrax in live zoo hippos poses a low unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP.
No measures are required for hippo semen.

Australia's proposed disease-specific biosecurity measures for anthrax for the import of live zoo hippos are:

1) For 20 days immediately before export the animal has not resided on any premises where clinical, epidemiological or other evidence of anthrax has occurred in any species during the previous 20 days, and the disease is compulsorily notifiable.

3.2 Borna disease virus

3.2.1 Background

Borna disease is caused by Borna disease virus, *Orthobornavirus bornaense* (BoDV), which is an RNA virus in the family *Bornaviridae.* BoDV is further divided into Borna disease virus 1 (BoDV-1) and Borna disease virus 2 (BoDV-2). BoDV-1 strains are responsible for rare outbreaks of invariably fatal neurological diseases that primarily affect horses and sheep, but with a demonstrated capacity to infect a broader host range, including humans (Briese et al. 2021). BoDV-2 has only been isolated once to date, from a single horse from Austria (Kanda et al. 2021; Nowotny et al. 2000). Although evidence of BoDV has been identified in a broader geographical distribution, the disease is mainly confined to endemic areas of central Europe (Briese et al. 2021; Nobach et al. 2020). Natural infection with BoDV or a BoDV-like agent has been identified in a pygmy hippo in a zoo collection in Germany (Schüppel, Kinne & Reinacher 1994).

Infection with Borna disease virus is not a WOAH listed disease but is nationally notifiable in animals in Australia and is listed as a category 4 disease within the EADRA (AHA 2022b). Australia is considered free of Borna disease virus (AHA 2023).

3.2.2 Technical information

Epidemiology

Borna disease is mainly identified in central Europe and is considered endemic in Germany, Austria, Switzerland and Liechtenstein, where sporadic cases or rare outbreaks occur (Hornig 2016; Nobach et al. 2020). However, the global distribution of BoDV and disease is still uncertain (Hornig 2016; Kinnunen et al. 2013). BoDV-1 RNA or antibody has been detected on several occasions outside of central Europe, either in the absence of known disease, or in association with rare case reports (Degiorgis et al. 2000; Hagiwara, Matoba & Asakawa 2009; Hagiwara et al. 2008; Kinnunen et al. 2013; Priestnall et al. 2011). However, these detections have often been accounted for by serological cross-reactions, likely laboratory contamination or from import of animals from infected zones, although the distribution of BoDV-1 is possibly broader than that of clinical disease (Dürrwald, Kolodziejek & Nowotny 2006; Kamhieh et al. 2006; Kamhieh et al. 2008; Kinnunen et al. 2013; Priestnall et al. 2011).

Although several aspects of the epidemiology of BoDV-1 remain elusive, there is a significant body of evidence to suggest that the bi-coloured white-toothed shew (*Crocidura leucodon*) is the natural reservoir host of BoDV-1 in central Europe. Infection in this species is persistent, asymptomatic and is distributed in both neural and extra-neural tissue, including several organs enabling long-term, intermittent shedding via secretions and excretions, including saliva, faeces and urine (Dürrwald et al. 2014; Nobach et al. 2015; Weissenböck et al. 2017). No other reservoir has been identified to date, although other species such as bank voles (*Clethrionomys glareolus*) have also been postulated as a potential reservoirs (Kinnunen et al. 2013). In contrast to the reservoir host, infections of other hosts such as horses, sheep and humans are currently thought of as incidental infections and are not known to play an important role in natural transmission (Briese et al. 2021; Nobach et al. 2020). In these hosts, the virus appears strictly neurotropic and confined to the nervous system by the immune response restricting further transmission (Nobach et al. 2020; Weissenböck et al. 2017). However, there is conflicting data suggesting viral RNA (but not infective virus) may be present in the secretions of some incidental hosts (Richt et al. 1993). The pathogenesis, epidemiology of field infections and stability of endemic areas is consistent with a lack of horizontal transmission in incidental hosts, and genetic evidence also supports the reservoir concept (Dürrwald et al. 2014; Kinnunen et al. 2013; Kolodziejek et al. 2005; Rubbenstroth et al. 2019). However, the detection of viral RNA in regions outside of central Europe such as Japan is not readily explained by current epidemiological knowledge of BoDV-1 (Hagiwara, Matoba & Asakawa 2009; Hagiwara et al. 2008; Kinnunen et al. 2013).

Transmission routes of BoDV are still largely unknown, but infection in animals is thought to establish via the olfactory route possibly due to contaminated feed, with spread to the central nervous system (CNS) via retrograde axonal transmission from olfactory nerve endings (Hornig 2016; Morales et al. 1988; Nobach et al. 2020). Zoonotic spillover with BoDV-1 has recently been identified in humans associated with immunosuppression, transplant-associated transmission, as well as in humans from endemic areas due to uncertain transmission routes but possibly from direct or indirect exposure to infected shrews (Niller et al. 2020). Although serology indicates many infections in animals are asymptomatic, reported incubation periods are variable ranging from 2 weeks to several months. Expression of clinical Borna disease in animals moved from endemic areas to non-endemic areas 2 to 5 months later suggest natural incubation periods may be prolonged, and demonstrate potential for movement of infected animals via international trade (Jacobsen et al. 2010; Priestnall et al. 2011).

Borna disease virus or a borna disease like virus has been identified by immunohistochemistry in a pygmy hippo that died in a German zoo (Schüppel, Kinne & Reinacher 1994). Large amounts of BoDV antigen were identified in association with a non-suppurative meningoencephalitis, despite the absence of central nervous system signs at death (Schüppel, Kinne & Reinacher 1994). No further records of Borna virus in hippos could be identified. Based on current knowledge of the biology of BoDV-1, it would appear hippos are likely to be incidental hosts where the virus is assumed to be strictly neurotropic presumably not involved in the transmission of BoDV-1.

As incidental hosts are not known to shed infective virus, it is presumed hippo semen is not a risk material for BoDV-1 (Briese et al. 2021).

Diagnosis

Clinical signs

Serological evidence indicates infection of incidental hosts is often asymptomatic. BoDV-1 is not considered cytopathic and is tightly neurotropic (Briese et al. 2021; Nobach et al. 2015). An immune mediated pathogenesis is responsible for the development of clinical signs of disease associated with mononuclear inflammation of the CNS (Briese et al. 2021; Nobach et al. 2020). Where clinical signs of disease occur, they are associated with non-specific neurological disorders depending on the region of the CNS affected, including ataxia, paresis, behaviour changes ranging from depression to aggression. Death within 1 to 4 weeks after the onset of clinical signs is common, but rarer chronic forms of recurrent disease may occur (Herden et al. 2013).

In the infected pygmy hippo cited in Schüppel, Kinne and Reinacher (1994), clinical signs of disease consistent with Borna disease (as recorded in other species) were absent. Oral mucosal lesions, emaciation and diarrhoea were the only clinical signs before death under general anaesthesia. A non-suppurative meningoencephalitis was identified via histopathology but this was not associated with nervous system signs.

Testing

Serology may be used to detect antibodies in serum and/or cerebrospinal fluid. However, antibodies may not be present, or they are sometimes detected late in the course of the disease and of low titre, likely due to the tightly neurotropic pathogenesis of BoDV-1 (Kinnunen et al. 2013). In addition, antibodies in the *Orthobornavirus* genus are broadly cross-reactive (Zimmermann et al. 2014) and positive results should be confirmed by detection of the virus. As such reliable ante-mortem diagnosis is difficult. Post-mortem, immunohistochemistry to demonstrate viral proteins, reverse transcription-PCR (RT-PCR) or virus isolation from tissues may be utilised to confirm BoDV infection (Herden et al. 2013; Kinnunen et al. 2013).

Treatment

No treatments are available for BoDV infection in animals.

Control

With the epidemiology of BoDV still largely uncertain, preventative measures are unknown. Vaccines were commonly utilised for sheep and horses in endemic areas until the 1990s but were discontinued due to post-vaccination viral shedding and lack of efficacy (Dürrwald et al. 2022; Herden et al. 2013).

3.2.3 Current biosecurity measures

No biosecurity measures are currently applied for BoDV in zoo bovids or zoo perissodactyls. Conditions for import of live horses and horse semen include 90 days premises freedom and absence of evidence of clinical disease at time of collection respectively. Borna disease is not WOAH listed and as such there are no recommendations in the WOAH Terrestrial Code.

3.2.4 Risk review

The following key points are relevant to the biosecurity risk of BoDV in live zoo hippos and their semen:

- Disease due to BoDV-1 is confined to restricted areas of central Europe, although detection of virus outside of this region indicates the virus might be more widely spread.

- The epidemiology of BoDV-1 is not well understood, however the bicoloured white-toothed shrew is implicated as the reservoir host in central Europe.
- BoDV, or a similar virus, has been detected in a pygmy hippo in a captive zoo collection in Germany in association with an asymptomatic non-suppurative meningoencephalitis.
- There is no evidence that hippos are important in the epidemiology of BoDV and pathological findings from the pygmy hippo are consistent with those found in incidental hosts.
- Incidental hosts, such as zoo hippos, are currently not thought to shed infectious virus to contribute to transmission.
- As incidental hosts are not known to shed infective virus, it is presumed semen is not a risk material for BoDV. This is supported by research indicating that infection is limited to the nervous system in incidental hosts.
- Zoo hippos do not mix with open herds of domestic livestock in Australia, however they may share enclosures with other mammalian species. Zoo hippos come into contact with humans e.g. keepers and veterinarians.
- BoDV causes a rare but often fatal neurological disease of a broad host range, including humans.
- BoDV is not present in Australia and is a nationally notifiable disease, listed as category 4 in the EADRA.

Based on the information above, the likelihood of entry and exposure for Borna disease virus in live zoo hippos is estimated as extremely low.

Based on the information above, the likelihood of entry and exposure for Borna disease virus in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of Borna disease virus is considered low.

For the likelihood of entry and exposure of extremely low combined with the likely consequence of establishment and spread of low using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with Borna disease virus in live zoo hippos of negligible which achieves Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of low using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with Borna disease virus in zoo hippo semen of negligible which achieves Australia's ALOP.

3.2.5 Conclusion

Based on the preceding information disease-specific risk management measures for Borna disease in live zoo hippos or their semen are not warranted.

3.3 Brucellosis (*Brucella abortus*, *Brucella melitensis* and *Brucella suis*)

3.3.1 Background

Brucellosis in animals is caused by bacteria in the family *Brucellaceae.* Many *Brucella* spp. are important causes of disease in domestic animals, humans and wildlife. The agents of concern covered in this chapter are *Brucella abortus* (bovine brucellosis), *B. melitensis* (caprine and ovine brucellosis) and *B. suis* (porcine brucellosis).

Brucella abortus, B. melitensis and *B. suis* are important production limiting bacteria of livestock and pathogens of zoonotic concern. Consequences of infection include abortion, endometritis, orchitis and infertility in livestock and an acute febrile illness in humans which may eventuate in chronic complications such as recurrent fevers, arthritis, endocarditis and orchitis (Corbel 2006; WOAH 2022a). These species are global in distribution, although several approved countries have either eradicated or never reported disease. *Brucella melitensis* has never occurred in animals in Australia and *B. abortus* was eradicated as part of the Brucellosis and Tuberculosis Eradication Campaign (BTEC) with freedom declared in 1989 (AHA 2022c). To maintain this status, it is essential to prevent the introduction of the organisms in imported animals and their products.

Brucella abortus, B. melitensis and *B. suis* are reported in several hosts, including cattle, sheep, goats, pigs, camelids as well as several wild animal species. However, preferred hosts are cattle in the case of *B. abortus,* sheep and goats in the case of *B. melitensis,* and pigs for most biovars of *B. suis* (Godfroid et al. 2013; Godfroid, Nielsen & Saegerman 2010; Spickler 2023). Serological exposure to *Brucella* spp. has been reported in free living common hippos but is unknown in pygmy hippos (Condy & Vickers 1972; De Vos & van Niekerk 1969; Guilbride et al. 1962; Rollinson 1962). Differentiating between smooth *Brucella* species (such as *B. abortus*, *B. melitensis* and *B. suis*) using serology is difficult due to shared common major surface antigens (smooth lipopolysaccharide); as such, these species are assessed together.

Infection with *B. abortus, B. melitensis* and *B. suis* are listed diseases of multiple species in the WOAH Terrestrial Code (WOAH 2023a). *Brucella suis* is endemic in feral pigs in some parts of Australia whilst *Brucella abortus* and *B. melitensis* are not present in Australia*. B. abortus, B. melitensis* and *B. suis* are nationally notifiable animal diseases in Australia (DAFF 2022b), and brucellosis in humans is a nationally notifiable disease (Department of Health and Aged Care 2023). Both *B. abortus* and *B. melitensis* are category 2 diseases under the EADRA, with the potential to cause major national socio-economic consequences through trade losses, market disruptions and severe production losses (AHA 2022c). *Brucella suis* is not listed in the EADRA.

3.3.2 Technical information

Epidemiology

The most epidemiologically important time for transmission of *Brucella* species is around parturition. Females either aborting or giving birth to live young may shed large amounts of bacteria in birth products, including placenta, foetal fluids and vaginal discharges. Subsequent ingestion of water or food contaminated with this material, or licking of genitalia, newborns or aborted products results in transmission to new hosts (AHA 2022c; Diaz Aparicio 2013; Poester, Samartino & Santos 2013). Although females typically abort only in their first pregnancy following infection, subsequent

pregnancies may continue to contribute to high level shedding of bacteria in the periparturient period with repeated uterine infections (Diaz Aparicio 2013).

Infection may also be transmitted to young through ingestion of infected colostrum or milk or in-utero (Diaz Aparicio 2013). Intermittent shedding in faeces and urine is also known to occur in infected animals but these modes are considered of lesser epidemiological significance for natural transmission (AHA 2022c; Diaz Aparicio 2013). Following environmental contamination, survival may be prolonged under optimal conditions, including up to 77 days in water and 108-174 days in liquid manure dependent on temperatures and exposure to light (AHA 2022c). Zoonotic transmission typically occurs via exposure to fluid or tissue from infected animals or consumption of infected unpasteurised dairy products (Corbel 2006).

Following infection, bacteria typically persist and replicate in phagocytic cells of the reticuloendothelial system, localising in organs rich in these cells, and have a strong tropism for reproductive organs (Gonzalez-Espinoza et al. 2021). Infection may be cleared following the acute phase, or chronic infections may establish which may persist for life (Ahmed, Zheng & Liu 2016; Gonzalez-Espinoza et al. 2021). Incubation periods are variable but may be prolonged, and some latently infected animals may not seroconvert, complicating diagnosis (Godfroid 2018; Olsen & Palmer 2014). The capacity for latency, incubation periods and infection durations in most wildlife hosts remain unknown, but infection dynamics are presumed to follow those of domestic animals (Rhyan 2013).

Although several mammalian hosts may be infected with *Brucella* spp., the major reservoir of *B. abortus* is domestic cattle, whilst *B. melitensis* is primarily a disease of goats and sheep (Godfroid et al. 2013; Godfroid, Nielsen & Saegerman 2010). Pigs (domestic or wild) are the primary hosts for biovars 1, 2 and 3 of *B. suis*, whilst biovars 4 and 5 are primarily found in wild cervids and rodents respectively (Spickler 2023). Biovar 2 of *B. suis* is also maintained in wild European hares (*Lepus europaeus*) (Spickler 2023). Close contact through co-grazing or contaminated food and water resources may provide for spillover into non-preferred hosts. Although multiple wild mammal species have been documented with serological exposure to *Brucella* spp., it is broadly considered that most wild mammals are typically infected as spillover from domestic livestock, and that species outside the preferred host range rarely maintain infection independently of preferred hosts and are not considered of epidemiological importance (Olsen & Palmer 2014; Simpson et al. 2021). However, some wild artiodactyls, including African buffalo (*Syncerus caffer*), bison (*Bison bison*), elk (*Cervus canadensis*) for *B. abortus* and alpine ibex (*Capra ibex*) in the case of *B. melitensis* are considered sustainable reservoirs in limited geographical regions (Godfroid 2002; Mick et al. 2014; Simpson et al. 2021). The ability of wildlife to transmit infection depends on factors such as abortion rates, behaviours during parturition and management practices (Simpson et al. 2021).

Serological exposure to *Brucella* spp. has been reported in wild common hippos in several studies, based on the results of serum agglutination tests. Apparent seroprevalence in these studies has ranged from 5.6 to 45.5 per cent in Zimbabwe, South Africa and Uganda (Condy & Vickers 1972; De Vos & van Niekerk 1969; Guilbride et al. 1962; Rollinson 1962). The serum agglutination tests used in these studies are susceptible to several limitations. This includes the inability to distinguish between different smooth *Brucella* spp. and liability to non-specific cross reactions (Guilbride et al. 1962). Studies using molecular techniques or culture could not be identified. To date, no field studies in

pygmy hippos could be identified, nor were studies of other wildlife in west Africa where this species primarily resides (Simpson et al. 2021). There are no known records of infection in captive hippos, and a retrospective study of mortality of captive pygmy hippos did not detect brucellosis (Flacke et al. 2016). It has been suggested that the aquatic habitat of hippos may contribute to the high prevalence of seropositivity in hippos, with prolonged survival of *Brucella* spp. in water, although there is limited further evidence available investigating or supporting this hypothesis (De Vos & van Niekerk 1969). Despite probable susceptibility to infection, disease associated with *Brucella* spp. exposure in hippos could not be identified, and De Vos and van Niekerk (1969) noted an absence of abortions and high fecundity in seropositive hippo populations, indicating a lack of detectable effect on reproduction in this species. Some authors consider hippos to be of minor importance to the epidemiology of disease in free-range settings (Godfroid 2002).

Records of *B. abortus, B. suis* and *B. melitensis* in captive zoo animals in approved countries are infrequent. Amongst artiodactyls, records exist in some captive Bovidae, Suidae and Cervidae managed at various scales, but there are no identified records from zoo hippos (Kreeger et al. 2004; Lignereux et al. 2022; Sánchez Romano et al. 2019; Sutherland-Smith 2014). Exposure of zoo hippos and other non-domestic animals in captive settings in approved countries is presumably restricted by limited mixing with natural hosts and modern biosecurity practices.

Semen is considered a risk material for *B. abortus*, *B. melitensis* and *B. suis* by the WOAH for bovids, sheep, camelids, pigs and cervids (WOAH 2022a). These *Brucella* species have been isolated from semen of infected livestock and some wildlife (Amin, Harndy & Ibrahim 2001; Campero et al. 1990; Frey et al. 2013). Bacteria may localise in the testes, epididymis and seminal vesicle from where it may be shed intermittently into the semen (Campero et al. 1990). There have been no studies to examine the capacity of hippo semen to be infected with *Brucella* spp.

Diagnosis

Clinical signs

There are no reports of clinical signs of *Brucella* infection in common hippos in the literature. Infections in wildlife species may be asymptomatic, or animals may present with similar clinical signs to those seen in domestic animals (Godfroid et al. 2013; Rhyan 2013). In domestic livestock, brucellosis is characterised by abortion and orchitis. Infected females may be clinically well, with signs absent until late pregnancy where abortion, birth of weak offspring, retained foetal membranes, reduced milk yields and metritis may occur. Typically, abortion occurs during the first pregnancy following infection only. In males, infection may result in orchitis. Swollen joints may be seen in chronically infected animals (Godfroid et al. 2013).

Testing

Diagnosing *Brucella* spp. infections in both domestic livestock and wildlife is challenging, particularly at the individual level. There are few validated tests for wildlife, and no known validated tests for diagnosis in hippos.

Serology is the most common test applied to wildlife. Serological tests include enzyme-linked immunosorbent assays (ELISAs), buffered *Brucella* antigen tests (Rose Bengal and buffered plate agglutination tests), serum agglutination tests and complement fixation tests (WOAH 2022a). As immunodominant antigens for *B. abortus, B. melitensis* and *B. suis* biovars are associated with the surface smooth lipopolysaccharide that is common to all 3 species and some other species within the

Brucella genus, many serological tests can't distinguish between these species (Godfroid, Nielsen & Saegerman 2010). In addition, serological cut off values are largely extrapolated, duration of antibody persistence in wildlife species are largely unknown, and as per domestic animals, its assumed that many individuals may not seroconvert despite infection (Dadar et al. 2021; Godfroid 2002; Godfroid, Nielsen & Saegerman 2010; Simpson et al. 2021). Furthermore, several of these tests are known to have cross-reactivity with other bacteria such as *Yersinia enterocolitica* O:9 (WOAH 2022a). As such, results of serological tests in wildlife where validation has not taken place should be interpreted with caution.

Tests to directly detect *Brucella* spp. are also available, and these are required for confirmation of infection status and identification to the species level (Godfroid, Nielsen & Saegerman 2010). These tests include culture, microscopic examination or molecular techniques, but their application is primarily restricted to confirmation of clinical cases, with sequestration in organs and intermittent shedding impairing detection and may necessitate lethal sampling (Dadar et al. 2021; OIE 2018b).

All identified research documenting *Brucella* spp. exposure in hippos has been based on agglutination tests. The serum agglutination test is no longer considered adequate for international trade by the WOAH (WOAH 2022a). No research using other diagnostic tests could be identified, and it was recognised by some of the authors of this research that non-specific cross-agglutination reactions or species other than *B. abortus* or *B. melitensis* may be responsible for these results (Guilbride et al. 1962).

Treatment

Brucella species are susceptible to several antibiotics. However, due to the intracellular nature and predilection for certain tissues, *Brucella* infection may persist in lymph nodes and other tissues following antibiotic administration and antibiotics are therefore not recommended (Khurana et al. 2021; Spickler 2018b).

Control

Although there are vaccines available for domestic livestock, currently there is no vaccine which is considered safe and effective for brucellosis in wildlife species (Godfroid et al. 2013; Jamil et al. 2022). Prevention of brucellosis involves practices to reduce the likelihood of introduction of infected animals to a population. The AUSVETPLAN stipulates eradication through destocking, test and slaughter are likely to be initiated if *B. abortus* or *B. melitensis* were detected in Australia (AHA 2018, 2022c).

3.3.3 Current biosecurity measures

Current biosecurity measures for brucellosis for various other commodities (both live animals and animal products) include country freedom, testing requirements and thermal treatments of susceptible commodities (e.g. milk). The WOAH Terrestrial Code recommendations for various domestic livestock species include country, zone or population freedom and testing (OIE 2018b).

3.3.4 Risk review

The following key points are relevant to the biosecurity risk of *B. abortus*, *B. melitensis* and *B. suis* in live zoo hippos and their semen:

- *Brucella abortus, B. melitensis* and *B. suis* have not been reported in zoo hippos in approved countries and are infrequently reported in other species in zoos in approved countries.
- Zoo hippos are sourced from and maintained in facilities that are under veterinary supervision and have health monitoring programs to monitor and investigate events such as abortion.
- A wide range of wildlife species, including common hippos, have been reported as serological reactors to *Brucella* spp. Serological testing for *Brucella* spp. has several limitations in wildlife, and with few exceptions, most wild mammals are typically spillover hosts and are not considered of epidemiological importance.
- There are no records of culture, molecular detection or clinical signs of *Brucella* spp. or brucellosis in zoo hippos.
- Infection with *B. abortus, B. melitensis* and *B. suis* may persist for years in susceptible species.
- *Brucella abortus, B. melitensis* and *B. suis* are primarily transmitted through direct or indirect contact with infectious material, generally from organisms shed during the periparturient period.
- Zoo hippos typically do not mix with open herds of domestic livestock in Australia, but may be exposed to natural hosts or reservoir species in captivity.
- Semen is a risk material for *B. abortus, B. melitensis* and *B. suis.*
- There have been no studies to examine the capacity of hippo semen to be infected with *B. abortus, B. melitensis* and *B. suis*.
- Infection with *B. abortus, B. melitensis* and *B. suis* are listed diseases of multiple species in the WOAH Terrestrial Code.
- Brucellosis is a disease of significant economic and zoonotic importance. *B. abortus* was eradicated from Australia as part of the BTEC. *B. melitensis* has never been reported in Australia. *Brucella suis* is endemic in feral pigs in some regions of Australia.
- Both *B. abortus* and *B. melitensis* have the potential to cause major national socio-economic consequences through trade losses, market disruptions and severe production losses.

Based on the information above, the likelihood of entry and exposure for *B. abortus*, *B. melitensis* and *B. suis* in live zoo hippos and their semen is estimated as very low.

The likely consequences of the establishment and/or spread of *B. suis* is considered low.

The likely consequences of the establishment and/or spread of *B. abortus* and *B. melitensis* is considered high.

For the likelihood of entry and exposure of very low, combined with the likely consequence of establishment and spread of low using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with *B. suis* in live zoo hippos and their semen of negligible which achieves Australia's ALOP.

For the likelihood of entry and exposure of very low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted

risk associated with *B. abortus* and *B. melitensis* in live zoo hippos and their semen of low which does not achieve Australia's ALOP.

3.3.5 Conclusion

As brucellosis (*B. abortus* and *B. melitensis*) in live zoo hippos and their semen poses a low unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP. No measures are required for *B. suis*.

Australia's proposed disease-specific biosecurity measures for brucellosis (*B. abortus* and *B. melitensis*) for the import of live zoo hippos are:

1) For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of brucellosis (*B. abortus* and *B. melitensis*) has occurred in any species during the 5 years prior to export and the disease is compulsorily notifiable.

Australia's proposed disease-specific biosecurity measures for brucellosis (*B. abortus* and *B. melitensis*) for the import of zoo hippo semen are:

1) For 12 months immediately before each semen collection the donor did not reside on any premises where clinical, epidemiological or other evidence of brucellosis (*B. abortus* and *B. melitensis*) has occurred in any species during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

3.4 External parasites (excluding screwworm fly)

3.4.1 Technical information

Epidemiology

External parasites of hippos include ticks, botflies and leeches. These parasites may be either primary or opportunistic pathogens of hippos. The various external parasites that have been identified in hippos are detailed in Table 4.

Detections have mostly occurred in free-ranging hippos, while parasitism in captive hippos is uncommon (Miller 2003). Evidence of disease resulting from external parasites is rarely found in both captive and wild hippos (Miller 2003). The biology of ectoparasites affecting hippos is often unclear, including epidemiology, prevalence, host range and significance. None of the species listed below have been identified in Australia.

The spread of ectoparasites via movement of their hosts is a well-recognised pathway for transmission into new areas (Barre & Uilenberg 2009; Burridge & Simmons 2003; Spickler 2022a).

A number of exotic ticks have been identified on wild or captive hippos, including *Amblyomma tholloni*, *Rhipicephalus simus, Rhipicephalus microplus*, and *Amblyomma variegatum*. *A. tholloni* preferentially parasitises elephants, while *R. simus, R. microplus* and *A. variegatum* prefer cattle and other ruminants. In general ticks (and tick-borne pathogens) have are known to occur in zoo populations (Hrnková et al. 2021) and it is possible that other tick species may also parasitise hippos even if they are not preferred hosts. The listed tick species are distributed around the world, and aside from direct consequences of parasitism (skin damage and secondary infections) they may also act as vectors of endemic and exotic diseases of concern, including heartwater (Mackenzie & Norval

1980; Spickler 2022a; Walker & Olwage 1987). The causative agents of serious human diseases (such as severe fever with thrombocytopenia syndrome (SFTS) virus and various spotted fevers) have also been detected in these tick species (Parola et al. 2013; Seo et al. 2021). These ticks may survive for extended periods of time in the environment (Spickler 2022b) and can be spread to new locations when attached to hosts by natural or human-mediated movements.

Hippos are also subject to parasitism by leeches. Both *Limnatis nilotica* and *Placobdelloides jaegerskioeldi* have been identified in free ranging hippos (Guilbride et al. 1962; Oosthuizen & Davies 1994). There are few reports of *P. jaegerskioeldi* however it appears to be unique to the hippo, infesting the rectum of mature animals (Oosthuizen & Davies 1994). *L. nilotica* on the other hand is non-specific and has been identified on several mammal species, including humans, cattle, dogs, goats, sheep, donkeys, camels, and common hippos (Arfuso et al. 2019; Guilbride et al. 1962). Leeches typically deposit eggs in cocoons which are attached to some form of substrate. Once emerged, immature parasitic leeches attach to suitable hosts where the feed. *P. jaegerskioeldi* are unique and migrate into the rectum of a hippo to develop (Oosthuizen & Davies 1994). Leeches are also known to act as vectors for disease such as *Trypanosoma* spp.

The hippo bot fly (*Rhinoestrus hippopotami*) is another parasite of concern however there have been few studies published. The common hippo is the only known host with no reports in pygmy hippos (Papavero 1977). Female *Rhinoestrus* flies (family Oestridae) typically deposit eggs on around the nostrils of their hosts and larvae migrate into the nasal cavities to develop (Colwell, Hall & Scholl 2006)). Pathology in hippos is unknown, however *Rhinoestrus* spp. infecting horses can cause significant morbidity and mortality (Colwell, Hall & Scholl 2006).

Diagnosis

Diagnosis of external parasites can be challenging. A visual examination and parasite screen can determine whether external parasites are present, and microscopic examination can be used for identification. Thorough inspection may require animals to be sedated or anaesthetised (Miller 2003). Parasites such as hippo bot fly or *P. jaegerskioeldi* may require more invasive techniques such endoscopy or postmortem examination for diagnosis.

Treatment

A number of acaricidal treatments exist, including organophosphates, pyrethroids and avermectins. Ivermectin has been used to successfully treat nasal bot fly infestations in other species (e.g. horses) (Colwell, Hall & Scholl 2006). Several anthelmintics have been tested against leeches. Niclosamide, levamisole and ivermectin have demonstrated effectiveness against *Limnatis nilotica* but their effectiveness against *P. jaegerskioeldi* is unknown (Bahmani et al. 2013; Bahmani et al. 2014).

Captive hippos have been treated safely using ivermectin, coumaphos or organophosphate dips (Miller 2003).

3.4.2 Current biosecurity measures

Under standard zoo conditions, animals must be effectively treated for internal and external parasites during PEQ.

3.4.3 Risk review

The following key points are relevant to the biosecurity risk of external parasites in live zoo hippos and their semen:

- There are limited studies on external parasites of hippos.
- Captive hippos are capable of hosting external parasites which are exotic to Australia.
- Ticks harboured by hippos may have a wide host range. Other reported external parasites reported on hippos, excepting the leech *Limnatis nilotica*, appear to be host specific.
- Infestations of ectoparasites have been reported in zoos globally.
- Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.
- Semen is not a risk material for external parasites of hippos.
- Zoo hippos do not mix with open herds of domestic livestock in Australia but may mix with other animals in zoo enclosures. However, given the mobile nature of parasites, direct contact may not be necessary for transmission or establishment and spread of an exotic ectoparasite species.
- Some ticks are subject to restrictions within Australia (e.g. cattle tick zone), but none of the external parasites listed are nationally notifiable.
- External parasites have not been implicated in causing disease in hippos. However, they may cause disease in other species of animals. Some external parasites (ticks) may act as vectors for exotic animal and human diseases such as heartwater and SFTS.
- Inspection and treatment of external parasites is generally effective, simple, cheap and safe to the live animal.
- Hippos may be given parasiticides to eliminate external parasites. Treatments are well established for ticks, but not for other external parasites of hippos. *Limnatis nilotica* may be susceptible to some acaricides and anthelmintics.

Based on the information above, the likelihood of entry and exposure for external parasites in live zoo hippos is estimated as moderate.

Based on the information above, the likelihood of entry and exposure for external parasites in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of external parasites is considered moderate.

For the likelihood of entry and exposure of moderate, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with external parasites in live zoo hippos of moderate which does not achieve Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall

unrestricted risk associated with external parasites in zoo hippo semen of negligible which achieves Australia's ALOP.

3.4.4 Conclusions

As the introduction of external parasites in live zoo hippos poses a moderate unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP. No measures are required for zoo hippo semen.

Australia's proposed disease-specific biosecurity measures for external parasites for the import of live zoo hippos are:

1) Within 24 hours prior to entering PEQ:

    a) Each hippo was examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and found to be visibly free of external parasites.

    Note: examined in this context means that all visible surfaces of the animals have been examined. This may require sedation or anaesthesia.

    AND

    b) Each hippo was treated with an external parasiticide effective against ticks. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

AND

2) Between 24 to 72 hours prior to export, each hippo was treated with an external parasiticide effective against ticks. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

AND

3) If PEQ extends beyond 30 days, repeat treatments to maintain sufficient coverage against ticks throughout the PEQ period were applied. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

Table 4 Summary of external parasite biology identified in hippos

External parasite	Susceptible species	Distribution	Life cycle	Disease effects	Controlled by
Amblyomma tholloni (Elephant tick)	African elephant (*Loxodonta africana*), also common hippos, rhinoceros, giant forest hog (*Hylochoerus meinertzhageni*) and warthog (Guilbride et al. 1962; King'Ori et al. 2022).	Distribution is limited primarily by the distribution of African elephants (King'Ori et al. 2022). Not reported in Australia.	3-host tick, larvae and nymphs may be present on cattle, sheep and goats, also reptiles (Petney, Horak & Rechav 1987).	Direct parasitism effects; vector of heartwater (Mackenzie & Norval 1980; Walker & Olwage 1987).	Acaricides
Amblyomma variegatum (Tropical bont tick)	Ruminants; one report in a pygmy hippo (Mbaya et al. 2008).	Africa. Not reported in Australia.	3-host tick, wide variety of hosts (Petney, Horak & Rechav 1987).	Vector of heartwater and African tick-bite fever (humans) (Hoy 2011). Potential vector of several rickettsial diseases causing spotted fevers in humans (Parola et al. 2013).	Acaricides
Rhipicephalus simus	Adults preferentially parasitise cattle, though have been detected on common hippos (Guilbride et al. 1962; Lorusso et al. 2013).	Found in southern Africa, isolated cases elsewhere. Not reported in Australia.	3-host tick (Ledger et al. 2019).	Potential vector of some human spotted fevers and *Theileria parva* (Ledger et al. 2019; Lounsbury 1903; Parola et al. 2013).	Acaricides
Rhipicephalus microplus (cattle tick)	Ruminants. One case study of *R. microplus* in common hippos (Santos et al. 2019).	Parts of Asia, Africa, Central and South America (Spickler 2022b). Not reported in Australia (Australian cattle tick now defined as *R. australis*) (Barker & Barker 2023).	One-host tick. Larvae can survive 3 to 4 months without feeding in environment (Spickler 2022b).	Vector for the severe fever with thrombocytopenia syndrome virus in humans (SFTS) (Han et al. 2022; Spickler 2022b).	Acaricides

External parasite	Susceptible species	Distribution	Life cycle	Disease effects	Controlled by
Limnatis nilotica	Opportunistic parasite. Detected on humans, cattle, dog, goat, sheep, donkey, camel, and common hippos (Arfuso et al. 2019; Guilbride et al. 1962).	Occurs in southern Europe, Middle East and north Africa (Arfuso et al. 2019). Not reported in Australia.	Typical lifecycle of aquatic leeches involves eggs deposited inside a cocoon on a substrate. Juveniles hatch and require 3 to 5 blood meals to reach maturity (Rajaei et al. 2014).	Significant infestations of leeches may result in anaemia. Leeches can also act as vectors for other pathogens, including *Trypanosoma* species (Guilbride et al. 1962).	Anthelminthics levamisole and ivermectin have demonstrated efficacy in killing leeches in laboratory studies (Bahmani et al. 2013; Bahmani et al. 2014).
Placobdelloides jaegerskioeldi	Thought to be species specific to common hippos (Oosthuizen & Davies 1994; Rietmann & Walzer 2014).	Found in Africa (Oosthuizen & Davies 1994). Not reported in Australia.	Life cycle not well defined as there are few documented reports. Mating likely occurs within the rectum of the hippo. However, hatching and juvenile development occurs in the external environment. Juveniles are likely transported to the hippo by mature adults where they may feed and migrate into the rectum once again (Oosthuizen & Davies 1994).	No pathological effects of infestations with *P. jaegerskioeldi* have been identified. Leeches are known to cause anaemia and act as vectors for some pathogens.	No specific treatments described for *P. jaegerskioeldi*. Levamisole and ivermectin have shown some effectiveness against other leech species (Bahmani et al. 2013; Bahmani et al. 2014).
Rhinoestrus hippopotami (bot fly)	Common hippos (Papavero 1977).	Central Africa: Cameroon, Sudan, Democratic Republic of the Congo, Uganda (Papavero 1977). Not reported in Australia.	No reports of *R. hippopotami* life cycle found. General bot fly life cycle: Single host life cycle. Eggs are typically laid in or on the ground or on the host. Once larvae emerge, they are consumed, and migrate and develop within the host. Mature larvae exit the host, pupate in ground, and emerge as adult flies. Flies lay eggs onto the host or ground.	No pathological effects have been reported in hippos. Heavy bot fly infestations in other species may cause irritation in infected tissues.	Ivermectin is commonly used for bot fly treatments.

3.5 Foot-and-mouth disease

3.5.1 Background

Foot-and-mouth disease virus (FMDV) is a highly contagious RNA virus in the *Aphthovirus* genus of the *Picornaviridae* family. It is responsible for the WOAH listed disease of multiple species, foot-and-mouth disease (FMD) (WOAH 2023a). FMDV is recognised as primarily affecting members of the order Artiodactyla (even toed ungulates). However, several species outside of this order have been shown to be susceptible to infection and/or disease under natural and experimental conditions (Bunn 2013; Thomson, Vosloo & Bastos 2003). As hippos are artiodactyls, it may be assumed that they would be susceptible to FMDV but there are no known reports of disease in either species of hippo.

FMDV virus is endemic in much of Africa, the Middle East and Asia, with sporadic outbreaks in nearby regions (Brito et al. 2017). In 2022, FMDV spread through Southeast Asia to several islands in Indonesia (WOAH 2024). FMDV is not currently present in approved countries.

FMDV is not present in Australia and is a nationally notifiable animal disease (DAFF 2022b). Recent modelling has suggested a large multistate outbreak of FMDV in Australia would cost the Australian economy $80 billion over 10 years (DAFF 2022a). FMDV is listed as a category 2 disease in the EADRA (AHA 2022b). Any report of FMDV in Australia, including in a zoo setting, would be expected to have an immediate impact on the Australian livestock industry through disruption to trade and animal movement.

3.5.2 Technical information

Epidemiology

There are 7 identified serotypes of FMDV which are serologically and immunologically distinct, with little cross-protection between serotypes and sometimes limited cross-protection between strains within a serotype (Jamal & Belsham 2013; Paton, Valarcher & Bergmann 2005). In addition, different strains and serotypes may vary in their infectivity for different species (Donaldson 1998). Several FMDV pools have been identified across endemic regions, each comprising of predominant serotypes and strains within distinct geographical areas (Brito et al. 2017). Hippo natural ranges overlap with several pools.

FMDV is found in ruptured vesicular fluid, and all body secretions or excretions, including respiratory aerosols, milk and semen of an infected animal (Alexandersen et al. 2003; Paton, Gubbins & King 2018). Transmission may occur via direct or indirect contact with infected animals or contaminated materials. Entry pathways include inhalation, ingestion or entry through cuts and abrasions of the skin or mucosa (Alexandersen et al. 2003; Paton, Gubbins & King 2018). The virus may remain infective in the environment for months in the environment under optimal conditions and can survive in water for up to 50 days (AHA 2022d; Bartley, Donnelly & Anderson 2002).

Reported incubation periods are variable, dependent on species, infectious dose, route and strain, and can range from 1 to 21 days in domestic animals while the WOAH Terrestrial Code defines it as 14 days (Alexandersen et al. 2003; Graves et al. 1971; OIE 2015b), and with recovery generally within 1 to 2 weeks. A carrier-state, with persistence of virus in the oropharynx for more than 28 days may occur in some animals (OIE 2015b). However, with the exception of African buffalo, there is no

evidence of wildlife carriers transmitting FMDV to other susceptible animals (Dawe et al. 1994; Sutmoller & Casas 2002; Weaver et al. 2013).

As artiodactyls, hippos may be susceptible to FMDV infection though there is limited evidence to support this (Bengis & Erasmus 1988; Dudley et al. 2015; Schaftenaar 2002; Thomson, Bengis & Brown 2008; Thomson, Vosloo & Bastos 2003). In southern Africa, serological surveys have not detected evidence of exposure in common hippo, including an unpublished study cited in Thomson, Vosloo & Bastos (2003) of serum from 877 hippos in Kruger National Park (Condy, Herniman & Hedger 1969; Overby & Zyambo 1983; Thomson, Vosloo & Bastos 2003). Pinto (2004) states hippos are susceptible to infection with FMDV experimentally, however primary literature could not be identified to support this. Records of a seropositive common hippo from west and central Africa were considered a false positive on the basis of failure to detect FMDV in this species in previous studies (Di Nardo et al. 2015). There are no known reports of infection or disease in common hippos during FMD epidemics in southern Africa. However, aside from Di Nardo et al (2015), research on common hippos outside of southern Africa is lacking, and no targeted FMDV research has been conducted on pygmy hippos. Common hippos are likely to only play a minor role, if any, in the epidemiology of FMD epidemics in southern Africa.

Although infrequent outbreaks have been reported, FMDV infection in non-domestic species within zoos are rare (Schaftenaar 2002). Although most wildlife are largely considered spillover hosts from FMDV infection in domestic livestock, and zoo animals have limited contact with domestic livestock herds, there are reports of zoo animals being infected during FMD outbreaks within Europe and Asia (Bunn 2013; Officer et al. 2014; Schaftenaar 2002; Weaver et al. 2013). Transmission sources were postulated to include personnel or visitors working with infected animals, but also contaminated feed, equipment or the introduction of infected animals (Schaftenaar 2002).

Semen is considered a risk material for FMDV in domestic livestock, with demonstrated transmission via artificial insemination and survival following frozen storage (Cottral, Gailiunas & Cox 1968; OIE 2015b). There are no known studies investigating FMDV transmission via hippo semen.

Diagnosis

Clinical signs and pathology may be suggestive but are insufficient for diagnosis. Laboratory testing is required for confirmation of diagnosis.

Clinical signs

Clinical signs of FMD specific to infection in hippos are unknown. Clinical signs in non-domestic and zoo artiodactyls are similar to those seen in domestic livestock and include anorexia, pyrexia, lameness and vesicles on oral and nasal mucous membranes and feet. Disease presentation may vary with age with higher mortality rates associated with cardiac involvement observed in young animals (Alexandersen et al. 2003; Arzt et al. 2011).

Testing

Laboratory diagnosis may be achieved through virus isolation or detection of FMDV nucleic acids or antigen in clinical specimens via various methods, including ELISAs and RT-PCRs (WOAH 2022b). Several serological assays have also been developed to indicate exposure, including virus neutralisation tests and ELISAs, some of which may be serotype specific (WOAH 2022b).

Treatment
There are no specific treatments for infection with FMDV (Spickler 2021).

Control
Countries that are free from FMD typically restrict import of products and live animals susceptible to FMDV. Control in endemic areas or outbreaks may include the use of vaccinations. Vaccinations do not provide complete protection against infection and provide limited to no cross-protection between strains and/or serotypes (Kamel, El-Sayed & Castaneda Vazquez 2019; Rodriguez & Grubman 2009).

3.5.3 Current biosecurity measures

Australia's biosecurity measures for FMDV across multiple commodities include country freedom. The WOAH Terrestrial Code recommendations for susceptible animals include country or zone freedom and testing.

3.5.4 Risk review

The following key points are relevant to the biosecurity risk of FMDV in live zoo hippos and their semen:

- FMD is a highly significant exotic viral disease which affects a broad range of artiodactyl species.
- Evidence of common and pygmy hippo susceptibility to disease and/or infection is limited. There have been no reports of FMD in hippos nor substantive evidence of FMDV infection in hippos and, with few exceptions, wildlife and zoo artiodactyls are largely considered spillover hosts due to outbreaks in domestic livestock.
- Registered or licenced zoos within the scope of this review will have health monitoring programs and are under veterinary supervision.
- FMDV may be transmitted by direct or indirect contact and may survive in the environment for an extended period.
- A carrier state has been documented, but with the exception of African buffalo, there is no evidence of other wildlife species being persistently infected and transmitting FMDV to other susceptible animals.
- Zoo hippos do not mix with open herds of domestic livestock in Australia, but may mix with other susceptible animals in captivity.
- There is evidence that FMDV can be transmitted via semen in other species.
- FMD is a disease of significant economic concern to Australia. A largescale outbreak of FMD in Australia has been estimated to cost the economy $80 billion over 10 years.
- Any report of FMDV in Australia, including in a zoo setting, would have an immediate and widespread impact on the Australian agricultural industry.

Based on the information above, the likelihood of entry and exposure for foot-and-mouth disease in live zoo hippos is estimated as negligible.

Based on the information above, the likelihood of entry and exposure for foot-and-mouth disease in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of foot-and-mouth disease is considered extreme.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of extreme using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with foot-and-mouth disease in live zoo hippos and their semen of very low which achieves Australia's ALOP.

3.5.5 Conclusion

Accordingly, based on the preceding information disease specific risk management measures for foot-and-mouth disease in live zoo hippos or their semen are not warranted.

3.6 Haemorrhagic septicaemia (*Pasteurella multocida* serotypes 6:B & 6:E)

3.6.1 Background

Haemorrhagic septicaemia (HS) is a highly lethal disease of cattle and water buffalo, caused by the B2 and E2 serotypes (Carter and Heddleston system; equivalent to 6:B and 6:E in Namioka-Carter system) of the bacterium *Pasteurella multocida,* a gram-negative anaerobic bacterium belonging to the family *Pasteurellaceae*. HS is widespread, being found in tropical and subtropical regions, including South-East Asia, India, the Middle East, regions of Africa, and southern and central Europe (OIE 2021a; Völker et al. 2014). Outbreaks of septicaemic pasteurellosis in wild and captive ruminants caused by the other *P. multocida* serotypes have been reported in countries free from HS, including Australia (Carrigan et al. 1991; OIE 2021a).

HS is a WOAH-listed disease (WOAH 2023a). HS is not present in Australia and is a nationally notifiable disease (AHA 2023; DAFF 2022b). HS is a category 4 disease in the EADRA with the potential to cause severe economic and production losses (AHA 2022b).

3.6.2 Technical information

Epidemiology

Pasteurella multocida is a pathogen causing a wide range of diseases, including HS, fowl cholera and atrophic rhinitis of swine (Almoheer et al. 2022). There are 2 forms of HS – the Asian form, caused by serotype B2, and the African form caused by serotype E2. Outbreaks of similarly appearing clinical disease associated with other subtypes of *P. multocida* have also been reported and may be referred to as septicaemic pasteurellosis, particularly in wild ungulates (Spickler 2019a). Outbreaks of HS occur sporadically and tend to be associated with high levels of physiological stress in hosts. *P. multocida* is shed in respiratory aerosols, saliva, urine, faeces and milk by both active carriers and clinical cases (Annas et al. 2014a). Transmission is through direct contact with infected animals, or indirect methods such as exposure to infected fomites or aerosols. Contaminated feed and vectors such as ticks and biting insects have also been implicated in transmission (Radostits et al. 2007). Close contact between animals is generally required for transmission by ingestion or inhalation. HS-causing strains of *P. multocida* have been identified in many tissues of clinically affected animals (Annas et al. 2014b; Bastianello & Jonker 1981; Khin, Zamri-Saad & Noordin 2010; Lane et al. 1992) and in the respiratory, gastrointestinal and urinary tracts of carrier animals (Annas et al. 2014a). Moist environmental conditions may prolong survival and the bacteria may persist in animal carcases for a few days (de Alwis 1999).

Epidemic outbreaks have been associated with high morbidity and mortality rates (OIE 2021e; Shivachandra, Viswas & Kumar 2011). HS has occurred in zoo populations of deer, zebra (*Equus quagga*), eland and *Bos* spp. with obvious clinical signs, including death, but overall the disease is rarely reported in zoos (Eriksen et al. 1999; Happy et al. 2013; Okoh 1980; Vellayan & Jeferry 2014).

The incubation period is generally 2 to 5 days but experimentally may be as short as a few hours (Spickler 2019a). Carrier status is an important epidemiological feature of HS (Annas et al. 2014a; de Alwis 1999). Not all animals are carriers and the number of carriers declines over the months following an outbreak. By 6 months the carrier rate is 5% or less (Spickler 2019a). Maximum duration of the carrier state is not known but up to 12 months has been reported (de Alwis 1999). Carriers may be latent or active. Latent carriers harbour the bacteria in the tonsils but are not known to shed the bacteria (Annas et al. 2014a; Bastianello & Henton 2004). Active carriers harbour the bacteria in the tonsils and nasopharynx and may actively secrete the organism for up to 6 weeks before returning to a latent state. Physiological stress is another important epidemiological feature (for example, concurrent disease, poor nutrition, high stocking density), and is known to induce latent carriers to become active and shed the bacteria (Moustafa et al. 2015). Young animals are more susceptible to disease than adults (Benkirane & De Alwis 2002).

Explosive outbreaks may occur in herds that have minimal immunity. Fatal epidemics of HS are reported in Kazakhstan in free-ranging saiga antelope (*Saiga tatarica*), resulting in deaths of tens of thousands of individuals within days. One significant epidemic occurred in the northern summer of 2015 resulting in the death of approximately 200,000 saiga antelope, and believed to be exacerbated by stress due to seasonal calving and adverse weather conditions (Fereidouni et al. 2019; Zhusypbvovich et al. 2016).

HS has been described in wild mammals, and either reported or suspected in African buffalo, bison, pigs, goats, sheep, eland, yak (*Bos grunniens*) and saiga antelope (Almoheer et al. 2022; Okoh 1980; Rimler 1992). HS has also been reported in a wide range of non-Bovidae species, including camels, cervids, donkeys, horses, pigs, elephants and poultry, although some of these may have been due to B1 or B3,4 serotypes (Bastianello & Henton 2004; de Alwis 1999). Cases of HS in hippos are unclear. An unknown *Pasteurellaceae* was associated with stillbirth of one captive pygmy hippo (deMaar, LaFrentz & Garner 2021) and *P. multocida* was associated with a case of enterotoxaemia and septicaemia and death in one captive pygmy hippo (Flacke et al. 2016), however the serogroup involved was not listed. No reports were found of HS in the common hippo, although an unknown *Pasteurella* sp. was associated with sudden death in a captive common hippo (Thiruthalinathan et al. 1996), and *P. multocida* has been detected in a case of septicaemia and death in a common hippo calf, but the serotype involved was not reported (Sridhar et al. 1993).

Scott Williams Consulting Pty Ltd (2017) did not locate any records of *P. multocida* (B:2 and E:2) being present in or transmitted by semen in cattle, and no evidence of presence or transmission in semen was located in the literature for domestic or wild artiodactyls.

Diagnosis

Diagnosis of HS is based on clinical signs, gross lesions, and patterns of morbidity and mortality, and confirmation requires isolation and characterisation of the pathogen (OIE 2021a)

Clinical signs

Infection may present as peracute, acute or subacute disease. Variable clinical signs are associated with HS, ranging from pyrexia, respiratory distress, nasal discharge and dependent oedema in the submandibular or brisket regions, to recumbency and sudden death. Peracute infection is characterised by sudden death. Acute and subacute infections are characterised by fever, anorexia, depression, profuse salivation and nasal discharge (Chung et al. 2015). Clinical signs may last from 1 to 10 days before the animal dies. Chronic clinical disease is not known to occur and survivors may become active or latent carriers (OIE 2021e; Spickler 2019a).

Testing

Limitations exist with diagnostics that rely on isolation of the causative agent as septicaemia occurs only at the terminal stages of the disease. The organism is also not consistently present in the nasal secretions or body fluids of sick animals (OIE 2021a). Serological tests are most commonly used to evaluate antibody response to vaccination rather than diagnostics, although carriers are reported to have high antibody levels (Bastianello & Henton 2004). Highly sensitive and specific surveillance results can be achieved using the indirect-ELISA with capsular antigens on serum or nasopharyngeal swabs (Afzal, Muneer & Akhtar 1992; Dawkins et al. 1990; Dziva et al. 2008; El-Jakee et al. 2016; Kharb 2015; Takada-Iwao et al. 2007).

Treatment

Antibiotic treatment may be effective if started in the earliest phase of disease.

Control

When administered at a herd level, vaccination is effective at reducing the incidence of disease (Zamri-Saad & Annas 2016). Differentiating infected from vaccinated animal markers and assays are in development for HS vaccines (Qureshi 2014; Qureshi & Saxena 2017).

Despite extensive work in cattle and bison, currently available vaccines have variable efficacy and duration (Khan et al. 2011; Qureshi & Saxena 2017; Tabatabaei et al. 2007). Live attenuated (Ahmad et al. 2014; Hodgson et al. 2005; Tabatabaei et al. 2007) and recombinant vaccines (Qureshi 2014) are available; live attenuated intranasal vaccines for B2 & E2 types are available and effective but may result in auto-vaccination of other in-contact animals (Rafidah et al. 2012).

No reports were found of vaccination of hippos against HS.

3.6.3 Current biosecurity measures

The WOAH Terrestrial Code does not stipulate biosecurity measures applicable to zoo hippos or their semen for HS. For live cattle and buffalo, the WOAH Terrestrial Code recommends country or zone freedom, or a combination of examination, testing and vaccination (OIE 1992). Australia has HS specific biosecurity requirements for the import of live animals, including live zoo bovids and live zoo elephants. Measures for live zoo bovids and live zoo elephants include country freedom or premises freedom and vaccination. No HS specific biosecurity measures are required for zoo bovid semen.

3.6.4 Risk review

The following key points are relevant to the biosecurity risk of HS in live zoo hippos and their semen:

- HS is endemic in tropical and subtropical regions, including South-East Asia, India, the Middle East, regions of Africa, and southern and central Europe.

- HS most commonly affects cattle and water buffalo but may have a wide host range. *Pasteurella* spp. and *P. multocida* (untyped) have been detected in both species of hippo, but without confirmation of the specific species or serotype it is unknown if those were serotypes specifically linked to HS.
- HS has occurred infrequently in zoos. Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.
- A carrier state is known to exist but declines following infection. By 6 months the carrier rate is 5 percent or less. Carriers that undergo sufficient stress become active shedders. Active carriers harbour the bacteria in the tonsils and nasopharynx and may actively secrete the organism for up to 6 weeks before returning to a latent state.
- Pre-export quarantine and post-arrival quarantine periods are required for live zoo hippos.
- Transmission may occur through several methods, including close exposure to infected animals, and exposure to infected fomites, feed, vectors and aerosols. However, close contact between animals is generally required for transmission by ingestion or inhalation.
- There is no evidence that semen poses a risk for transmission of HS between hosts.
- Zoo hippos do not mix with open herds of domestic livestock in Australia, although may be held with other susceptible species in mixed exhibits.
- HS infection is acute with high mortality and morbidity rates. Naïve herds experience significant mortality and would likely be detected.
- HS is a nationally notifiable animal disease and listed as category 4 disease in the EADRA. An uncontrolled outbreak in Australia would cause significant production losses in livestock industries and loss of export markets.

Based on the information above, the likelihood of entry and exposure for haemorrhagic septicaemia in live zoo hippos is estimated as very low.

Based on the information above, the likelihood of entry and exposure for haemorrhagic septicaemia in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of haemorrhagic septicaemia is considered moderate.

For the likelihood of entry and exposure of very low, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with haemorrhagic septicaemia in live zoo hippos of very low which achieves Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with haemorrhagic septicaemia in zoo hippo semen of negligible which achieves Australia's ALOP.

3.6.5 Conclusion

Accordingly, based on the preceding information, disease specific risk management measures for haemorrhagic septicaemia in live zoo hippos or their semen are not warranted.

3.7 Infectious bovine rhinotracheitis and infectious pustular vulvovaginitis

3.7.1 Background

Varicellovirus bovinealpha1 (Bovine alphaherpesvirus-1) (BHV-1) is a member of the *Alphaherpesvirinae* subfamily within the *Orthoherpesviridae* family of viruses. It is primarily a pathogen of domestic cattle (*Bos* spp.), responsible for the WOAH listed-diseases of Bovidae: infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) (WOAH 2023a). BHV-1 has also been detected in several other domestic and wild artiodactyls suggesting a broader range of competent hosts. Several studies have identified a high prevalence of BHV-1 antibodies in common and pygmy hippos (Doyle & Heuschele 1983; Hedger & Hamblin 1978; Kaminjolo & Paulsen 1970; Rampton & Jessett 1976).

BHV-1 and the diseases IBR and IPV occur worldwide, including in Australia. However, evidence indicates that the highly virulent and abortigenic strains of BHV-1 found in many approved countries, where they cause significant economic loss, are not present in Australia (AHA 2023; Graham 2013; Gu & Kirkland 2003). Some approved countries, including Denmark, Finland, Sweden and Austria, have eradication programs or have eradicated BHV-1 (Iscaro et al. 2021; WOAH 2024). IBR and IPV are not notifiable animal diseases in Australia and not classified in the EADRA (AHA 2022b; DAFF 2022b).

3.7.2 Technical information

Epidemiology

BHV-1 primarily causes respiratory disease (IBR), genital disease (IPV) and abortions. The virus can be divided into 3 subtypes BHV-1.1, BHV-1.2a and BHV-1.2b. Only strains of subtype BHV-1.2b have been identified in Australia (AHA 2023). BHV-1.2b is not considered abortigenic and is broadly regarded as less pathogenic than subtype BHV-1.1 and BHV-1.2a, typically resulting in subclinical or mild clinical signs (Gu & Kirkland 2003; Muylkens et al. 2007). Although subtype disease associations have been reported, with BHV-1.1 associated with respiratory disease (IBR) and BHV-1.2 with genital disease (IPV), these are not exclusive associations, and route of infection may be more important in determining disease presentation (Gu & Kirkland 2003; Muylkens et al. 2007; Nettleton & Russell 2017). BHV-1 associated disease is rare in Australia due to presence of mild strains of BHV 1.2b and the predominance of pasture-based grazing systems (AHA 2016).

BHV-1 transmits primarily via direct mucosal contact (typically via the upper respiratory tract and genitals) with large amounts of virus shed in nasal secretions, vaginal secretions and semen (Muylkens et al. 2007; Nettleton & Russell 2017). The virus is moderately stable in the environment and may transmit via fomites, including feed and equipment on which it can remain viable for several weeks depending on environmental conditions (Gibbs & Rweyemamu 1977). Aerosol spread may also occur within a herd, but this is limited to short distances (within metres) (Mars et al. 2000).

In domestic cattle, incubation periods are typically 2 to 6 days, but longer periods have been identified (Jones & Chowdhury 2010; Muylkens et al. 2007). The WOAH Terrestrial Code defines the

incubation period for IBR and IPV as 21 days in cattle (OIE 1998a). Infective virus is shed for 5 to 14 days during acute respiratory infections, after which it establishes a life-long latent infection in the ganglia of the trigeminal nerve or, for genital infections, in the sacral spinal ganglia in natural hosts (Gu & Kirkland 2008; OIE 2017b; Winkler, Doster & Jones 2000). BHV-1 may also develop latent infections in lymphoid tissue (Winkler, Doster & Jones 2000). Periodic reactivation of latent infections may occur under periods of stress such as transport, corticosteroid treatments or parturition, leading to intermittent shedding throughout life. The virus is highly contagious and introduction of an infected animal carrying virulent strains into a naïve herd can result in 100% morbidity (Wiseman et al. 1980).

The natural hosts of BHV-1 are domestic cattle, but the virus has a demonstrated capacity to infect a broader range of artiodactyls. Aside from cattle, natural infections of BHV-1 have been detected in both domestic and wild hosts, including roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), mink, ferrets, Indian gazelle (*Gazella bennettii*), sheep, goats, water buffalo and pigs (Crandell, Schwartz & Lawhorn 1987; Fusco et al. 2015; Hemmatzadeh et al. 2016; Kálmán & Egyed 2005; Porter, Larsen & Cox 1975). Some early reports of BHV-1 detection in wildlife have since been reclassified as other closely related species of herpesvirus such as bubaline herpesvirus 1 (Gu & Kirkland 2008; Scheffer et al. 2017; St George & Philpott 1972). In addition to DNA detection or virus isolation, BHV-1 antibodies have been detected in a wide range of hosts, including elephants, capybaras (*Hydrochoerus hydrochaeris)*, mink, ferrets, African buffalo, deer, antelope, South American tapir (*Tapirus terrestris*), white rhinoceros (*Ceratotherium simum*), and giraffe (Anderson & Rowe 1998; Barnard 1998; Bhat, Manickam & Kumanan 1997; Hedger & Hamblin 1978; Medici, Mangini & Fernandes-Santos 2014; Rampton & Jessett 1976). However due to serological cross-reactivity, interpretations of host-capacity for BHV-1 infection are likely limited in the absence of virus isolation or molecular testing (EFSA 2017; Pastoret et al. 1988).

Serological evidence of exposure to BHV-1 or a closely related virus has been demonstrated in both common and pygmy hippos. Thirty-two of 79 common hippos sampled in the Luangwa Valley, Zambia, 181 of 188 common hippos in Uganda and 64 of 70 common hippos from various southern African states had demonstrable antibodies to BHV-1 on the basis of virus neutralisation tests following heat inactivation (Hedger & Hamblin 1978; Kaminjolo & Paulsen 1970; Rampton & Jessett 1976). Hedger and Hamblin (1978) note similar neutralisation titres in hippos to cattle in the region, although no clinical signs were associated with any animal in the study. Seropositivity has also been noted in a single pygmy hippo from a captive collection in the United States (Doyle & Heuschele 1983). BHV-1 is not known to have been isolated or detected via molecular methods from hippos.

Infection with other related *Alphaherpesvirinae* cannot be excluded on the basis of the serological results reported in hippos. Cross-reactivity has been hypothesised to account for the widespread detection of BHV-1 seropositive responses in many wildlife species (Ek-Kommonen, Pelkonen & Nettleton 1986; Pastoret et al. 1988; Thiry et al. 2006). To date, no *Alphaherpesvirinae* are known to have been detected in hippos based on isolation or molecular work which could account for cross-reactivity, although antibodies to BHV-2 have also been identified in common hippos (Plowright & Jessett 1971). With evidence limited to serological detection alone, there is uncertainty as to whether hippos can be infected with BHV-1 or if they may play a role in disease epidemiology.

The capacity for viral latency, reactivation and shedding titres are important considerations in determining the role of hosts in transmission of BHV-1 (EFSA 2017). Aside from cattle, few species have a demonstrated capacity to act as latent carriers of BHV-1. Latency with reactivation of shedding has been demonstrated to a variable extent in sheep and goats (Engels et al. 1992; Hage et al. 1997; Six et al. 2001). In contrast, experimental studies of cervid species have indicated despite infection following high challenge, shedding of virus occurs at low titres and there is no evidence of latent infections forming in reindeer (*Rangifer tarandus*) and red deer (Thiry et al. 2006). Detection of viral DNA in water buffalo suggests the capacity for latency in this species, but reactivation could not be demonstrated experimentally (Scicluna et al. 2010), and similarly latency of BHV-1 or a similar virus has been demonstrated in wildebeest although transmission to sentinel steers could not be demonstrated (Karstad et al. 1974; Mushi & Karstad 1979; Mushi et al. 1979). However, field studies suggest water buffalo herds may be of epidemiological importance in some regions (Fusco et al. 2015). Overall, current evidence indicates that despite the capacity to infect a broader host range, the only evidence for potential epidemiological importance in BHV-1 transmission is restricted to species within the Bovidae family, although the role of hippos has not been examined (EFSA 2017; Muylkens et al. 2007; Nettleton & Russell 2017).

Serosurveys of zoo animals have indicated rare exposure to BHV-1 or related viruses. Seropositivity has been detected in several captive artiodactyls, including deer, antelopes, giraffe and pygmy hippos, including in approved countries (Doyle & Heuschele 1983; Frolich et al. 2006; Mahmoud 2015; Probst, Speck & Hofer 2011). BHV-1 is not known to have been isolated or detected through molecular methods from captive zoo animals in approved countries, nor is there known evidence of disease associated with serological detections.

Semen is a known risk material for BHV-1 transmission, with several studies indicating the presence and transmission of viable virus in cattle semen, including frozen semen, and that shedding in semen may occur prior to development of an antibody response (van Oirschot 1995). There is no data on pathogens in semen of hippos.

Diagnosis

Clinical signs

Clinical disease has not been recognised in common or pygmy hippos nor in wildlife with the exception of wild wildebeest administered steroids when bought into captivity that developed pustular lesions on genitalia (Karstad et al. 1974). In cattle, many infections are subclinical, but where signs occur, they are typically manifested as an acute respiratory and/or venereal disease. This includes rhinitis with vesicles, pustules or erosions of the nasal mucosa or genitalia, conjunctivitis, tracheitis, fever, drop in milk production, with disease sometimes complicated by secondary bacterial infections leading to pneumonia, particularly in intensely managed cattle. BHV-1.1 and BHV-1.2a are also abortigenic, and strains of BHV-1.1 are associated with more severe clinical signs (Graham 2013; Jones & Chowdhury 2010; Muylkens et al. 2007). Signs typically resolve within 2 weeks in the absence of secondary complications (Jones & Chowdhury 2010; Nettleton & Russell 2017).

Testing

Clinical signs are not pathognomonic and may be absent, necessitating laboratory diagnosis. PCR on nasal or genital swabs is preferred for diagnosis of acute cases, but these have limited suitability to confirm freedom of infection due to latency (OIE 2017b). PCR enables differentiation from other antigenically related herpesviruses and can be applied to semen (Gu & Kirkland 2008; OIE 2017b).

Serological methods to identify sub-clinically infected animals include ELISAs and viral neutralisation tests. Some of these tests are known to display serological cross-reactivity with other *Alphaherpesvirinae* species and are not sufficiently discriminatory to enable species identification due to shared common antigens (Lyaku, Nettleton & Marsden 1992; Martin et al. 1990; Nixon, Edwards & White 1988; OIE 2017b; Pastoret et al. 1988; Thiry et al. 2006). Seroconversion following acute infection typically takes 7 to 14 days in cattle and antibody responses may disappear during latency (OIE 2017b).

Treatment
There are no specific treatments for BHV-1 infection in animals.

Control
Vaccines are available for domestic cattle. Live attenuated and killed vaccines are not fully efficacious against infection, but aid in reducing clinical signs, viral shedding and reduce transmission (OIE 2017b). Quarantine with antibody testing to confirm freedom can be utilised to prevent introduction of latently infected cattle into a herd (Nettleton & Russell 2017).

3.7.3 Current biosecurity measures

The WOAH Terrestrial Code does not stipulate biosecurity measures applicable to zoo hippos or their semen for BHV-1. For live cattle and their semen, the WOAH Terrestrial Code recommendations include combinations of herd freedom, examination, testing and vaccination. Australia's biosecurity measures for BHV-1 in zoo bovids includes lifetime residency with country freedom, premises freedom with individual testing, or premises freedom with herd-screening programs in place. In addition, zoo bovid semen may be tested by PCR.

3.7.4 Risk Review

The following key points are relevant to the biosecurity risk of BHV-1 in live zoo hippos and their semen:

- BHV-1 and the diseases IBR and IPV occur worldwide, including in Australia.
- The highly virulent and abortigenic strains of BHV-1 found in many approved countries, where they cause significant economic loss, are not present in Australia.
- BHV-1 endemic strains present in Australia (BHV-1.2b) are not subject to regulatory control. Therefore, only the risk associated with exotic strains (BHV-1.1 and BHV-1.2a) will be considered further.
- BHV-1 is primarily a disease of domestic cattle. A range of non-domestic species may be infected, though their epidemiological importance is largely thought to be limited outside of the Bovidae family.
- Natural hosts of BHV-1 may become lifelong latent carriers of BHV-1, with periodic reactivation of infection leading to intermittent viral shedding. This may be precipitated by periods of stress.
- Serological evidence of BHV-1 infection has been widely detected in common hippos in range states, without signs of disease. There is one report of BHV-1 seropositivity in a captive pygmy hippo with no reports of disease. However, positive test results could be due to cross-reactions with antibodies to other alphaherpesviruses, of which there are a number that affect various

artiodactyl species. There is no evidence that common or pygmy hippos play an epidemiological role in BHV-1 transmission.

- Semen is a known risk material for BHV-1 transmission, with several studies indicating the presence and transmission of viable virus in cattle semen. There are no studies on pathogens that can be transmitted by zoo hippo semen.
- Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.
- Transmission is primarily via direct contact, with limited aerosol and fomite spread.
- Zoo hippos do not mix with open herds of domestic livestock in Australia. However other wildlife species could share enclosures with hippos, including zoo bovids.
- BHV-1 is a WOAH listed disease of Bovidae that can cause serious production losses.

Based on the information above, the likelihood of entry and exposure for exotic strains of BHV-1 in live zoo hippos is estimated as extremely low.

Based on the information above, the likelihood of entry and exposure for exotic strains of BHV-1 in zoo hippo semen is estimated as extremely low.

The likely consequences of the establishment and/or spread of exotic strains of BHV-1 is considered moderate.

For the likelihood of entry and exposure of extremely low, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with exotic strains of BHV-1 in live zoo hippos or in zoo hippo semen of negligible which achieves Australia's ALOP.

3.7.5 Conclusion

Accordingly, based on the preceding information, disease specific risk management measures for BHV-1 in live zoo hippos or their semen are not warranted.

3.8 Internal parasites

3.8.1 Technical information

Epidemiology

Internal parasites of concern in hippos include nematodes (roundworms), cestodes (tapeworms), trematodes (flukes) and monogeneans. Several of these helminths have been identified in free-ranging common hippos, but there is limited information about the internal parasites of pygmy hippos (Table 5). Some of these helminths have been implicated in disease in hippos and/or other species, but most have been incidental findings. Additionally, although some of these helminths have been identified in other animals in Australia, many are currently considered exotic.

The biology of helminths identified in hippos remain largely unknown, including life cycles, host ranges and health impact (Table 5). Additionally, there is little information available on helminth diversity or burdens in zoo hippos, although parasitism is not considered to be a common clinical problem in captive hippos (Miller 2003). The only known helminth recorded in pygmy hippos is an unspecified hookworm from a captive collection in west Africa (Flacke & Decher 2019). Few studies

are reported using molecular taxonomic approaches in hippos, and as such species identification and host ranges may be tentative.

Trematodes are particularly common in free-ranging common hippos, presumably due to their aquatic habitat (Junker, Horak & Penzhorn 2015). This includes *Fasciola* spp., which cause important production limiting diseases of livestock and have been associated with cholangitis with bile duct abscesses or calcification in free-ranging common hippos (Cowan, Thurlbeck & Laws 1967; McCully, van Niekerk & Kruger 1967). Both *F. gigantica* and *F. nyanzae* as well as unidentified species have been detected in free-ranging common hippos throughout their range states, whilst a single case due to an unknown species was associated with mortality in a captive common hippo (Akanbi et al. 2021; Bargues et al. 2022; Cowan, Thurlbeck & Laws 1967; Dinnik & Dinnik 1961; Hammond 1972; Mas-Coma, Valero & Bargues 2009; McCully, van Niekerk & Kruger 1967; Rietmann & Walzer 2014; Schols et al. 2021). Although *F. nyanzae* is suggested to be a species-specific parasite of common hippos (Dinnik & Dinnik 1961), *F. gigantica* may parasitise a broad host range, primarily artiodactyls, but can also infect humans causing acute or chronic hepatobiliary diseases (Bargues et al. 2022). The lifecycles of *Fasciola* spp. are complex and prolonged (taking up to 17 weeks for *F. gigantica*), requiring life stages in various lymnaeid snails as intermediate hosts (OIE 2022). In the definitive host, prepatent periods of 3 to 11 weeks follow ingestion of infective *Fasciola* larvae. Although both *F. gigantica* and *F. nyanzae* are exotic to Australia, several lymnaeid snails that are competent intermediate hosts for other *Fasciola* spp. are present.

Other trematodes reported in hippos include stomach flukes and *Ogmocotyle* spp. (Graber, Blanc & Delavenay 1980; Leiper 1910; McCully, van Niekerk & Kruger 1967; Schols et al. 2021; Sey 1991; Sey & Graber 1979). The biology of the species identified in hippos are poorly understood. Broadly, these genera require intermediate hosts which are typically planorbid or lymnaeid snails (Pfukenyi & Mukaratirwa 2018). Adults reside in the stomach or gastrointestinal tract, and in some instances have been associated with enteritis in free-ranging common hippos (McCully, van Niekerk & Kruger 1967), whilst others have been incidental findings. In other species, these genera may cause production losses in livestock in the presence and migration of moderate-heavy infections with immature fluke (Huson, Oliver & Robinson 2017). Host ranges of these species vary, with some only reported in common hippos (e.g. *Gigantoatrium gigantoatrium, Sellstrema sellsi,* and several *Nilocotyle* and *Ugandocotyle* species), whilst others have been reported in other artiodactyl and non-artiodactyl hosts (e.g. *Cotylophoron cotylophorum, Buxifrons buxifrons* and *Carmyerius* spp.) (Mitchell et al. 2023; Sey 1991).

Schistosomes (*Schistosoma edwardiense, S. hippopotami* and unspecified species) have been associated with endocarditis and vasculitis, and are commonly found in free-ranging hippos in natural range states (Cowan, Thurlbeck & Laws 1967; McCully, van Niekerk & Kruger 1967; Morgan et al. 2003), whilst a case has been identified in an imported pygmy hippo from an unspecified location (Miller 2003). In humans, schistosomiasis poses a major public health burden, although the species definitively identified in common hippos (*S. edwardiense, S. hippopotami*) have not been identified in humans or other species. Other *Schistosoma* spp. vary in their host specificity. Many are host specific, whilst some such as *S. japonicum* may be found in a broad host range with domestic livestock and wild animals serving as reservoirs for human infection (He, Salafsky & Ramaswamy 2001). Broadly, schistosomes exhibit a 2-host life cycle, requiring aquatic snail species as intermediate hosts from genera, including *Oncomelania*, *Bulinus* and *Biomphalaria* (Lu et al. 2018). In

the snail, cercariae develop and are released into water which then penetrate the definitive host skin. Adult worms inhabit the bloodstream, producing eggs that are shed in either the urine or faeces. Snail species in Australia have not yet been identified as transmitting any of the *Schistosoma* parasites (Lu et al. 2018).

Cestodes recorded in hippos include *Echinococcus* spp. and *Moniezia* spp. *Moniezia expansa* is present in Australia, whilst other *Moniezia* species of hippos are not known to have been described to the species level with disease associations unknown (Guilbride et al. 1962; Sandground 1936). Both *Echinococcus granulosus,* and *E. felidis* have been reported in common hippos (Halajian et al. 2017; McCully, van Niekerk & Kruger 1967). These species have 2-host life cycles, consisting of a broad intermediate host range that includes several artiodactyls, and carnivores as definitive hosts. Although *E. granulosus* is present in Australia, *E. felidis* is exotic, and has only been detected in sub-Saharan Africa. Hippos serve as an intermediate host for both species and without access to definitive hosts (lion or hyena for *E. felidis* and canids for *E. granulosus*) they are likely to be dead end hosts (Romig, Ebi & Wassermann 2015).

Little is known about the biology of nematode species identified in hippos beyond initial species identification. Detection, including in zoos, have frequently been incidental findings, although some species have been identified in association with disease such as *Stephanofilaria thelazoides* which was identified in association with ulcerated skin lesions (Boomker et al. 1995). None of the species identified in hippos are known to have been detected in Australia, host ranges are unknown and most presumably have a direct life cycle with some exceptions.

Common hippos are the only known mammalian host of a monogenean, *Oculotrema hippopotami* (Colitz & Montiani-Ferreira 2022). Most polystomes are host-specific and *O. hippopotami* has only been detected in common hippos throughout their range states as well as some captive hippos from unspecified locations (Du Preez & Kok 1997) The species exhibits a direct life cycle, with adults attached to the ocular conjunctiva, where they have been associated with conjunctivitis.

Diagnosis

Most internal helminths can be diagnosed through either faecal floats or sedimentation techniques to examine eggs and/or larvae, dependent on species. Sedimentation of faeces is typically required to detect patent trematode infections. Intermittent shedding necessitates repeated examination, and newly infected animals may have negative results on these tests. Some of the helminths described in hippos are not known to shed eggs and/or larvae in faeces, necessitating alternate diagnostics such as visual inspection (*Oculotrema* spp.) or smears and/or skin biopsies of suspicious lesions (*Stephanofilaria* spp.).

Treatment

Zoo hippos have been treated safely with ivermectin, albendazole, oxfendazole, fenbendazole and levamisole for helminths (Miller 2003). However, some of these anthelmintics may have a limited spectrum of activity against cestodes or trematodes. Although not known to have been investigated in hippos, in livestock, the effectiveness of anthelmintics against *Fasciola* spp. is dependent on life stages of the fluke in the definitive host, with only some anthelmintics effective at any given life stage. Triclabendazole is generally considered effective at all life stages, whilst albendazole, closantel, nitroxynil are only effective at later life stages (8-12 weeks, drug dependent). Fenbendazole and oxfendazole have some ovicidal activity but have no effect on adult flukes. (NSW DPI 2017; Rojo-

Vazquez et al. 2012). Drug combinations may result in synergism for effectiveness at earlier life stages, however repeat treatment may be necessary for drugs less efficient against early immature flukes. Resistance is a problem for several of these drugs in different geographical areas. Similarly, the efficacy of compounds against stomach flukes in domestic livestock is unknown, limited or variable. Only some compounds such as oxyclozanide are considered efficacious (Huson, Oliver & Robinson 2017; Rojo-Vazquez et al. 2012; Rolfe & Boray 1988).

3.8.2 Current biosecurity measures

Standard zoo conditions require effective treatment of internal and external parasites during PEQ.

3.8.3 Risk review

The following key points are relevant to the biosecurity risk of internal parasites in live zoo hippos and their semen:

- Helminths recorded in hippos include trematodes, monogeneans, cestodes and nematodes.
- Trematodes are particularly common in free-ranging common hippos.
- There is limited information available regarding helminths of pygmy hippos.
- Helminths are not considered to be a common clinical problem in zoo hippos.
- Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.
- Several helminths recorded in hippos are exotic to Australia and have a broad host range. Others are host-specific or have an unknown host specificity. Prepatent periods for most helminths recorded in hippos are unknown.
- Lifecycles of some helminths of hippos require access to intermediate or definitive hosts, which may be restricted in captivity or are not present in Australia. Intermediate hosts are not well characterised for many of the helminths identified in hippos.
- The monogenean recorded in hippos is host specific and is reported as causing few clinical issues.
- For the cestodes reported in hippos that are considered exotic to Australia, hippos serve as intermediate hosts only, and the ability to continue life cycles in captivity would be restricted.
- The life cycle of schistosomes require aquatic snail species as intermediate hosts. Snail species in Australia have not yet been identified as transmitting any of the *Schistosoma* parasites.
- Exotic nematode and trematodes (excluding schistosomes) reported in hippos have the potential to continue life cycles in Australia based on their mode of transmission and/or availability of known intermediate hosts.
- Semen is not a risk material for the identified helminths of hippos.
- Several production limiting diseases of livestock have been associated with the species or genera of helminths recorded in hippos.
- Zoo hippos may be given anthelminthics to eliminate internal parasites, although only a limited range of anthelmintics have been applied to hippos.

- Some anthelmintics may have a limited spectrum of activity against cestodes or trematodes.

Based on the information above, the likelihood of entry and exposure for internal parasites in live zoo hippos is estimated as moderate.

Based on the information above, the likelihood of entry and exposure for internal parasites in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of internal parasites of hippos is considered low.

For the likelihood of entry and exposure of moderate, combined with the likely consequence of establishment and spread of low using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with internal parasites in live zoo hippos of low which does not achieve Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of low using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with internal parasites in zoo hippo semen of negligible which achieves Australia's ALOP.

3.8.4 Conclusion

As the introduction of internal parasites in live zoo hippos poses a low unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP. No measures are required for zoo hippo semen.

Australia's proposed disease-specific biosecurity measures for internal parasites for the import of live zoo hippos are:

1) Within 24 hours prior to entering PEQ, each hippo was treated with a broad spectrum anthelmintic or combination of anthelmintics effective against nematodes and trematodes. The product name, active ingredients, dose rates and dates of treatments must be recorded on the veterinary certificate.

AND

2) Between 24 to 72 hours prior to export, each hippo was treated with a broad spectrum anthelmintic or combination of anthelmintics effective against nematodes and trematodes. The product name, active ingredients, dose rates and dates of treatments must be recorded on the veterinary certificate.

AND

3) If PEQ extends beyond 30 days, repeat treatments to maintain sufficient coverage against nematodes and trematodes throughout the PEQ period were applied. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

Table 5 Summary of internal parasite biology identified in hippos

Helminth	Susceptible species	Distribution	Life cycle	Disease effects	Controlled by
Fasciola spp. (*F. gigantica, F. nyanzae*, unidentified species)	*F. nyanzae*: common hippos. *F. gigantica*: artiodactyls primarily (incl hippos), humans.	*F. nyanzae*: Common hippo range-states (sub-Saharan Africa). *F. gigantica*: Africa, Asia, eastern Europe, and some Pacific islands, including Hawaii (OIE 2022). Exotic to Australia.	Complex, requiring life stages in various lymnaeid snails used as intermediate hosts. Prepatent periods following ingestion of infective larvae range from 3 to 11 weeks in the definitive host (OIE 2022).	*F. gigantica* may cause production limiting diseases of livestock due to ill thrift, liver disease and mortalities and may cause hepatobiliary diseases in humans (Bargues et al. 2022; OIE 2022).	Some anthelmintics, with variable efficacy at different life stages.
Schistosomes (*Schistosoma edwardiense, S. hippopotami,* unspecified species)	*S. edwardiense, S. hippopotami:* common hippos.	Common hippo range-states (sub-Saharan Africa).Additionally identified in an imported pygmy hippo from an unspecified location (Miller 2003).	Two host life cycles, requiring aquatic snail species as intermediate hosts. Snail species in Australia have not yet been identified as transmitting any of the *Schistosoma* parasites (Lu et al. 2018).	Endocarditis and vasculitis reported in hippos (Cowan, Thurlbeck & Laws 1967; McCully, van Niekerk & Kruger 1967).	Praziquantel has been used to control schistosomes in domestic livestock (Wang et al. 2006).
Other trematodes, including stomach flukes (*Buxifrons* spp., *Gigantocotyle* spp., *Carmyerius* sp., *Nilocotyle* spp. *Sellstrema* spp., *Ugandocotyle* spp.) and *Ogmocotyle* spp.	Variable. Some have only been detected in common hippos, whilst others have been reported from several artiodactyl and non-artiodactyl hosts.	Common hippo range-states (sub-Saharan Africa). Some such as *Cotylophoron cotylophorum* have been reported throughout Africa, Asia, North, Central and South America.	Poorly understood. Broadly, these genera have complex lifecycles, requiring life stages in various lymnaeid or planorbid snails used as intermediate hosts (Pfukenyi & Mukaratirwa 2018).	Some have been associated with enteritis in hippos (McCully, van Niekerk & Kruger 1967). In other species, these genera may cause significant production losses in livestock (Huson, Oliver & Robinson 2017).	Limited, variable or unknown efficacy for many anthelmintics. Compounds containing oxyclozanide considered efficacious.
Oculotrema hippopotami (Colitz & Montiani-Ferreira 2022)	Common hippos only (Du Preez & Kok 1997).	Common hippo range-states (sub-Saharan Africa) (Rubtsova et al. 2018; Vanhove et al. 2022). Additionally reported in imported captive hippos from unspecified locations (Miller 2003).	Direct lifecycle, eggs released into the water develop into oncomiracidium which then attach to the host eye (Vanhove et al. 2022).	Conjunctivitis (Colitz & Montiani-Ferreira 2022).	Records of treatment could not be identified. Monogeneans are not known to infect other mammals.

Helminth	Susceptible species	Distribution	Life cycle	Disease effects	Controlled by
Echinococcus granulosus, E. felidis (Halajian et al. 2017; McCully, van Niekerk & Kruger 1967)	Broad intermediate host range, including artiodactyls, humans. Definitive host for *E. felidis* is reported as the lion or hyena and canids for *E. granulosus* (Romig, Ebi & Wassermann 2015).	*Echinococcus granulosus* is present in Australia. *E. felidis* is not known to have been described outside of Africa.	Two host life cycle with a carnivore definitive host and various intermediate hosts.	Cyst formation has been documented in the liver and lung of common hippos (McCully, van Niekerk & Kruger 1967).	Treatment in intermediate hosts is typically restricted to surgery for high-value animals (Spickler 2020).
Moniezia expansa, unspecified *Moniezia* species (Guilbride et al. 1962; Sandground 1936).	*M. expansa* capable of parasitising several artiodactyls, including sheep, goats and cattle (Guo 2017).	*M. expansa* is found worldwide, including in Australia.	Two host lifecycle, requiring mites as intermediate hosts (Guo 2017).	Not reported for hippos, production losses reported in livestock (Coles 2002).	Treatment in hippos has not been described. Praziquantel, albendazole, fenbendazole and niclosamide have been applied in livestock (Coles 2002).
Mammomonogamus hippopotami, Loxodontofilaria hippopotami, Stephanofilaria thelazoides, Leiperiatus hopkeni, Toxacara hippopotami, Capillaria spp., unspecified hookworms (Boomker et al. 1995; Flacke & Decher 2019; Leiper 1910; Maung 1975; Pabutta et al. 2021; Rietmann & Walzer 2014; Walzer & Stadler 2015).	Unknown host ranges.	Common hippo range-states (sub-Saharan Africa) as well as in zoo hippos in captive collections from unspecified locations (Miller 2003).	Poorly understood. Many are likely to have a direct life cycle (e.g. *Toxacara* spp.) others such as *Stephanofilaria* complete part of their life cycle within a haematophagous insect. Prepatent periods are unknown, but in other species, prepatent periods may be as short as 3 weeks (e.g. *Toxacara* spp.).	Frequently incidental.	Several anthelmintics (Miller 2003).

3.9 *Mycobacterium tuberculosis* complex (*M. bovis, M. caprae, M. tuberculosis*)

3.9.1 Background

The *Mycobacterium tuberculosis* complex (MTBC) comprises a group of bacteria in the *Mycobacterium* genus of the family *Mycobacteriaceae*, responsible for tuberculosis of animals and humans. Members of the MTBC include *M. africanum*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii* and *M. tuberculosis* (WOAH 2022c). These bacteria are non-spore forming, acid fast, facultative intracellular bacteria.

Tuberculosis is an infectious, chronic granulomatous disease. Any member of the MTBC complex may cause tuberculosis and all mammal species are susceptible to tuberculosis (WOAH 2022c). However, there are identified preferred host associations within the complex. *M. tuberculosis* and *M. africanum* are primarily human pathogens, *M. microti* primarily a rodent pathogen, and *M. pinnipedii* primarily a pathogen of marine mammals (WOAH 2022c). *M. bovis* and *M. caprae* are associated with various artiodactyls but the primary natural host is considered cattle for *M. bovis*, whilst *M. caprae* has a strong association with goat herds in some regions of Europe (Aranaz et al. 2003; Rodriguez et al. 2011; Spickler 2019b). Zoonotic infections may occur with any member of the MTBC complex (WOAH 2022c).

Infection with the *Mycobacterium tuberculosis* complex is a WOAH listed disease of multiple species (WOAH 2023a). For the purposes of the WOAH Terrestrial Code, MTBC is considered to be infection with *M. bovis, M. caprae* and *M. tuberculosis* but excludes vaccine strains (OIE 2017a). These variants are considered the most important due to their broader host range and public health impact (WOAH 2022c). All 3 of these species are nationally notifiable diseases of animals in Australia, whilst tuberculosis (due to MTBC excluding vaccine strains) is a zoonosis and a nationally notifiable disease of public health concern in Australia (DAFF 2022b; Department of Health and Aged Care 2023). Bovine tuberculosis is currently listed as a category 4 disease in the EADRA (AHA 2022b).

The agents of greatest significance for Australian agriculture are *M. bovis* and *M. caprae*, both of which cause tuberculosis in several livestock species but are exotic to Australia (AHA 2022a; WOAH 2024). Natural infections of both species have been identified in domestic and non-domestic artiodactyls and other mammalian animals, in which infection may be maintained. *Mycobacterium tuberculosis* is maintained in humans but may occasionally infect animals. *M. tuberculosis* is present in humans in Australia, although incidence rates are one of the lowest in the world (Meumann et al. 2021). Only *M. bovis* has been identified in hippos to date (Kerr et al. 2022).

Mycobacterium bovis and *M. caprae* are present in some approved countries (WOAH 2024). *M. bovis* was eradicated from Australia after a 27 year campaign costing approximately $840 million, declaring freedom in accordance with the WOAH Terrestrial Code in 1997 (More, Radunz & Glanville 2015). To maintain this status, it is essential to prevent the introduction of the organisms in imported animals and their products.

3.9.2 Technical information

Epidemiology

Tuberculosis is a global disease, although several countries are considered free of MTBC in animals (AHA 2022a; WOAH 2024). The major causes of tuberculosis in animals varies with geographical

region, with *M. bovis* the primary pathogen of livestock in western Europe, Africa and the Americas, and *M. caprae* a major cause of small ruminant, cattle and wildlife infections in some regions of south and central Europe (Nigsch et al. 2018; Rodriguez et al. 2011; WOAH 2022c). In addition, *M. tuberculosis* is of increasing importance in some epidemiological settings and species such as captive elephants or livestock in contact with infected handlers (Rajbhandari et al. 2022; Romero et al. 2011).

Although domestic cattle are considered the primary natural host, *M. bovis* has a broad host range and all mammals are considered susceptible to infection (Spickler 2019b; WOAH 2022c). Wildlife reservoirs of *M. bovis* include white-tailed deer (*Odocoileus virginianus*) and bison in North America, brushtail possums (*Trichosurus vulpecula*) in New Zealand, badgers (*Meles meles*) in the United Kingdom and Ireland, and African buffalo and lechwe (*Kobus lechwe*) in Africa (Clifton-Hadley et al. 2008; Kaneene & Pfeiffer 2006; Spickler 2019b). Several other wildlife species may have the potential to act as reservoirs of *M. bovis*, but their role in the epidemiology of the disease has not been confirmed. *M. caprae* was first isolated from goats, but similarly to *M. bovis* has a broad host range (Aranaz et al. 2003; Spickler 2019b). Infection has been reported in cattle, sheep, pigs (domestic and wild), red deer and several zoo animals (Pate et al. 2006; Rodriguez et al. 2011). Purported wildlife reservoirs for *M. caprae* include red deer and wild boar (Fink et al. 2015; Welz et al. 2023).

Transmission of the MTBC complex occurs through inhalation, ingestion or direct contact through mucous membranes or breaks in the skin. The bacteria are shed from the respiratory tract and in excretions and secretions, including saliva, faeces, urine, vaginal and uterine discharges. Animals with gross tuberculous lesions communicating with airways or the intestinal tract are likely to excrete large numbers of bacteria into the environment, and infected individuals may shed bacteria in the absence of clinical signs (Constable et al. 2017; Cousins et al. 2004).

For *M*. *bovis*, a single colony forming unit is sufficient to cause disease via the respiratory route and the size of the infectious dose received may influence disease severity (Dean et al. 2005; Menzies & Neill 2000). The incubation periods of *M. bovis* and *M. caprae* may be prolonged and difficult to detect. Not all infected animals go on to develop clinical disease, which is influenced by the individual's ability to mount a successful cell-mediated immune (CMI) response to the infection.

Mycobacterium bovis has been diagnosed in free-ranging common hippos (Kerr et al. 2022). In a retrospective study of culled free-ranging common hippos in Kruger National Park and surrounding areas in South Africa, 7 out of 60 individuals were test-positive to MTBC based on various methods (serology via the Dual Path Platform (DPP) Vet TB assay (n=4); quantitative PCR (qPCR) via the Xpert MTB/RIF Ultra qPCR assay (n=5) and culture (n=1)). Positive culture results were obtained from one individual from both lung and thoracic lymph node samples, with molecular typing confirming this as *M. bovis* SB0121, which is commonly identified in African buffalo in the region (Kerr et al. 2022). This individual had no evidence of gross lesions at necropsy nor clinical signs noted prior to culling (Kerr et al. 2022).

Aside from Kerr et al. (2022), there has been limited examination of MTBC in wild hippo. Tschopp et al. (2010) looked for evidence of tuberculosis in free-ranging Ethiopian wildlife, but only tested one common hippo, which was negative both on serology and culture of lymph nodes. Makondo et al. (2014) attempted tissue culture of 7 common hippos in Tanzania but did not recover any acid-fast bacteria. No studies examining wild pygmy hippos could be identified. There is no evidence of a

significant epidemiological role for hippos and MTBC, but they are susceptible to infection. It is assumed that transmission, shedding, etc., are similar to that described for other susceptible species.

Single or multiple cases of MTBC have frequently been reported in susceptible zoo wildlife (Lecu & Ball 2015; Montali, Mikota & Cheng 2001). Suspicion of MTBC has been raised in several zoo hippos but definitive infection is unknown (Bouts et al. 2009; Flacke et al. 2015; Lindau 1982; Mann et al. 1981). Bouts et al. (2009) identified repeated positive serology and comparative intradermal skin tests for MTBC in a pygmy hippo during the post-arrival quarantine period at a zoo in the United Kingdom. *M. interjectum*, a non-tuberculosis mycobacterium, was subsequently isolated from bronchial lavage samples but did not clearly account for the comparative intradermal skin tests nor serological results (Bouts et al. 2009). No macroscopic or microscopic lesions consistent with tuberculosis were noted at necropsy and MTBC was unable to be cultured (Bouts et al. 2009). Additionally, case reports of tuberculosis in a single common zoo hippo and pygmy zoo hippo exist, although confirmatory diagnostic testing in these cases could not be identified beyond gross or histological evidence (Flacke et al. 2015; Lindau 1982).

There are no identified studies of MTBC in hippo semen. In other species, semen from infected donors can pose a transmission risk, either through intrinsic infection (bacteria is present in seminal fluid), or extrinsic infection (tuberculosis lesions in the prepuce contaminate the seminal sample) and can survive in frozen semen (Niyaz Ahmed, Khan & Ganai 1999; Scott Williams Consulting Pty Ltd 2017). Although uncommon, miliary tuberculosis and chronic testicular tuberculosis have been reported in the testes of bulls (Hein & Tomasovic 1981).

Diagnosis

Clinical signs

Tuberculosis has a chronic, variable, and often subclinical course (Cousins et al. 2004; Ncube et al. 2022). In non-domestic animals, infection is often very advanced before clinical signs are detectable (Montali, Mikota & Cheng 2001; Ncube et al. 2022; Thomas et al. 2021). If clinical signs develop, they are generally non-specific and include ill-thrift, weakness, chronic wasting and eventually death. In confirmed infection of hippos, no clinical signs (or evidence of gross disease) were noted on necropsy (Kerr et al. 2022).

Testing

Diagnostics for MTBC fall into 3 categories: direct, CMI, and serological (humoral) techniques. Diagnosis is complex as many tests lack both sensitivity and specificity, and shedding of organisms is intermittent. Test specificity and sensitivity depend on the test used, the stage of the disease, the distribution of infection and other confounding factors. Most diagnostic tests have not been validated for non-domestic animals and there are no known validated diagnostic tests for hippos. Tests based on immunological response (both cell-mediated and humoral) may vary in reliability with host species and should be interpreted carefully. Many tests for *M. bovis* or *M. caprae* may show cross-reaction between other MTBC members, and other species of mycobacteria (including the atypical mycobacteria).

Investigation of possible mycobacterial infection in hippos, based on ante-mortem testing, has rarely been conclusive. Modalities applied have included direct (culture, PCR, histopathology and acid-fast staining), CMI (tuberculin skin tests), and humoral test methods (Bouts et al. 2009; Flacke et al. 2015; Kerr et al. 2022; Lindau 1982; Mann et al. 1981). Kerr et al. (2022) provided the first definitive

evidence of *M. bovis* infection in common hippos, utilising a combination of serology (DPP Vet TB assay), molecular techniques (Xpert MTB/RIF Ultra qPCR assay) and culture. One individual was positive across all methods, but 6 other individuals had positive results to one or more test-methods. Gross necropsy did not reveal obvious macroscopic pathology (Kerr et al. 2022).

Repeat testing and the use of tests that target different parts of the immune response is a recommended strategy to overcome the limitations of MTBC diagnostic tests (Lecu & Ball 2015; Thomas et al. 2021). Knowledge of herd health and institutional history aid in the interpretation of diagnostic tests for tuberculosis in artiodactyls (both domestic and non-domestic, including hippos). Many zoos in approved countries have ongoing MTBC screening programs utilising a range of recommended or 'in-house' protocols.

Direct tests include culture, microscopy and molecular techniques. Microscopy of smears with acid-fast staining, or histopathology, can be used for presumptive diagnosis but has low sensitivity and may detect non-tuberculosis mycobacteria (Thomas et al. 2021; WOAH 2022c). Culture is the gold standard for confirmatory diagnosis (Thomas et al. 2021). However, culture may take up to 12 weeks for a result (WOAH 2022c). There is often difficulty in obtaining suitable specimens for culture and the test has low sensitivity (Lécu & Ball 2011; Thomas et al. 2021). Molecular techniques to detect mycobacterial DNA in biological samples is increasingly being used as an alternative to culture, due to rapid turn around and increased sensitivity over culture methods (WOAH 2022c). Several commercial and in house assays have been developed, including the Xpert MTB/RIF Ultra qPCR assay which has been applied to hippos (Kerr et al. 2022; WOAH 2022c).

CMI modalities applied to hippos include tuberculin skin tests. Other blood-based methods of measuring CMI are not known to have been applied in hippos. Tuberculin skin tests (TST) may be either single (intradermal injection of bovine purified protein derivative (PPD) tuberculin only) or comparative tests (injection of both bovine PPD tuberculin and avian tuberculin at a site approximately 15cm away) (WOAH 2022c). These tests rely on a delayed hypersensitivity response to tuberculin antigens, with the reaction measured 48 to 72 hours after injection (Thomas et al. 2021; WOAH 2022c). The comparative test compares the response to avian tuberculin with the response to mammalian tuberculin and helps rule out false positives from non-specific reactions due to exposure to atypical mycobacteria. Due to the aquatic environment of hippos with access to multiple routes of nontuberculosis mycobacteria exposure, comparative TSTs are preferred (Bouts et al. 2009), although improve specificity at the cost of sensitivity (WOAH 2022c). The preferred site of injection is variable dependent on species, but the caudo-lateral ear is reportedly the most common site for TST administration reported in hippos, which may require general anaesthesia for administration (Bouts et al. 2009; Ziccardi et al. 2000). Generally, CMI modalities are more reliable earlier in the course of the disease and become less sensitive once advanced pathology, and greater infectivity, occur due to anergy of the CMI response (de la Rua-Domenech et al. 2006; Modise 2012).

The TSTs rely on a local inflammatory cell response which may be low or absent due to concurrent immunosuppression, latent infections, a state of anergy associated with advanced or generalised disease, recently acquired infections, tegument cellular organisation for that species, or environmental factors (de la Rua-Domenech et al. 2006; Grobler, De Klerk & Bengis 2002; Lécu & Ball 2011; Thomas et al. 2021). False negatives may also occur due to incorrect injection technique, subjectivity in the interpretation, or the use of suboptimal reagent concentrations (de la Rua-

Domenech et al. 2006). Additionally, after a TST is performed, it is recommended that an interval of time is allowed to elapse before the next test, to reduce the possibility of desensitisation by antigen overload. The recommended minimum intervals vary between host species and test type, with minimums of 42 days stipulated for cattle and 120 days for cervids in the WOAH Terrestrial Manual (WOAH 2022c). False positives may be due to previous exposure to injection adjuvant or non-specific reactions to contaminants or exposure to atypical or saprophytic mycobacteria (Bushmitz et al. 2009; de la Rua-Domenech et al. 2006). Further limitations include variable sensitivity and specificity and the need to immobilise certain animals in order to both administer and read the test (Lécu & Ball 2011; Thomas et al. 2021). In addition, TSTs have been noted to be unpractical in some pachyderms and have a high false negative rate in elephants (Thomas et al. 2021; WOAH 2022c). These tests have not been validated in hippos along with many other non-domestic artiodactyls, and criteria for classification as negative or suspect exist for few non-domestic species.

Humoral methods reported to have been applied in hippos include ELISA techniques, lateral flow tests and multi-antigen print immunoassay (MAPIA). In contrast to CMI methods, humoral techniques become more reliable as pathology develops, as humoral response is limited in early stages with minimal pathology (De la Rua-Domenech et al. 2006; Modise 2012). Consequently, their sensitivity is typically lower than CMI based tests in recently infected individuals. The sensitivity and specificity of these tests varies depending on the antigens selected and host species amongst other factors (Thomas et al. 2021). These techniques have been used for the diagnosis of MTBC in many non-domestic artiodactyls and have the advantage of being simple, rapid, and low cost (de la Rua-Domenech et al. 2006; Lecu & Ball 2015; Thomas et al. 2021). They may be used in conjunction with CMI tests to increase sensitivity and specificity, and to assist in interpretation of results in anergic individuals (Lecu & Ball 2015; Thomas et al. 2021; WOAH 2022c). Although some of these humoral based tests have been applied to both common and pygmy hippos (DPP Vet TB Assay; TB STAT-PAK; MAPIA), none are known to have been validated in either hippo species (Bouts et al. 2009; Kerr et al. 2022).

Treatment

Mycobacterial diseases in any species are difficult to treat successfully. A variety of chemotherapeutic methods have been attempted, usually to preserve or prolong the life of valuable animals. Ante-mortem verification that treatment has eliminated all of the organisms is difficult to prove definitively. No records of treatment in zoo hippos could be identified.

Control

MTBC in domestic livestock is typically addressed by isolation, quarantine, testing and culling strategies. In zoos, risk is managed through isolation, compartmentalisation, pre- and post- transfer testing and health checks of individual animals; routine screening; investigation of sick individuals and post-mortem examination of deceased animals.

3.9.3 Current biosecurity measures

Australia's biosecurity measures for MTBC across several zoo taxa (zoo bovids, zoo elephants, zoo perissodactyls) include premises freedom combined with testing, or country freedom.

3.9.4 Risk review

The following key points are relevant to the assessment of the biosecurity risk of MTBC in live zoo hippos and their semen:

- *M. bovis, M. caprae* and *M. tuberculosis* are widely distributed throughout the globe with a broad host range. Infections with these *Mycobacterium* are frequently reported in captive wildlife.
- *M. bovis* is the only MTBC agent identified in free-range common hippos. No cases have been definitively described in zoo hippos or free-range pygmy hippos, although suspicious case records exist.
- Zoo hippos within the scope of this review are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision. MTBC screening programs may exist in some zoos.
- Asymptomatic infections are common amongst susceptible species and no clinical signs have been reported in hippos known or suspected to be infected with MTBC. MTBC infections have extended incubation periods and may be lifelong.
- Transmission primarily occurs via respiratory aerosols. It can also occur via ingestion, contaminated respiratory secretions, pasture and other fomites.
- Shedding of MTBC *Mycobacterium* is known to occur in the absence of clinical signs.
- Agents of the MTBC may be transmitted via semen and may remain viable in frozen semen. The capability for this to happen in hippos is unknown.
- Zoo hippos do not mix with open herds of domestic livestock in Australia. However, they do come into contact with other susceptible animals in captivity, and humans through husbandry and provision of veterinary services.
- Infection with *M. bovis*, *M. caprae* and *M. tuberculosis* are WOAH listed and nationally notifiable animal diseases.
- MTBC bacteria are zoonotic and tuberculosis (due to the mycobacterium tuberculosis complex, excluding vaccine strains) is a nationally notifiable disease in humans.
- *M. bovis* and *M. caprae* are exotic to Australia. Australia's campaign to eradicate *M. bovis* took 27 years and cost $840 million.
- An outbreak of *M. bovis* or *M. caprae* in Australia would likely have considerable impact domestically and internationally. International trade pathways for valuable domestic industries would likely be interrupted and significant public investment would be required for national eradication of bovine tuberculosis should it become established in domestic species.
- Treatment of tuberculosis is not reliable at eliminating infection.
- Diagnostic testing is complex. A wide variety of tests exist each with its own limitations, particularly in non-domestic species, including hippos. There is no validated diagnostic test for hippos, and many existing tests may be unreliable and/or impractical for many zoo species. Multiple tests are recommended to improve diagnosis accuracy.

Based on the information above, the likelihood of entry and exposure for *M. tuberculosis* complex in live zoo hippos and their semen is estimated as low.

The likely consequences of the establishment and/or spread of *M. tuberculosis* complex is considered high.

For the likelihood of entry and exposure of low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with *M. tuberculosis* complex in live zoo hippos and their semen of moderate which does not achieve Australia's ALOP.

3.9.5 Conclusion

As the introduction of *M. tuberculosis* complex in live zoo hippos and their semen poses a moderate unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP.

Australia's proposed disease-specific biosecurity measures for MTBC for the import of live zoo hippos are:

1) Option 1:

   a) For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

      AND

   b) The animal received 2 separate skin tests for tuberculosis in the 210 days prior to export, with negative results. The tests were performed a minimum of 90 days apart from each other and one was performed during PEQ. Each test was either a TST or CTST. Each test was read 72 hours post-inoculation.

      Note: where multiple skin tests for tuberculosis have been performed, all skin tests must be performed at least 90 days apart.

OR

2) Option 2:

   a) For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

      AND

   b) The animal was tested for tuberculosis using 2 different tests, performed during the 30 days immediately before export, with both tests returning negative results. One test was either a TST or CTST and the test was read 72 hours post-inoculation. The second test was a serological test performed on a blood sample taken during this period using the TB STAT-PAK or another serological test approved by the department.

      Note: where multiple skin tests for tuberculosis have been performed, all skin tests must be performed at least 90 days apart.

Australia's proposed disease-specific biosecurity measures for MTBC for the import of zoo hippo semen are:

1) Option 1:

    a) For 12 months immediately before each semen collection the donor animal did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

    AND

    b) The donor animal received 2 separate skin tests for tuberculosis in the 210 days prior to each semen collection, with negative results. The tests were performed a minimum of 90 days apart from each other and one was performed in the 30 days immediately before each semen collection. Each test was either a TST or CTST. Each test was read 72 hours post-inoculation.

    Note: where multiple semen collections were performed and the testing timeframes for each overlap, all skin tests for tuberculosis must be performed at least 90 days apart.

OR

2) Option 2:

    a) For 12 months immediately before each semen collection the donor animal did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

    AND

    b) The donor animal was tested for tuberculosis using 2 different tests, performed during the 30 days immediately before each semen collection, with both tests returning negative results. One test was either a TST or CTST and the test was read 72 hours post-inoculation. The second test was a serological test performed on a blood sample taken during this period using the TB STAT-PAK or another serological test approved by the department.

    Note: where multiple semen collections were performed and the testing timeframes for each overlap, all skin tests for tuberculosis must be performed at least 90 days apart.

3.10 New World and Old World screwworm

3.10.1 Background

Screwworm fly myiasis is an external parasitic disease of mammals, caused by the larvae of 2 species of flies, New World screwworm, *Cochliomyia hominivorax* and Old World screwworm, *Chrysomya bezziana*. Both species are members of the family *Calliphoridae*, subfamily *Chrysomyinae*. Screwworms are the larvae of the flies that feed on living flesh causing extensive tissue damage, which in severe infestations may be fatal (OIE 2019).

Cochliomyia hominivorax is presently found in some regions of South and Central America and the Caribbean. It has never been reported in Canada and was initially eradicated from the United States in the 1980s (Hall 1991). However, an incursion of New World screwworm occurred in Florida in 2016

to 2017, primarily affecting Key deer (*Odocoileus virginianus clavium*). The United States declared freedom again in 2017 after a successful eradication program (USDA 2017).

Chrysomya bezziana is found throughout parts of Africa, the Middle East, the Indian subcontinent, China, South-East Asia and some Pacific nations (OIE 2019). Old World screwworm was detected in a variety of zoo animals in Singapore in 2017 (WOAH 2018). *Chrysomya bezziana* has not been reported in European countries or New Zealand.

New World screwworm and Old World screwworm are WOAH-listed diseases (WOAH 2023a). They are absent from Australia and are listed as nationally notifiable animal diseases, classified as category 2 diseases in the EADRA (AHA 2022b; DAFF 2022b).

3.10.2 Technical information

Epidemiology

Screwworm flies lay eggs in the open wounds or orifices of warm-blooded mammals. After hatching, larvae then feed on the host tissue causing extensive damage, which can result in high morbidity and mortality rates within the host population (Allan 2001). The flies prefer warm, moist conditions with temperatures of 16 to 30°C and larvae usually spend 4 to 7 days on the host before dropping off to pupate within the soil (Rodriguez & Raphael 2008). At tropical temperatures larvae may hatch from the eggs within 24 hours of being laid. The life span of a male fly is up to 14 days. A 10 day lifespan is common for a female with some living up to 30 days or more. The life cycle of a single fly may vary with temperature; at tropical temperatures it may be less than 21 days whilst at low temperatures maturation may take 2 to 3 months (Spickler 2016).

Both species of flies can affect all warm-blooded animals, including humans. Infestations in birds are rare (Spickler 2016). Oral myiasis has been reported in a captive common hippo, although the species of larva was not identified (Rossi Jr et al. 2009). *C. hominivorax* and *C. bezziana* have similar climatic requirements. Australia is the only continent with a suitable climate where screwworm fly has not established. Several native species have been shown to be susceptible, becoming infested in other countries, including red kangaroos and wallabies (AHA 2020).

Modelling suggests that climatic conditions would limit screwworm fly survival to the northern areas of Australia should it be introduced and become established (Fruean & East 2014), however climate change may alter this modelling. Infested wounds in humans and animals are the most likely source of introduction, followed by the importation of adult flies.

Semen is not a risk material for screwworm fly.

Diagnosis

Clinical signs

Screwworms may not be obvious in wounds in the first 2 days after infestation but by day 3 larvae can be readily seen. The navels of newborns and vulval and perineal regions of their dams are common sites of infestation however any open would is susceptible and larvae readily infest mucous membranes (Spickler 2016).

Infested wounds will commonly enlarge with serosanguinous discharge. Wounds may emit a foul odour. Infested animals may have decreased appetite, appear depressed and separate from herds (Spickler 2016).

Testing

Identification of adult flies confirms the presence of screwworm fly in a region, but identification of larvae from clinical cases through morphological identification and/or molecular methods is required to confirm individual animal infestation. Larvae should be collected from the deepest part of the wound to avoid collecting non-screwworm larvae which may be present in the shallower wound tissue (OIE 2019).

Treatment

Treatment of infested wounds usually consist of physical debridement of the wound followed by the application of topical insecticides (James et al. 2014; Spradbery 1994). Topical insecticides should be applied at 2 to 3-day intervals until the wound has healed.

Subcutaneous administration of ivermectin at a dose of 200 µg per kg caused 100% mortality of screwworm larvae up to 2 days old in cattle (Spradbery et al. 1985). Oral administration of afoxolaner has been reported to be effective in treating screwworm myiasis in dogs (Cutolo et al. 2021).

Control

Cochliomyia hominivorax was eradicated from the southern United States, Libya and Mexico by treating wounds of all infested animals with insecticidal smears and releasing billions of sterile flies in a program known as the Sterile Insect Technique (SIT). Trials have shown that SIT can also be used to control *C. bezziana*, which poses the greater risk to Australia (Spradbery et al. 1989). As documented in the AUSVETPLAN manual, Australia's response to screwworm fly incursion would include initial suppression of screwworm fly populations through a variety of management strategies, followed by SIT, the only proven method of eradication (AHA 2020).

At 200 µg per kg body weight, the residual protection of ivermectin provided against Old World screwworm was a minimum of 14 days in pen trials and 16 to 20 days in field trials with cattle with castration and branding wounds (Perkins 1987; Spradbery et al. 1985).

A capsule formation of ivermectin and a spray-on formulation of dicyclanil protected against screwworm fly strikes in sheep for 12 weeks (James et al. 2014).

Wardhaugh, Mahon and Ahmad (2001) evaluated pour-on formulations of doramectin, eprinomectin and moxidectin and a sustained-release (SR) bolus of ivermectin against Old World screwworm in cattle in Malaysia. Doramectin and eprinomectin provided 7 to 14 and 3 to 7 days protection respectively while no activity, even at 3 days, was shown for moxidectin.

Topical 1% fipronil was also shown to have preventative effects against New World screwworm myiasis in cattle for up to 17 days (Lima et al. 2004).

3.10.3 Current biosecurity measures

Australia's biosecurity measures for screwworm fly myiasis in other live animals include country freedom or premises freedom in conjunction with inspection regimes. The WOAH Terrestrial Code recommendations include country freedom or inspection for external parasites, treatment of infested wounds and prophylactic treatment for the transport of domestic and wild mammals (OIE 1998b).

Australia's response to an incursion of screwworm fly is outlined in the AUSVETPLAN (AHA 2020).

3.10.4 Risk review

The following key points are relevant to the biosecurity risk of screwworm fly in live zoo hippos and their semen:

- All warm-blooded mammals are potential hosts for screwworm infestation.
- Oral myiasis has been reported in a captive common hippo, although the species of larva was not identified.
- Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.
- Adult screwworm flies lay eggs in the wounds or orifices of warm-blooded animals. Larvae eat living tissue for 4 to 7 days before dropping off the host to pupate in the soil or other substrate.
- The length of a screwworm fly life cycle may vary with temperature. In hotter temperatures it may be less than 21 days.
- Semen is not a risk material for transmission.
- Adult screwworm flies can travel considerable distance.
- High morbidity and mortality are known to occur in host populations.
- A single detection of screwworm fly in an Australian zoo would likely have minimal impact nationally. However, should adult flies emerge and establish more broadly, outbreaks would have considerable domestic and native animal welfare affects as well as significant economic and trade impacts. Control of flies would be challenging.
- Infestation with New World and Old World screwworm are WOAH listed diseases of multiple species. The WOAH Terrestrial Code recommends the application of risk mitigation measures for New World and Old World screwworm to the international movement of all domestic and wild animals from screwworm fly infested countries, including country freedom or inspection for external parasites, treatment of infested wounds and prophylactic treatment.

Based on the information above, the likelihood of entry and exposure for screwworm fly in live zoo hippos is estimated as extremely low.

Based on the information above, the likelihood of entry and exposure for screwworm fly in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of screwworm fly is considered high.

For the likelihood of entry and exposure of extremely low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with screwworm fly in live zoo hippos of very low which achieves Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with screwworm fly in zoo hippo semen of negligible which achieves Australia's ALOP.

3.10.5 Conclusion

Based on the preceding information, no disease specific risk mitigation measures additional to WOAH Terrestrial Code requirements are required for live zoo hippos; as such only the WOAH Terrestrial code requirements will be applied (OIE 1998b). Disease specific management measures are not warranted for zoo hippo semen.

In accordance with the WOAH Terrestrial Code, Australia's proposed disease-specific biosecurity measures for New World and Old World screwworm for the import of live zoo hippos are:

1) Option 1:

    a) For 60 days immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of screwworm-fly (*Cochliomyia hominivorax* or *Chrysomya bezziana*) occurred during the 12 months prior to export and the disease is compulsorily notifiable.

OR

2) Option 2:

    a) Within 24 hours prior to entering PEQ:

        i) All hippos in the consignment were examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and all animals were found to be visibly free from infested wounds.

        Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

        AND

        ii) All wounds (if present) are prophylactically treated with an officially approved oily larvicide at the recommended dose.

        Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

        AND

        iii) All animals were then treated, immediately after inspection, with a product officially approved for the control of New World or Old World screwworm, under supervision of an Official Veterinarian and in accordance with the manufacturer's recommendations.

        Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

    AND

    b) Between 24 to 72 hours prior to export:

        i) All hippos in the consignment were examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and found to be visibly free from infested wounds.

Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

AND

ii) All wounds (if present) are prophylactically treated with an officially approved oily larvicide at the recommended dose.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

AND

iii) The hippo was then treated, immediately after inspection, with a product officially approved for the control of New World or Old World screwworm, under supervision of an Official Veterinarian and in accordance with the manufacturer's recommendations.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

AND

c) Within 72 hours of arrival all hippos in the consignment were examined by the veterinarian in charge of post-arrival quarantine and found to be visibly free from infested wounds.

Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

3.11 Rabies virus

3.11.1 Background

Rabies virus is a member of the *Lyssavirus* genus (*Rhabdoviridae*), responsible for the disease rabies. Rabies virus (*Lyssavirus rabies*) is considered the most important *Lyssavirus* species for both animal and public health (Muller et al. 2022). Rabies virus (RABV) causes a progressively fatal encephalitis that affects all species of mammals and is zoonotic. No records of RABV in either species of hippo were identified during this review; however they are assumed to be susceptible to infection.

Lyssavirus reservoir hosts are typically bats, however RABV has adapted to a number of non-chiropteran reservoir hosts. Species within the *Canidae, Procyonidae, Herpestidae, Mephitidae, Viverridae* and *Mustelidae* families, and New World bats are considered the primary urban and sylvatic reservoirs for RABV (Fooks et al. 2017; Müller & Freuling 2020). The role of species within these cycles is dependent on local factors, including RABV strain, species distributions and interactions, and control programs. Dogs represent the most important reservoir for public health as more than 99% of human cases reported are the result of exposure to infected domestic dogs (WHO 2023). Other mammals typically act as dead-end hosts.

Infection with RABV is a WOAH listed disease of multiple species (WOAH 2023a). It is present in most countries worldwide, with some island countries, territories and states considered free. It is not present in Australia, is a nationally notifiable animal disease and a nationally notifiable disease of public health concern (DAFF 2022b; Department of Health and Aged Care 2023). Rabies is currently listed as a category 1 disease in the EADRA (AHA 2022b).

3.11.2 Technical information

Epidemiology

Rabies virus is shed in the saliva of infected animals, with transmission occurring directly primarily via bites and/or saliva contamination of breaks in the skin or mucous membranes. Rarer transmission modes have been documented, including consumption of infected carcasses and aerosol spread (Fisher, Streicker & Schnell 2018; Fooks et al. 2017).

Rabies virus is neurotropic. Once inoculated RABV replicates locally and then travels via peripheral neurons via retrograde transport to the central nervous system and eventually to the brain (Fooks et al. 2017). From the brain, the virus can further disseminate to peripheral tissue via nerves, including salivary glands where replication and intermittent shedding into saliva occurs, enabling onward transmission (Fooks et al. 2017). In domestic dogs, shedding in saliva has been documented to occur up to 13 days prior to the onset of clinical signs (Fekadu, Shaddock & Baer 1982). The incubation period of RABV is highly variable in all mammals. Dependent factors include infectious dose, site of inoculation, species and strain. Prolonged incubation periods of several years have been reported, but more typically incubation periods range from 10 days to several months, although incubation periods in naturally acquired infections of wildlife are largely unknown (Müller & Freuling 2020; Muller et al. 2022). For the purposes of international trade, the WOAH considers the incubation period to be 6 months, as most animals that are infected with RABV will go on to develop rabies during this time (WOAH 2023c).

Infection dynamics are often described as urban, where transmission occurs between domestic animals and humans, or sylvatic, whereby RABV is transmitted between wildlife species. Different variants of RABV are adapted to specific reservoir hosts, leading to host-specific maintenance cycles (Brunker & Mollentze 2018). Several different species of carnivores and New World bats may act as reservoir or maintenance hosts globally, establishing sylvatic and urban cycles of RABV circulation. Capacity to act as a reservoir host is determined by host pathobiological constraints such as dissemination to salivary glands, host ecology, including host densities and bite contact behaviours as well as specific viral determinants (Fisher, Streicker & Schnell 2018; Gilbert 2018; Müller & Freuling 2020). In contrast, in spillover hosts, infection is typically dead-end, although further horizontal transmission has been rarely reported. Aside from a purported role of kudu as a potential reservoir host for RABV in Namibia, artiodactyls are largely considered spillover hosts and are incapable of maintaining independent cycles of infection (Gilbert 2018; Muller et al. 2022; Scott et al. 2013).

There have been no identified reported cases of RABV in either species of hippo. However, due to the broad mammalian host range for RABV, it is assumed both species of hippo are susceptible to RABV infection. Artiodactyls are considered to have a moderate risk of natural infection (Hanlon 2013). It is likely that hippo, similarly to most artiodactyls and other herbivorous hosts, would be dead-end hosts, and in most circumstances die without further transmission of RABV. Specific information on incidence rates of RABV in zoo mammals could not be identified in this review and although it is considered to be rare, several spillover events of rabies in zoos in diverse species have been recorded (David et al. 2007; Grome et al. 2022). Prevention of contact between zoo animals and reservoir host species is generally not feasible. Higher contact rates of humans with individual captive mammals through provision of husbandry or post-mortem procedures may increase the likelihood of human exposure (Grome et al. 2022; Kenny et al. 2001).

There is no evidence for RABV transmission via semen or embryos (AHA 2021b).

Diagnosis

Clinical signs

The initial clinical signs of rabies are variable and non-specific, but once clinical signs develop the disease invariably results in death of the infected host, typically within days (Fisher, Streicker & Schnell 2018; Muller et al. 2022). Diagnosis on clinical signs alone is not possible. Clinical signs described in artiodactyls include unusual docility, salivation, aggression, abnormal bellowing, incoordination and other signs of neurological disease (Hudson et al. 1996; Muller et al. 2022; Swanepoel 2004). There are no known cases of rabies in hippos and as such clinical signs are unknown. However, it is presumed clinical presentation would be similar to that of other artiodactyls.

Testing

Reliable diagnostic tests for RABV in live animals are not available. Post-mortem diagnosis is primarily achieved through direct fluorescent antibody tests, PCR assays, and rapid immunohistochemistry tests typically applied to brain tissue, although other tissue types such as salivary glands have also been tested with variable sensitivity and specificity (WOAH 2023d).

Treatment

There are limited treatment options for infection with RABV. In humans post exposure prophylaxis is possible with the World Health Organization (WHO) providing guidelines for treatment, however post-exposure treatment is not recognised in animals. This includes washing of the exposure site, vaccination and administration of rabies immunoglobulin (Fooks et al. 2017). Euthanasia is generally the only management option for animals which have been exposed to RABV or display clinical signs of rabies (Brown et al. 2011).

Control

A range of highly effective, safe and thermostable inactivated veterinary vaccines exist for use in domestic carnivores and herbivores. Application of these vaccinations to non-domestic animals in zoos is dependent on species, captive management type, regulatory controls and local epidemiological situations, with vaccines utilised in hippos in some captive settings.

3.11.3 Current biosecurity measures

Australia's current biosecurity measures for RABV for live animals include, but are not limited to, premises freedom, country freedom and (for domestic dogs and cats) vaccination. The WOAH Terrestrial Code recommendations include country, zone or establishment freedom for international movements of all mammals (WOAH 2023c).

3.11.4 Risk review

The following key points are relevant to the biosecurity risk of rabies virus in live zoo hippos and their semen:

- Rabies virus is endemic in several approved countries.
- Zoo hippos are sourced from and maintained in facilities that have health monitoring programs and are under veterinary supervision.

- There are no known reports of rabies in either species of hippo. However, RABV is known to be able to infect all mammals. Therefore, hippos are considered to be able to be infected.
- Rabies may have an extended incubation period.
- Transmission of RABV from animal to animal occurs via direct contact (saliva entering bites or pre-existing skin wounds), and rarely via other routes (consumption or aerosols).
- It is likely that hippo, similarly to most artiodactyls and other herbivorous hosts, would in most circumstances die without further transmission of RABV.
- There are no reports of transmission of RABV in semen of any herbivore species.
- Zoo hippos do not mix with open herds of domestic livestock in Australia. However, zoo hippos may share enclosures with other mammalian species, and enclosures can potentially be accessed by small mammals (e.g. rodents, possums, etc). Zoo hippos also come into contact with humans (e.g. keepers and veterinarians).
- Rabies virus is a nationally notifiable animal disease and a nationally notifiable disease in humans. The detection of a case of rabies in Australia would have significant animal and public health repercussions.
- Infection with RABV is a WOAH listed disease of multiple species, and the WOAH recommends the application of risk management measures for RABV to the international movement of all mammals.

Based on the information above, the likelihood of entry and exposure for rabies virus in live zoo hippos is estimated as extremely low.

Based on the information above, the likelihood of entry and exposure for rabies virus in zoo hippo semen is estimated as negligible.

The likely consequences of the establishment and/or spread of rabies virus is considered high.

For the likelihood of entry and exposure of extremely low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with rabies virus in live zoo hippos of very low which achieves Australia's ALOP.

For the likelihood of entry and exposure of negligible, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with rabies virus in zoo hippo semen of negligible which achieves Australia's ALOP.

3.11.5 Conclusion

Based on the preceding information, no disease specific risk management measures for rabies virus additional to WOAH Terrestrial Code requirements are warranted for live zoo hippos; as such only the WOAH Terrestrial code requirements will be applied (WOAH 2023c). Disease specific management measures are not warranted for zoo hippo semen.

Australia's proposed disease-specific biosecurity requirements for infection with rabies virus for the import of live zoo hippos are:

1) Option 1:
    a) The animal showed no clinical sign of rabies the day prior to or on the day of export.

       AND

    b) The animal was kept since birth or at least 180 days prior to export in a country considered free (as assessed by the Australian Department of Agriculture, Fisheries and Forestry) from infection with rabies virus.

OR

2) Option 2:
    a) The animal showed no clinical sign of rabies the day prior to or on the day of export.

       AND

    b) For 180 days immediately before export the animal has not resided on any premises in the country of export where clinical, epidemiological or other evidence of rabies virus has occurred during the 12 months prior to export and the disease is compulsorily notifiable.

3.12 Rift Valley fever

3.12.1 Background

Rift Valley fever (RVF) is a transboundary zoonotic disease caused by the mosquito-borne *Phlebovirus riftense* (syn. Rift Valley fever virus (RVFV)) in the family *Phenuiviridae*. RVF is primarily a disease of ruminants, causing significant production losses through mortality and abortions, but also causes an influenza-like illness in humans and occasionally a haemorrhagic fever with encephalitis. The virus is historically endemic in several countries in sub-Saharan Africa (Arishi et al. 2000; Gould & Higgs 2009). In recent decades RVFV has extended its geographic range to north and west African countries, the Arabian Peninsula and the Indian Ocean archipelagos of Mayotte and Comoros, whilst serologically positive animals have been detected in Turkey and some countries of the Middle East (Fakour, Naserabadi & Ahmadi 2017; Gur et al. 2017; Linthicum, Britch & Anyamba 2016).

RVFV infects a broad species range and has been reported in several artiodactyl families. Serological evidence of RVFV infection has been reported in common hippos, but disease is not known from either species of hippo.

RVF is a zoonosis but is not a nationally notifiable disease in humans in Australia. RVF is a WOAH listed disease of multiple species (WOAH 2023a). It is not present in Australia, is a nationally notifiable animal disease and is classified as a category 2 disease in the EADRA (AHA 2022b, 2023; DAFF 2022b).

3.12.2 Technical information

Epidemiology

RVF is a disease of multiple host species. Transmission of RVFV in animals occurs primarily via the bite of infected mosquitos. Once infected, mosquitos amplify and can maintain infective virus for life, whilst some species may vertically transmit RVFV to offspring (Bergren et al. 2021). Several mosquito genera may contribute to transmission, but *Aedes* and *Culex* spp. are considered the major biological

vectors (Linthicum, Britch & Anyamba 2016; Pepin et al. 2010). Preliminary studies suggest several mosquito species in Australia are likely to be competent vectors for RVFV (Turell & Kay 1998). Other arthropods (midges, ticks and other haematophagous insects) may also facilitate transmission, either mechanically or biologically, but their role in natural epidemiology of infection is currently unknown (Mansfield et al. 2015; Pepin et al. 2010). The virus appears to be ecologically flexible with a distribution across diverse ecological zones.

Although RVFV has been detected in various excretions from mammalian hosts, including milk and aborted materials, non-vector-borne transmission in animals is supported by limited field evidence and is not currently considered to be of major epidemiological importance (Pepin et al. 2010; Wilson et al. 2018; Wright et al. 2019). In humans, exposure to infective tissue and secretions is the main route of infection, although vector-borne transmission may also occur (Pepin et al. 2010; Wright et al. 2019). Vertical transmission has been recorded in livestock and human case-reports, and viral replication in the placenta has been identified in experimental studies of rodents (Antonis et al. 2013; Wright et al. 2019).

Incubation periods of RVFV are short based on laboratory experiments of livestock and other species, typically ranging from 12 hours to 6 days, with variability dependent on several host and agent factors (AHA 2021c; Gerdes 2004a; Pepin et al. 2010). Viraemia is typically brief, lasting up to 7 days across most domestic livestock, although viraemic durations of up to 13 and 17 days have been reported in experimental infections of rhesus macaques (*Macaca mulatta*) and black rats (*Rattus rattus*) respectively (Nielsen et al. 2020; Rostal et al. 2017; Stoek et al. 2022). Although viraemia is of short duration, some vertebrates, including domestic livestock species may act as amplifying hosts, facilitating transmission and epidemic propagation through generation of high levels of viraemia. Infective doses required for mosquitos vary by species, but may be as low as $10^{1.3}$ plaque forming units per ml (Tantely, Boyer & Fontenille 2015). The length of viraemia and titres reached in natural infections of wild mammals is largely unknown with only sporadic viral isolation in these species. However, experimental evidence indicates several non-domestic species may be capable of producing high viraemic levels considered capable of infecting mosquitos, including several rodent species, African buffalo and rhesus macaques (Olive, Goodman & Reynes 2012; Rostal et al. 2017). The WOAH Terrestrial Code defines the infective period for RVF in animals as 14 days for regulatory purposes (OIE 2016). There is no known carrier state in vertebrate hosts, and once recovered, long-lasting humoral immunity is generated.

RVFV epidemiology is tightly linked to local ecological factors, although many aspects of the epidemiology remain to be elucidated. In eastern and southern Africa, a feature of RVF is its occurrence as cyclical epidemics separated by quiescent periods of 5 to 15 years or longer (Gerdes 2002). Broadly, in these regions, epidemics are strongly linked to rainfall and flooding events, which facilitate the mass hatching and emergence of floodwater breeding mosquitos, predominantly *Aedes* spp. (Gerdes 2002; Manore & Beechler 2015). Following emergence, infection and amplification of virus in susceptible domestic and wild ruminants and recruitment of secondary mosquito populations result in propagation of an epidemic (Pepin et al. 2010). RVFV antibody prevalence will determine whether the number of susceptible hosts in the region are able to support an epidemic, despite conducive environmental conditions (Linthicum, Britch & Anyamba 2016). In contrast, in semi-arid environments in west Africa, RVFV outbreaks have been linked to years of rainfall deficit, whilst in

other regions such as irrigated areas, year-round persistence of RVFV may be facilitated (Rolin et al 2013).

Maintenance of RVFV through inter-epidemic periods is uncertain. Current evidence indicates both long term survival in mosquito eggs via transovarial transmission and cryptic cycling in vertebrate hosts may contribute to viral maintenance, with low level cycling in a multi-species vertebrate reservoir system supported by detection of viral antibodies in several hosts, both wild and domestic, outside of epidemic periods (Beechler et al. 2015; Bergren et al. 2021; Chevalier et al. 2010; Linthicum, Britch & Anyamba 2016; Manore & Beechler 2015; Olive, Goodman & Reynes 2012; Rostal et al. 2017). Dissemination of RVFV is generally over short distances, however long-distance movement through translocation of infected animals or mosquitoes is thought to account for the recent geographic expansion of RVFV, although the precise mechanisms for this are unknown (Linthicum, Britch & Anyamba 2016; Mansfield et al. 2015).

Serological evidence of RVFV exposure has been identified in common hippos in 2 studies. Weinbren and Hewitt (1959) opportunistically tested 70 common hippo sera collected during a culling program in eastern Africa, identifying 52 positive reactors, although it was uncertain whether these results indicated exposure to RVFV. In addition, serological records of exposure exist from Kruger National Park in South Africa, with an unknown number of presumed common hippos tested (Young 1970). Beyond these studies, there have been no further identified studies on common hippos nor pygmy hippos. As such, the contribution of hippos to RVFV epidemiology is uncertain. No known records of disease exist in association with RVF epidemics in hippos, virus is not known to have ever been isolated from hippos, and the ability of hippos to generate sufficient viraemia to enable infection of vectors is unknown. The peri-aquatic habitat of hippos may facilitate vector exposure and there is blood-meal evidence of competent RVFV vectors feeding preferentially on hippos (Crabtree et al. 2013; Ogola et al. 2023; Omondi et al. 2015). The current AUSVETPLAN response considers that hippos may be important in disease epidemics within a zoo environment (AHA 2021c).

WOAH considers semen of ruminants a risk for RVFV transmission (OIE 2016), although definitive evidence for presence of infectious virus in semen could not be identified. However, RVFV antigen has been identified in sheep testes, in bull semen from 1 to up to 3 weeks post vaccination with a live-attenuated vaccine, and in humans 4 months post symptom development (Odendaal et al. 2019). With no hippo specific research available, it is assumed the risk of RVFV transmission in semen of livestock is also relevant to hippos.

Diagnosis

Clinical signs

In domestic species, there is a large amount of variability in individual susceptibility to RVF dependent on age, species, prior immunity and infectious doses amongst other factors. Some individuals may have asymptomatic infections, whilst severe clinical disease associated with high mortality rates of up to 100% in young animals may ensue (Gerdes 2004b). When evident, clinical signs are largely non-specific, and include fever, weakness, conjunctivitis, nasal discharge and anorexia. However, abortion storms and mortalities in young animals concurrent with human cases of disease are characteristic of RVF outbreaks (WOAH 2023e). Clinical signs and pathology associated with RVF in either species of hippo have not been described. However, where reported, disease in other wild artiodactyls are similar to those of domestic livestock (Olive, Goodman & Reynes 2012).

Testing

Diagnostics tests for RVFV include virus isolation, demonstration of viral antigens and serological methods, with a combination of different diagnostic approaches required depending on testing purposes.

Serological diagnostics include IgM and IgG ELISAs, and viral neutralisation assays such as plaque-reduction neutralisation tests (PRNT) (WOAH 2023e). However, there is no information on the application or validation of ELISAs for hippo serum. Virus neutralisation tests have previously been applied to hippo sera, but application of these tests such as the PRNT is currently limited by the requirement for high containment biosafety facilities amongst other biosafety requirements (Weinbren & Hewitt 1959; WOAH 2023e).

For confirmation of clinical cases, diagnostics are largely focused on virus isolation or antigen detection. Viral RNA may be detected in blood during the brief viraemic periods (Ikegami 2012; Mansfield et al. 2015). RT-PCR protocols have been developed to detect viral antigen (WOAH 2023e). Virus culture and isolation can be performed on whole blood or serum from the acute stage of disease or on tissues collected at post-mortem such as liver, lymph nodes, spleen or abortion products. Histopathology followed by immunostaining of the liver may also be applied to demonstrate viral antigen in tissue (WOAH 2023e).

Treatment

No specific treatments are available for RVFV infection in animals.

Control

Control measures applied in endemic countries include use of insecticides, vaccination and prevention of slaughter of susceptible animals during epidemics to minimise the risk of transmission to humans (AHA 2021c). An AUSVETPLAN response strategy has been developed for RVF with eradication through a stamping out policy preferred (AHA 2021c). RVF is difficult to control and countries with a history of infection in live animals are very likely to remain infected with RVFV (Gerdes 2004a).

3.12.3 Current biosecurity measures

In other commodities, including live zoo bovids and their semen and zoo perissodactyls, Australia's current biosecurity measures for RVF include country freedom. The WOAH Terrestrial Code recommendations for susceptible live animals include country or zone freedom, vector protection and vaccination.

3.12.4 Risk review

The following key points are relevant to the biosecurity risk of RVF in live zoo hippos and their semen:

- RVFV is capable of infecting a broad range of hosts and is a WOAH-listed disease of multiple species. It is primarily a disease of ruminants but has been reported in several wild artiodactyls.
- There is limited serological evidence of infection in wild common hippos, but antigen detection or viral isolation in hippos is not known to have occurred and clinical disease is unknown.
- Mosquitoes are considered the main vectors for transmission of RVFV in animals. The peri-aquatic habitat of hippos may increase vector exposure and there is blood-meal evidence of

competent vectors feeding on hippos. However, the ability of hippos to develop a viraemia and at a sufficient level for transmission of RVFV to vectors is unknown.

- The viraemic period is short (lasting up to 7 days across most domestic livestock). No RVFV carrier-state has been identified in mammals, including hippos.
- Transmission is possible through live animals and semen.
- Several mosquito species in Australia are likely to be competent vectors for RVFV and vector exclusion is unlikely to be feasible in a zoo setting.
- RVF is a zoonosis and infection of humans may occur through contact with infective materials.
- RVF outbreaks may cause significant disease, with fatalities in livestock and humans.
- Should RVF enter, spread and establish in Australia, eradication is likely to be challenging.
- Countries with a history of infection in live animals typically remain infected with RVFV.

Based on the information above, the likelihood of entry and exposure for Rift Valley fever in live zoo hippos is estimated as very low.

Based on the information above, the likelihood of entry and exposure for Rift Valley fever in zoo hippo semen is estimated as very low.

The likely consequences of the establishment and/or spread of Rift Valley fever is considered high.

For the likelihood of entry and exposure of very low combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with Rift Valley fever in live zoo hippos and their semen of low which does not achieve Australia's ALOP.

3.12.5 Conclusion

As the introduction of Rift Valley fever in live zoo hippos and their semen poses a low unrestricted risk measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP.

Australia's proposed disease-specific biosecurity measures for Rift Valley fever for the import of live zoo hippos are:

1) For 90 days immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of RVF has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

Australia's proposed disease-specific biosecurity measures for Rift Valley fever for the import of zoo hippo semen are:

1) For 90 days immediately prior to each semen collection the donor animal was continuously resident in a country where no clinical, epidemiological or other evidence of RVF has occurred in any species during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

3.13 Surra (*Trypanosoma evansi*)

3.13.1 Background

Trypanosoma evansi is a flagellated protozoan in the *Trypanozoon* subgenus of the Trypanosomatidae family. It is a WOAH listed disease of multiple host species, with the largest host range and geographical distribution of any pathogenic trypanosome (Desquesnes et al. 2013a; WOAH 2023a). It causes production limiting and economically important diseases of camels, horses, buffalo and cattle and is known by several names, including surra, Mal de Caderas and El Gafar, with affected species varying dependent on the geographical region (Desquesnes et al. 2013a; OIE 2021d). There are no known case reports in hippos, but it is broadly considered that nearly all mammals are susceptible to infection (Desquesnes et al. 2013b).

Trypanosoma evansi is present in countries within north and west Africa, the Middle East, Russia, Asia and South America (WOAH 2024). Aside from presence in the offshore territories of some approved countries (Canary Islands) it is not currently present in approved countries. However, outbreaks in continental Spain (Tamarit et al. 2010) and France (Desquesnes et al. 2008) have occurred in the last 2 decades associated with import of infected camels from the Canary Islands.

Trypanosoma evansi is not present in Australia and is a nationally notifiable animal disease (DAFF 2022b). It is classified as a category 4 disease under the EADRA, as a production loss disease with possible international trade impacts and local market disruptions (AHA 2022b). Aside from livestock production losses, experimental studies have demonstrated that some species of Australian native fauna might be particularly susceptible to *T. evansi* resulting in potentially significant ecological consequences if it became established (Reid 2002; Reid et al. 2001).

3.13.2 Technical information

Epidemiology

Trypanosoma evansi is thought to have evolved from *T. brucei* and is considered by several authors as a subspecies of *T. brucei*, along with *T. equiperdum,* the causative agent of dourine. Differentiating between these species is difficult unless using specific molecular diagnostic methods (Desquesnes et al. 2022a; Desquesnes et al. 2013b; Lai et al. 2008; OIE 2021d; Wen et al. 2016).

Transmission occurs principally by mechanical vectors. Major vectors are tabanids and *Stomoxys* spp., although other vectors may also play a role, including vampire bats in South America (Desquesnes et al. 2013a; Hoare 1965). Following feeding on an inoculated host, survival on vector mouth parts is short lived. Transmission is most efficient when feeding is interrupted, and the vector resumes the blood meal on a new host within 30 minutes (Desquesnes et al. 2013a; Krinsky 1976). The restricted timeframe for transmission results in predominantly intra-herd transmission except during periods of resource shortages favouring congregation, or co-grazing (Desquesnes et al. 2013a). Other possible routes of transmission include peroral, transplacental and perinatal transmission via colostrum and milk (Campigotto et al. 2015; Desquesnes et al. 2013a; Narnaware et al. 2016; Raina et al. 1985). Initial infection may resolve or progress to persistent infection with periodic parasitaemia and latent carrier states (Desquesnes et al. 2013b; Hoare 1972). Although many animals demonstrate no clinical signs, in hosts that develop disease, incubation periods are variable, generally ranging from 5 to 60 days although may be as long as 3 months in some species (AHA 2021d).

All mammals are considered susceptible to *T. evansi* and infection has been recorded in a large range of domestic animals and wildlife across several familial groups in both wild and captive settings (Aregawi et al. 2019; Kasozi et al. 2021; Mbaya, Aliyu & Ibrahim 2009). There are rare documented human cases, however it is not currently considered a major zoonosis (Sengupta et al. 2022; Van Vinh Chau et al. 2016). Although multiple hosts can become infected, not all infected animals play a role in transmission as mechanical transmission requires a minimum level of parasitaemia, and susceptibility of some hosts to rapidly fatal disease may preclude ongoing transmission (Desquesnes et al. 2009; Mahmoud & Gray 1980). The epidemiological role played by different host species is variable over time and space, influenced by several factors, including breed, strain of *T. evansi* and environmental stressors (Desquesnes et al. 2013a). Horses, camels, and bovines are amongst the more important domestic reservoir hosts identified, whilst cats and dogs are suggested to not play a role in ongoing transmission (Desquesnes et al. 2013a; OIE 2021d). Capybaras and vampire bats are identified wild reservoir hosts, but the role of other wildlife in maintaining transmission is not known (Aregawi et al. 2019). If infection were to establish in Australia, some native fauna may be susceptible to disease and act as reservoir hosts, with experimental evidence indicating high level parasitaemia and death within 60 days in agile wallabies (*Macropus agilis*) and dusky pademelons (*Thylogale brunii*) following inoculation with *T. evansi* (Reid et al. 2001).

Although recorded in secondary literature (Kasozi et al. 2021), primary records of *T. evansi* in hippos in either captive or wild settings could not be identified in this review. However, the focus on tsetse-transmitted trypanosomes in hippo natural ranges has resulted in a lack of research around *T. evansi* in wild mammals in this region (Aregawi et al. 2019). Records of *T. brucei* exist for hippos on the basis of morphological identification (Dillmann & Awan 1972) or molecular identification (Anderson et al. 2011), and hippos subsequently could reasonably be assumed susceptible to *T. evansi*, whilst identified vectors are known to feed on hippos (Odeniran et al. 2019). However, the capacity to generate sufficient parasitaemia to play an important role in the epidemiology of infection is currently unknown, and susceptibility to disease development is also unknown.

Trypanosoma evansi DNA has been detected in semen during experimental infection studies of sheep (Da Silva et al. 2016) and has been associated with poor semen quality (Ogundele et al. 2016), but viable organisms are not known to have been demonstrated in semen, and there is currently no known evidence of successful venereal transmission in any species. It is therefore assumed hippo semen is not a risk material for *T. evansi.*

Diagnosis

Clinical signs

There is variability in susceptibility to clinical disease at the individual and species-level, due to either strain or host effects (Desquesnes et al. 2013b; Hoare 1972). Broadly, infection may be asymptomatic, or result in acute, subacute or chronic presentations. Clinical signs of surra are non-specific and include progressive emaciation, oedema, anaemia, fevers (recurrent and linked to parasitaemia levels in chronic cases) and late-stage neurological signs, including ataxia and progressive paresis. In highly susceptible animals such as dogs, infection is typically fatal if untreated and will eventuate in death within weeks or months. In less susceptible hosts, chronic presentations may continue for several years (Desquesnes et al. 2013b). Abortions and stillbirths may occur in pregnant animals (Narnaware et al. 2016). Mortality rates may be high if susceptible animals are

moved from non-endemic to endemic areas, or when the parasite is first introduced into a non-endemic region (Hoare 1972; Payne et al. 1991).

Testing

As clinical signs are not pathognomonic, definitive diagnosis requires laboratory testing. However, cyclical parasitaemia and shared characteristics with other *Trypanozoon* species complicates diagnosis. Several testing options are available with variable test sensitivity and specificity. Morphological identification through examination of blood smears may be used if parasitaemia is sufficient, but typically requires parasite concentrations of greater than 10^5 trypanosomes per ml (Desquesnes 2017). This may be improved through enrichment techniques such as the haematocrit centrifuge technique, but detection limits are still 50 to 200 trypanosomes per ml (OIE 2021d). In addition, morphological identification cannot differentiate from other members of the *Trypanozoon* subgenus (OIE 2021d). Polymerase chain reaction has the greatest analytical sensitivity, with a detection limit of 1 to 10 trypanosomes per ml, dependent on extraction methods and primers used (OIE 2021d). Several serological tests, including the card agglutination test for *T. evansi* (CATT) and ELISAs have been developed. The CATT, which mainly targets IgM, is the only commercially available test and is most useful for diagnosing active or recent infections but exhibits low sensitivity in some animal species such as pigs and cattle (Desquesnes et al. 2022a; OIE 2021d). ELISAs have been developed and are most likely to correctly identify uninfected animals but the required species-level validation is not known to have occurred for hippos (OIE 2021d). However, due to shared surface antigens, serological tests are cross reactive with other *Trypanosoma* spp. (Desquesnes et al 2022).

Treatment

Trypanocidal drugs are available for treatment of sick animals to eliminate parasites, however their application to hippos is unknown.

Control

Prevention of *T. evansi* in non-endemic regions relies on restricting the importation of infected animals. Control through vector management is of limited feasibility and efficacy for mechanical vectors and no vaccines are currently available.

3.13.3 Current biosecurity measures

Australia's biosecurity measures for *T. evansi* in zoo animals include country freedom for zoo perissodactyls, whilst zoo bovids require country freedom and lifetime residency requirements or diagnostic testing. There are currently no recommendations in the WOAH Terrestrial Code.

3.13.4 Risk review

The following key points are relevant to the risk of *T. evansi* in live zoo hippos and their semen.

- *T. evansi* is a listed disease of multiple species in the WOAH Terrestrial Code. It can infect multiple host species, with the largest host range and geographical distribution of any pathogenic trypanosome.
- There are no records of *T. evansi* in zoo or wild hippos, although it is presumed they are susceptible given their susceptibility to *T. brucei*. As such, there is no evidence that hippos play an epidemiological role in *T. evansi* infection in any setting.

- *T. evansi* can cause chronic latent infections in several species. Clinical signs may not be present, and diagnostic testing can be challenging.
- Outbreaks of *T. evansi* have occurred in association with the movement of infected animals from endemic regions.
- *T. evansi* is principally transmitted by mechanical vectors. Suitable vectors required for transmission are present in Australia.
- Mechanical transmission requires replication and sufficient levels of parasitaemia within host animals and can only occur for short periods. Transmission via mechanical vectors occurs primarily within the same herd or within species in close proximity.
- Zoo hippos do not mix with open herds of domestic livestock in Australia but they may be held with other susceptible species in captivity.
- There is currently no evidence demonstrating successful transmission of *T. evansi* via semen in any species.
- Some Australian marsupial species have been demonstrated to be susceptible to *T. evansi* infections in experimental studies, resulting in fatal infection characterised by high parasitaemia.
- It is listed as a category 4 disease under the EADRA. An uncontrolled outbreak would cause production losses in the beef and dairy industries and ongoing costs in the horse industry.
- Introduction of the parasite into naïve areas has resulted in high levels of infection, morbidity and mortality.
- Significant ecological consequences may occur if infection were to become established in Australia.

Based on the information above, the likelihood of entry and exposure for *T. evansi* in live zoo hippos is estimated as low.

Based on the information above, the likelihood of entry and exposure for *T. evansi* in zoo hippo semen is estimated as extremely low.

The likely consequences of the establishment and/or spread of *T. evansi* is considered high.

For the likelihood of entry and exposure of low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with *T. evansi* in live zoo hippos of moderate which does not achieve Australia's ALOP.

For the likelihood of entry and exposure of extremely low, combined with the likely consequence of establishment and spread of high using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with *T. evansi* in zoo hippo semen of very low which achieves Australia's ALOP.

3.13.5 Conclusion

As the introduction of *T. evansi* in live zoo hippos poses a moderate unrestricted risk measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP. No measures are warranted for zoo hippo semen.

Australia's disease-specific proposed biosecurity measures for *Trypanosoma evansi* for the import of live zoo hippos are:

1) For 12 months immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of *Trypanosoma evansi* has occurred in any species during the 12 months prior to export and infection is compulsorily notifiable.

3.14 Tsetse-associated trypanosomes (*T. brucei, T. congolense, T. simiae* and *T. vivax*)

3.14.1 Background

Trypanosomes are protozoan parasites which may infect the blood, lymph or tissue of hosts. Several species are known to cause disease in domestic animals, wildlife and humans. The species *Trypanosoma brucei* (*T. brucei* sspp. excluding *T. evansi* and *T. equiperdum*), *T. congolense*, *T. simiae* and *T. vivax* (tsetse-associated trypanosomes) cause trypanosomosis (tsetse-associated). Cattle, small ruminants, pigs, equids, cats and dogs, amongst other vertebrate hosts, are susceptible to infection, which in livestock results in anaemia, loss of body condition and emaciation. The disease is a major cause of production losses in endemic areas (Desquesnes et al. 2022a). In addition, *T. brucei* sspp. are responsible for human African trypanosomiasis, also known as sleeping sickness, for which various species of domestic and wild animals are suggested as a potential reservoir.

Tsetse-associated trypanosomes are primarily transmitted by haematophagous arthropods, especially by flies from the genus *Glossina* (commonly referred to as tsetse). Although tsetse are absent from Australia, insect vectors capable of mechanical transmission are present.

Infection with *T. brucei* (*T. brucei* sspp. excluding *T. evansi* and *T. equiperdum*)*, T. congolense*, *T. simiae* and *T. vivax* is a WOAH listed disease of multiple species (WOAH 2023a). The disease complex is not present in approved countries. It is absent from Australia and trypanosomosis (tsetse-associated) is a nationally notifiable animal disease, but is not currently classified in the EADRA (DAFF 2022b).

3.14.2 Technical information

Epidemiology

Tsetse-associated trypanosomes are transmitted to vertebrate hosts via inoculation during feeding from biological and/or mechanical vectors. The biological vector for trypanosomes considered in this chapter is the tsetse. Subsequently the spatial distribution for these species of trypanosomes is dependent on the distribution of tsetse which is found in regions of sub-Saharan Africa. However, mechanical transmission by other biting insects has been demonstrated and has facilitated the establishment of *T. vivax* outside of the tsetse belt into Asia, South and Central America, and may contribute to maintenance in areas of Africa where tsetse have been eradicated (Desquesnes et al. 2022a; Fikru et al. 2012).

Only *T. vivax* has been able to establish outside of Africa, where in the absence of tsetse, most authors consider it to replicate exclusively within the vertebrate host. There is no clear evidence for the role of other biological vectors. *Stomoxys* and tabanid flies are considered the main mechanical vectors of *T. vivax* outside of sub-Saharan Africa, although given the right conditions (influenced by sufficient host-parasitaemia, host contact, vector densities, size of mouth parts and suitable feeding

ecology), any haematophagous biting insect may act as a mechanical vector (Desquesnes 2004). Although mechanical transmission under experimental conditions have also been demonstrated for other species, including *T. congolense* and *T. brucei* sspp., its importance in the natural epidemiology of these species is currently thought to be minimal (Desquesnes et al. 2009; Desquesnes & Dia 2003; Desquesnes & Dia 2004; Radwanska et al. 2018). Experimental studies and modelling have suggested that low host parasitaemia of *T. congolense* infections (4 to 25 times lower than *T. vivax* in experimental studies of cattle) results in inefficient mechanical transmission and may explain why *T. congolense* does not persist outside of the tsetse belt (Desquesnes et al. 2009).

Whilst infection of tsetse is lifelong, the survival of tsetse-associated trypanosomes in mechanical vectors is short lived. Reported survival durations for *T. vivax* are up to 30 minutes on mouthparts and 5 to 7 hours in the gut (Desquesnes et al. 2013a; Desquesnes & Dia 2004). Interrupted feeding with subsequent host switching within minutes facilitates trypanosome spread via mechanical vectors. The potential for delayed mechanical transmission is limited by vector feeding strategies. Typical time between blood meals in tabanids (5 to 7 days) exceeds trypanosome survival in the gut, although *Stomoxys* may facilitate delayed mechanical transmission for up to 48 hours due to shorter periods between feeding (Desquesnes et al. 2013a). Due to these restrictions, transmission via mechanical vectors occurs primarily within the same herd unless various factors favour mixing of animals (Desquesnes et al. 2013a). Peroral, iatrogenic (needles, surgical instruments), perinatal and transplacental routes of transmission have also been described (Batista et al. 2022; Desquesnes 2004; Melendez, Forlano & Figueroa 1993; Silva et al. 2013).

Tsetse-associated trypanosomes can infect a wide range of wild and domestic species. These parasites are well-adapted to a variety of wild host species, most of which are considered highly tolerant to infection, and may serve as reservoirs of infection for vectors and livestock (Connor & Van den Bossche 2004; Kasozi et al. 2021). Once infected, a vertebrate host may be infected for life. Chronic infection is characterised by parasitaemia typically below the detectable limit of several diagnostic tests (Desquesnes et al. 2022a; Desquesnes et al. 2022b). Periodic flares of parasitaemia due to escape of immune control may subsequently enable further transmission and disease development. Evasion of the immune system may be triggered by the parasite population cyclically developing new surface antigens (Desquesnes et al. 2022a). Although wild hosts are frequently asymptomatic, for the purposes of the WOAH Terrestrial Code the incubation period of tsetse-associated trypanosomes is considered to be 90 days (OIE 2021b).

Morbidity and mortality rates are variable dependent on species, strain, host immune status and age. *T. vivax* and *T. congolense* are considered more pathogenic for livestock than *T. brucei* sspp. In susceptible livestock, mortality may be up to 50 to 100% in some herds, but in endemic regions although morbidity remains high, mortality is low when treatment is applied (Desquesnes 2004; Spickler 2018a). High morbidity and mortality may occur when susceptible species are introduced into endemic areas, or spread of competent infected vectors or hosts into regions where animals are naive (Desquesnes 2004; Spickler 2018a).

Trypanosome species relevant to this chapter have been reported in common hippos throughout their natural geographic ranges (Anderson et al. 2011; Dillmann & Awan 1972; Guilbride et al. 1962; Moloo 1980). However, *T. brucei* and *T. vivax* are the only species known to have been confirmed by molecular work (Anderson et al. 2011). Reported prevalence in common hippos is low, although this

may reflect chronic infections with low parasitaemia undetectable by diagnostic tests due to natural tolerance. Dillmann and Awan (1972) demonstrated that blood smear examination of hippos has poor sensitivity when compared with mouse inoculation, probably reflective of low parasitaemia. Although the epidemiological involvement of common hippos in trypanosome diseases has not been demonstrated to be any more significant than other wild vertebrates, there is a demonstrated feeding preference of tsetse for this host (Clausen et al. 1998; Moloo 1980). No reports were found on trypanosomes in pygmy hippos, nor was presence in captive hippos.

Although there is evidence that trypanosome DNA can be detected in the semen of infected livestock (Bezerra et al. 2018; Couto et al. 2022), there is currently no evidence demonstrating successful transmission by this route, including in hippos. The WOAH Terrestrial Code considers semen a safe commodity for *T. brucei, T. congolense*, *T. simiae* and *T. vivax* (OIE 2021b).

Diagnosis

Clinical signs and pathology are not pathognomonic and laboratory testing is necessary for diagnosis.

Clinical signs

Clinical signs of trypanosomosis (tsetse-associated) include fever, oedema, abortion, decreased fertility, emaciation and, frequently, anaemia in livestock (OIE 2021c). Clinical disease may range from peracute (including death within a week) to chronic (more common) and may persist for years in cattle (Connor & Van den Bossche 2004).

Clinical signs due to trypanosomosis (tsetse-associated) have not been reported in hippos. Non-domestic vertebrates may be resistant to disease caused by species found in their natural range, and able to carry long-term infections without evidence of clinical disease (Kasozi et al. 2021; Reichard 2002).

Testing

Due to fluctuating parasitaemia, diagnostic testing to determine infection status, particularly those based on parasite detection, can be problematic. Microscopic examination of blood films for the parasite may be utilised, although the value of blood film examination in hippos may be limited due to low parasitaemia (Dillmann & Awan 1972). The microhaematocrit centrifugation technique may improve diagnostic sensitivities over blood film examination but is only considered capable of detection when parasitaemia is above 50 to 100 trypanosomes per ml (Desquesnes et al. 2022b). Morphological differentiation of species on the basis of microscopic examination is not possible. Polymerase chain reaction may also be utilised to detect *Trypanosoma* nucleic acids and is considered to have a higher analytical sensitivity than the microhaematocrit centrifugation technique (Desquesnes et al. 2022b). However, the low parasitaemia intensity of chronic infections may still be below the level of detection (Desquesnes et al. 2022b). Serological tests include an indirect fluorescent antibody test and ELISAs, but cross reactivity with other trypanosomes may occur, and species validation is required (Desquesnes 2017; Desquesnes et al. 2022b).

Treatment

A number of trypanocidal drugs may be used for the treatment of trypanosomosis (tsetse-associated) in affected livestock. Examples include diminazene, homidium and isometamidium for use in cattle, sheep and goats (Connor and Van den Bossche 2004).

There are no documented cases of trypanosome infection treatment in hippos.

Control
Measures such as eradication of tsetse fly and prophylactic or metaphylactic administration of trypanocidal drugs are used in countries where the disease is endemic or at risk of spread.

3.14.3 Current biosecurity measures

Australia's biosecurity measures for trypanosomosis (*T. vivax*) in zoo perissodactyls and zoo bovids include country freedom and testing. The WOAH Terrestrial Code recommends an absence of clinical signs of disease, lifetime residency in a free country, zone or compartment and protection from vectors during transport when importing susceptible animals from countries, zones or compartments free from infection with tsetse-associated trypanosomes.

3.14.4 Risk review

The following key points are relevant to the biosecurity risk of tsetse-associated trypanosomes in live zoo hippos and their semen:

- Infection with *Trypanosoma brucei, T. congolense, T. simiae* and *T. vivax* is a WOAH listed disease of multiple species.
- The disease complex is not present in approved countries.
- Tsetse-associated trypanosomes can infect a wide host range. They have been reported at low prevalence in common hippos in range states (within the tsetse zone in Africa). There is no information on trypanosomes in pygmy hippos but they are also assumed to be susceptible to infection.
- Records of tsetse-associated trypanosome infection in captive hippos could not be identified.
- Tsetse-associated trypanosomes may cause life-long infections. Clinical signs are not pathognomonic, and in trypanotolerant species are likely to be absent.
- Tsetse-associated trypanosomes are primarily transmitted by haematophagous arthropods, especially by tsetse, which are the main biological vector for transmission. Tsetse are absent from Australia, but other mechanical vectors are present.
- Hippos are preferred blood meal sources for tsetse but they have not been shown to play a more important role than other wild hosts in the epidemiology of tsetse-associated trypanosomes in endemic regions.
- *T. vivax* can spread via competent mechanical vectors and mechanical vectors for *T. vivax* are present in Australia (e.g. *Stomoxys* and tabanid flies).
- There is limited evidence to support the importance of mechanical transmission in the epidemiology of other tsetse-associated trypanosomes.
- Mechanical transmission requires replication and sufficient levels of parasitaemia within host animals and can only occur for short periods. Transmission via mechanical vectors occurs primarily within the same herd or within species in close-proximity.
- Parasitaemias in wild common hippos are postulated to be low possibly reflecting trypanotolerance or chronic infections.

- There is currently no evidence demonstrating successful transmission of tsetse-associated trypanosomes via semen.
- Zoo hippos do not typically mix with open herds of domestic livestock in Australia but may be exposed to other susceptible animals in captive collections.
- Trypanosomosis (tsetse-associated) is a nationally notifiable animal disease. Tsetse-associated trypanosomes are a major cause of production losses in livestock. High morbidity and mortality may occur where animals are naïve.

Based on the information above, the likelihood of entry and exposure for *T. vivax* in live zoo hippos is estimated as low, while the likelihood of entry and exposure for other tsetse-associated trypanosomes in live zoo hippos is estimated as very low.

Based on the information above, the likelihood of entry and exposure for tsetse-associated trypanosomes in zoo hippo semen is estimated as extremely low.

The likely consequences of the establishment and/or spread of tsetse-associated trypanosomes is considered moderate.

For the likelihood of entry and exposure of low, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with *T. vivax* in live zoo hippos of low which does not achieve Australia's ALOP.

For the likelihood of entry and exposure of very low, combined with the likely consequence of establishment and spread of moderate, using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with other tsetse-associated trypanosomes in live zoo hippos of very low which achieves Australia's ALOP.

For the likelihood of entry and exposure of extremely low, combined with the likely consequence of establishment and spread of moderate using the risk matrix (Figure 3), estimates an overall unrestricted risk associated with tsetse-associated trypanosomes in zoo hippo semen of negligible, which achieves Australia's ALOP.

3.14.5 Conclusion

As the introduction of *T. vivax* in live zoo hippos poses a low unrestricted risk, measures to manage the associated biosecurity risks are required to reduce the risk to achieve Australia's ALOP. No measures are warranted for other tsetse-associated trypanosomes in live zoo hippos, and no measures are warranted for zoo hippo semen.

Australia's proposed disease-specific biosecurity measures for *Trypanosoma vivax* for the import of live zoo hippos are:

1) For 12 months immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of *Trypanosoma vivax* has occurred in any species during the 12 months prior to export and infection is compulsorily notifiable.

4 Biosecurity measures for zoo hippos and their semen from approved countries

The biosecurity measures described in this policy review are proposed for the importation of live zoo hippos and their semen from approved countries.

4.1 Baseline measures for live zoo animals

There are baseline risk management measures common to most current import policies for live zoo animals, including:

1) The animal must be resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before export, unless otherwise approved by the department. The residency requirement may be achieved in more than one approved country or holding institution if specifically authorised by the department and the conditions for each country of residence and holding institution were met.
2) The premises of origin (zoo or wildlife park) must provide separation from other animal populations, be under veterinary supervision and have a documented health monitoring program that would be effective in monitoring for the diseases of biosecurity concern identified in this review.

   The required outcome of veterinary supervision is up to date and regular knowledge of the animals, their health status, and the general health status of the institution that allows a veterinarian to sign off on these records.

   The required outcome of separation is a sufficient distance or other barriers to maintain a distinct animal health status with regards to the diseases in this policy.

   The required outcome of a health monitoring program is the regular monitoring, ongoing surveillance, and veterinary oversight to ensure that the health status of animals and an institution is known and monitored over time (e.g. post-mortem records for deceased animals, disease testing programs). This underpins official certification.
3) The animal must be held in pre-export quarantine for at least 30 days and isolated from all other animals not eligible for export to Australia, during which it is inspected at least daily for signs of disease, and treated and tested for diseases in accordance with Australian entry requirements.
4) For the 30 days immediately before export, the animal showed no clinical signs or other evidence of infectious or contagious diseases, including the diseases retained for risk review in this policy.
5) During the pre-export quarantine period or the 90 days immediately prior, the animal was not under any quarantine restrictions (aside from pre-export quarantine).
6) The pre-export quarantine facility has acceptable documented standards of how it will meet Australian requirements.

7) Immediately following arrival in Australia, the animal must be transported to an approved arrangement site which has been audited and approved by the department, in a manner that ensures no direct exposure to animals of a lesser biosecurity status en-route, and must undergo a period of post-arrival quarantine of at least 30 days.

8) The receiving institution must be approved under relevant Australian state or territory legislation to hold the species being imported.

The period of clinical health may be longer under some disease's risk management requirements as a disease specific risk management measure.

4.2 Baseline measures for zoo animal semen

General risk measures relevant to semen are:

1) The donor animal must be resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before collection, unless otherwise approved by the department. The residency requirement may be achieved in more than one approved country or holding institution if specifically authorised by the department and the conditions for each country of residence and holding institution were met.

2) The premises of origin (zoo or wildlife park) must provide separation from other animal populations, be under veterinary supervision and have a documented health monitoring program that would be effective in monitoring for the diseases of biosecurity concern identified in this review.

   The required outcome of veterinary supervision is up to date and regular knowledge of the animals, their health status, and the general health status of the institution that allows a veterinarian to sign off on these records.

   The required outcome of separation is a sufficient distance or other barriers to maintain a distinct animal health status with regards to the diseases in this policy.

   The required outcome of a health monitoring program is regular monitoring, ongoing surveillance, and veterinary oversight to ensure that the health status of animals and an institution is known and monitored over time (e.g. post-mortem records for deceased animals, disease testing programs). This underpins official certification.

3) The donor animal was not under quarantine restriction for the 90 days immediately prior to each semen collection, on the day(s) of each semen collection and for the 30 days immediately after each semen collection.

4) For the 30 days immediately before each semen collection, the donor animal showed no clinical signs or other evidence of infectious or contagious diseases, including the diseases retained for risk review in this policy.

5) The donor animal showed no signs of infectious or contagious disease at the time of each semen collection and for the 30 days immediately after.

6) The receiving institution must be approved under relevant Australian state or territory legislation to hold the relevant donor and recipient hippo species.

The period of clinical health may be longer under some disease's risk management requirements as a disease specific risk management measure.

4.3 Additional considerations for this policy

Additional baseline requirements

The operational and quarantine facilities requirements apply to all zoo hippos and their semen.

In addition, the WOAH Terrestrial Code specifies minimum risk mitigation standards for the international movement of live animals and their germplasm, for specific diseases. Where specific disease chapters within the WOAH Terrestrial Code stipulated measures applicable to the movement of all wild mammals and/or germplasm, these requirements were considered part of the baseline requirements for import under this policy. The relevant chapters within the WOAH Terrestrial Code are New World screwworm (*Cochliomyia hominivorax*) and Old World screwworm (*Chrysomya bezziana*), and infection with rabies virus. These chapters stipulate risk mitigation measures applicable to the movement of live zoo hippos, and those measures subsequently form part of this policy.

Explanatory notes

The residency periods and timing of tests in section 4.4 and section 4.5 are based on recommendations in the WOAH Terrestrial Code (where applicable) and are amended for consistency and clarity of certification.

For disease agents of biosecurity concern that have no recommendations in the WOAH Terrestrial Code for the periods of premises residency and/or disease freedom, the periods are based on the epidemiology and information detailed in the relevant sections in chapter 3.

Where the term 'approved' appears in conditions (e.g. 'an approved test') this refers to the approval by the department unless otherwise specified. Where possible, examples have been given of approved tests but importers may need to contact the department to check if other tests have been assessed as 'approved'.

The terminology for quarantine periods has changed since the release of the *Biosecurity Act 2015*. The legislation uses the phrase 'pre-arrival quarantine' for the period in the exporting country (off-shore) and the phrase 'post-entry quarantine' for the period in Australia. However, for consistency with other zoo policies the traditional terms 'pre-export quarantine' (PEQ) and 'post-arrival quarantine' (PAQ) are used in these conditions.

Where a test or multiple tests are required as part of risk mitigation for a specific disease, results of all relevant tests performed must be included in the final certification and all must comply with the requirements. For example, where an import requirement requires a single negative test result, any positive test result would render the animal ineligible (even if a negative test result is also obtained separately).

Equivalence

In accordance with Australia's international obligations under the SPS Agreement, the principle of equivalence applies to these biosecurity measures. Where the competent authority of an exporting country can objectively demonstrate that alternative biosecurity measure(s) to those required by the

department would provide an equivalent level of sanitary protection, the department will consider relevant submissions.

Submissions for equivalence must present evidence that the overall biosecurity risk management outcome can be met. The outcomes required for each disease may vary depending on its epidemiology, sensitivity and specificity of available tests, environmental factors in both the exporting country and Australia, and any other relevant matters. For example, a positive test result may not necessarily prevent import of an animal for a disease where a carrier state does not exist and where evidence can be provided that the animal is not currently infected.

Evidence to support a proposal for equivalence may include peer-reviewed literature (e.g. a new diagnostic test), health management records and records of post-mortem examinations that demonstrate freedom from a specific disease.

Proposals for equivalence require assessment on a case-by-case basis. They are usually examined after the permit-application stage. The time and resources required to assess a proposal for equivalence will vary depending on how far the proposal deviates from general policy. As a guide, the time frame for assessment of equivalence is expected to be several weeks. Large deviations from policy (e.g. where country-freedom has been proposed as the sole risk management measure) may take a prolonged amount of time or may not be possible to assess within the framework of an individual application.

4.4 Biosecurity measures for imports of live zoo hippos from approved countries

4.4.1 Documentation

Each zoo hippo must travel with an original international veterinary certificate that conforms to Article 5.10.2. of the WOAH Terrestrial Code, signed by an Official Veterinarian of the country of export.

An Official Veterinarian means a veterinarian authorised by the Veterinary Authority of the country of export to perform certain official tasks associated with animal health and/or public health, and inspections of commodities and, when appropriate, to certify in conformity with the certification procedures in chapter 5.2 of the WOAH Terrestrial Code.

The veterinary certificate must:

1) Be written in English and a language understood by the Official Veterinarian of the country of export.
2) Meet the requirements of the 4.4.3 certification section of this review and state that all the pre-export quarantine requirements have been met.
3) Provide unique identification for each zoo hippo, including the International Organization for Standardization (ISO) microchip number, a physical description, species, sex and age.
4) Include the name and address of the zoological or wildlife park of origin.
5) Include the name and address of the exporter and importer and identify the import permit against which it was issued.

6) Include the dates of pre-export quarantine of each zoo hippo.

7) Include original laboratory reports along with the dates of sampling for any tests required, the type of test used and the test results.

The Official Veterinarian must:

1) Scan and confirm as well as document the microchip number of each zoo hippo during the pre-export quarantine period and restate the relevant microchip number for each separate veterinary certificate.

2) Provide a veterinary certificate that is specific to the group of zoo hippos it covers.

3) Sign, date and stamp (with the stamp of the Veterinary Authority (Official Veterinarian stamp)) each page of the veterinary certificate and all attached documents (e.g. laboratory reports) that form part of the extended veterinary certification.

4) Endorse each page of copies of supporting documents with the date, signature and Official Veterinarian stamp.

5) Record their name, signature and official contact details on the veterinary certificate.

4.4.2 Pre-export quarantine requirements

Pre-export quarantine

A minimum pre-export quarantine period of 30 days applies.

Any variation from the pre-export quarantine requirements must have been specifically authorised by the Department of Agriculture, Fisheries and Forestry.

Location

The pre-export quarantine facility must be located within a government registered, licensed zoological institution or wildlife park that must provide separation from other animal populations, is under veterinary supervision and in which the animals held in the premises are subject to a health monitoring program that is capable of addressing Australia's biosecurity requirements.

1) The required outcome of veterinary supervision is up to date and regular informed knowledge of the animals, their health status, and the general health status of the institution that allows a veterinarian to sign off on these records.

2) The required outcome of separation is a sufficient distance or other barriers to maintain a distinct animal health status with regards to the diseases in this policy.

3) The required outcome of a health monitoring program is the regular monitoring, ongoing surveillance, and veterinary oversight to ensure that the health status of animals and an institution is known and monitored over time.

This underpins official certification.

Facilities

1) The pre-export quarantine facility must meet the country and premises requirements specified in the certification section (section 4.4.3).

2) The entire pre-export quarantine facility must be surrounded by physical and procedural barriers that provide sufficient security to isolate the zoo hippos in pre-export quarantine from all other animals except those that meet all the conditions in these biosecurity measures.

   The required outcome is that quarantined animals are protected from disease transmission, which includes direct contact, direct and indirect aerosol transfer, fomite transfer (e.g. footwear, feed, water).

3) Buildings holding hippos in the pre-export quarantine facility must be constructed so that they can be cleaned and disinfectant applied effectively, and must be maintained in good order.

4) The pre-export quarantine facility is serviced by a water supply that is not in direct communication with any other animal facility. The water is clean and not pumped directly from a natural watercourse without prior treatment.

5) The institution where the pre-export quarantine facility is located must utilise a separate area for the cleaning and disinfection of vehicles for transporting zoo hippos, and facilities for the safe loading and unloading of zoo hippos.

6) The institution where the pre-export quarantine facility is located must have a dedicated area to facilitate veterinary examination and collection of samples as needed, and must ensure biosecurity requirements are maintained should it be necessary to utilise these facilities for animals in pre-export quarantine.

Operation

1) The pre-export quarantine facility must have current approval from the Department of Agriculture, Fisheries and Forestry and the Veterinary Authority of the exporting country before commencement of pre-export quarantine.

2) The Department of Agriculture, Fisheries and Forestry may audit the approved pre-export quarantine facility.

3) All pre-export quarantine operations and procedures must be detailed in Standard Operating Procedures (SOPs), consistent with a risk-based approach and approved by the Department of Agriculture, Fisheries and Forestry.

4) The Official Veterinarian must inspect the pre-export quarantine facility within 72 hours before commencement of pre-export quarantine and must ensure that the facility was cleaned and disinfectant applied to their satisfaction.

5) Pre-export quarantine must be under the supervision of the Official Veterinarian.

6) The pre-export quarantine period commences from the time the last zoo hippo in the export consignment has entered the pre-export quarantine facility and all zoo hippos have been examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian.

7) All equipment used in feeding, handling and treating zoo hippos in pre-export quarantine must be new or cleaned and disinfected before entry, and must be used only in the facility during pre-export quarantine.

8) During pre-export quarantine, the facility must be occupied only by animals of the export consignment.

If other animals are present then all animals must demonstrate equivalent health status to the export consignment. This includes testing and providing the results of these tests to the department to demonstrate this.

9) Only personnel specifically authorised by the Official Veterinarian are permitted entry to the pre-export quarantine facility. Details of all visitor entries must be recorded and maintained.

10) All veterinary visits, health problems, tests, test results, treatments and reasons for removal from the pre-export quarantine facility of any animal, must be reported to the Official Veterinarian within 24 hours, and to the Department of Agriculture, Fisheries and Forestry within 48 hours. The sole exceptions to this are inspections, visits and treatments required for certification.

11) A detailed health record must be kept for each zoo hippo and be available to the Official Veterinarian and to the Department of Agriculture, Fisheries and Forestry on request.

12) Zoo hippos that leave the facility during pre-export quarantine for any reason not authorised by the Department of Agriculture, Fisheries and Forestry cannot re-join the consignment during pre-export quarantine.

4.4.3 Certification

The following must be met, and be certified to as such by the Official Veterinarian:

1) During pre-export quarantine:
    a) The zoo hippo(s) were not vaccinated.
    b) All zoo hippos in the pre-export quarantine facility remained free from evidence of infectious or contagious disease and the zoo hippo(s) were inspected at least daily for signs of disease.
    c) The zoo hippo(s) were isolated for at least 30 days prior to export.
    d) All samples for testing were taken by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian.
    e) All testing was conducted in a laboratory approved by the Veterinary Authority in the country of export.
    f) For any topical treatments administered, sufficient time must elapse (in accordance with manufacturer instructions or as otherwise approved by the department) to achieve drug efficacy following administration before the hippo has access to an aquatic environment.
2) The animal has been resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before export.
    a) The herd of origin (including any mammals sharing the same enclosure) has remained closed during the previous 12 months.

    Note: A closed herd in this context means that new mammals were not introduced to the collection (including mammals that were part of the collection for a time and were removed for a period prior to reintroduction).
3) During the pre-export quarantine period or the 90 days immediately prior, the animal was not under any quarantine restrictions (aside from pre-export quarantine).

4) All of the following risk management measures have been met:

a) Anthrax:

i) For 20 days immediately before export the animal has not resided on any premises where clinical, epidemiological or other evidence of anthrax has occurred in any species during the previous 20 days and the disease is compulsorily notifiable.

b) Brucellosis (*B. abortus* and *B. melitensis*):

i) For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of brucellosis (*B. abortus* and *B. melitensis*) has occurred in any species during the 5 years prior to export and the disease is compulsorily notifiable.

c) External parasites:

i) Within 24 hours prior to entering pre-export quarantine:

A Each hippo was examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and found to be visibly free of external parasites.

Note: examined in this context means that all visible surfaces of the animal(s) have been examined. This may require sedation or anaesthesia.

AND

B Each hippo was treated with an external parasiticide effective against ticks. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

AND

ii) Between 24 to 72 hours prior to export, each hippo was treated with an external parasiticide effective against ticks. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

AND

iii) If pre-export quarantine extends beyond 30 days, repeat treatments to maintain sufficient coverage against ticks throughout the pre-export quarantine period were applied. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

d) Internal parasites:

i) Within 24 hours prior to entering pre-export quarantine, each hippo was treated with a broad spectrum anthelmintic or combination of anthelmintics effective against nematodes and trematodes. The product name, active ingredients, dose rates and dates of treatments must be recorded on the veterinary certificate.

AND

ii) Between 24 to 72 hours prior to export, each hippo was treated with a broad spectrum anthelmintic or combination of anthelmintics effective against nematodes and trematodes. The product name, active ingredients, dose rates and dates of treatments must be recorded on the veterinary certificate.

AND

iii) If pre-export quarantine extends beyond 30 days, repeat treatments to maintain sufficient coverage against nematodes and trematodes throughout the pre-export quarantine period were applied. The product name, active ingredients, dose rates and dates of treatment must be recorded on the veterinary certificate.

e) *Mycobacterium tuberculosis* complex:

i) Option 1:

A For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

AND

B The animal received 2 separate skin tests for tuberculosis in the 210 days prior to export, with negative results. The tests were performed a minimum of 90 days apart from each other and one was performed during pre-export quarantine. Each test was either a TST or CTST. Each test was read 72 hours post-inoculation.

Note: where multiple skin tests for tuberculosis have been performed, all skin tests must be performed at least 90 days apart.

OR

ii) Option 2:

A For 12 months immediately before export the animals did not reside on any premises where clinical, epidemiological or other evidence of infection with Mycobacterium tuberculosis complex has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

AND

B The animal was tested for tuberculosis using 2 different tests, performed during the 30 days immediately before export, with both tests returning negative results. One test was either a TST or CTST and the test was read 72 hours post-inoculation. The second test was a serological test performed on a blood sample taken during this period using the TB STAT-PAK or another serological test approved by the department.

Note: where multiple skin tests for tuberculosis have been performed, all skin tests must be performed at least 90 days apart.

(The veterinary certificate must indicate the option that applies).

f) New World screwworm and Old World screwworm:

i) Option 1:

A For 60 days immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of screwworm-fly (*Cochliomyia hominivorax* or *Chrysomya bezziana*) occurred during the 12 months prior to export and the disease is compulsorily notifiable.

OR

ii) Option 2:

A Within 24 hours prior to entering pre-export quarantine all hippos in the consignment were examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and all animals were found to be visibly free from infested wounds.

Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

AND

B Within 24 hours prior to entering pre-export quarantine all wounds (if present) are prophylactically treated with an officially approved oily larvicide at the recommended dose.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

AND

C Within 24 hours prior to entering pre-export quarantine all animals were then treated immediately after inspection with a product officially approved for the control of New World or Old World screwworm, under supervision of an Official Veterinarian and in accordance with the manufacturer's recommendations.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

AND

D Between 24 to 72 hours prior to export all hippos in the consignment were examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian and found to be visibly free from infested wounds.

Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

AND

E Between 24 to 72 hours prior to export all wounds (if present) are prophylactically treated with an officially approved oily larvicide at the recommended dose.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

AND

F Between 24 to 72 hours prior to export the hippo was then treated, immediately after inspection, with a product officially approved for the control of New World or Old World screwworm, under supervision of an Official Veterinarian and in accordance with the manufacturer's recommendations.

Note: officially approved in this context means officially approved by the exporting country and agreed by DAFF.

(The veterinary certificate must indicate the option that applies).

g) Rabies virus:

i) Option 1:

A The animal showed no clinical sign of rabies the day prior to or on the day of export.

AND

B The animal was kept since birth or at least 180 days prior to export in a country considered free (as assessed by the Australian Department of Agriculture, Fisheries and Forestry) from infection with rabies virus.

OR

ii) Option 2:

A The animal showed no clinical sign of rabies the day prior to or on the day of export.

AND

B For 180 days immediately before export the animal has not resided on any premises in the country of export where clinical, epidemiological or other evidence of rabies virus has occurred during the 12 months prior to export and the disease is compulsorily notifiable.

(The veterinary certificate must indicate the option that applies).

h) Rift Valley fever:

i) For 90 days immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of Rift Valley fever has occurred during the 5 years prior to export and the disease is compulsorily notifiable.

i) Surra (*Trypanosoma evansi*):

i) For 12 months immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of *Trypanosoma evansi* has occurred in any species during the 12 months prior to export and infection is compulsorily notifiable.

j) *Trypanosoma vivax*:

i) For 12 months immediately before export the animal was continuously resident in a country where no clinical, epidemiological or other evidence of *Trypanosoma vivax* has occurred in any species during the 12 months prior to export and infection is compulsorily notifiable.

5) General inspection and transport:

a) The zoo hippo was examined by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian within 72 hours before leaving the pre-export quarantine facility for the port of export and was found to be:

i) Free from evidence of infectious or contagious disease.

ii) Visibly free of external parasites.

iii) Healthy and fit to travel.

b) Vehicles and transport containers used for transporting zoo hippos from the pre-export quarantine facility to the port of export, and to Australia, were new or were cleaned and disinfected to the satisfaction of the Official Veterinarian before entering the pre-export quarantine facility to load the zoo hippos.

c) The zoo hippo was sealed in its travel container with tamper-evident seals before leaving the pre-export quarantine facility for the port of export. The seal number is recorded on the veterinary certificate.

d) Arrangements are in place to ensure that the zoo hippo had no contact with other animals prior to departure of the vessel or aircraft except those that meet all the conditions in these biosecurity measures.

4.4.4 Transport

1) Exporters or their agents must have detailed plans to cover procedures, including contingency plans, for transporting the animal from pre-export quarantine until arrival in Australia.

2) Animals must be consigned to Australia by a route approved by the Department of Agriculture, Fisheries and Forestry.

3) Animals must travel in a container recommended for that particular species under the International Air Transport Association (IATA) Live Animal Regulations.

4) The use of hay or straw as bedding during transport is not permitted. Treated wood shavings, sterilised peat and soft board can be used.

5) Animals must remain isolated from all animals except those that meet all the conditions described in these biosecurity measures, during transport from the pre-export quarantine facility until arrival in Australia.

6) Insect netting must be carried on the flight at all times for contingencies. There must be sufficient insect netting to cover all travel containers completely. Insect netting must be in good condition to minimise entry of insect vectors into the travel containers.

Transit and transhipment

1) Animals must transit or tranship only at an approved airport. Any transhipment requires the prior approval of the Department of Agriculture, Fisheries and Forestry. Animals are not to leave the airport and must not be removed from their travel containers during transit or transhipment.

2) Animals must remain on board the aircraft at approved transit airports. Cargo doors can be opened at approved transit airports to allow for unloading or loading of freight. Immediately after the cargo hold doors are closed, a knockdown aerosol insecticide must be sprayed throughout the cargo hold, in the manner recommended by the manufacturer.

3) In cases where animals in travel containers are to be unloaded, before opening the cargo door, the travel containers must be completely covered in netting to prevent insect access to the animals. The netting must remain in place until the animals are reloaded onto an aircraft. Immediately after the animals are reloaded onto an aircraft and the cargo hold doors are closed,

a knockdown aerosol insecticide spray must be sprayed throughout the cargo hold in the manner recommended by the manufacturer. The insect netting must not be removed until 30 minutes after spraying.

Delayed take off and unscheduled landings

1) Exporters or their agents must have contingency plans for the management of delayed take off and unscheduled landings.

2) If the aircraft lands at any airport other than in an approved country, the department must be informed immediately, and the animal must not proceed to Australia without approval from the department. The decision as to whether the animal can continue to travel to Australia, and additional biosecurity measures that may be required, will be made by the Department of Agriculture, Fisheries and Forestry on a case-by-case basis after assessing the risks.

Arrival in Australia

1) Importers or their agents must have a plan developed in consultation with the Department of Agriculture, Fisheries and Forestry to cover post-arrival procedures. The plan must include roles and responsibilities for their staff, vehicles for transporting animals to the approved arrangement site (AA site) and road transport arrangements, including contingency plans for vehicle and equipment failures.

2) Vehicles for transporting the animals from the port of entry to the AA site must be cleaned and disinfected to the satisfaction of the Australian government biosecurity officer before loading the animals. The Department of Agriculture, Fisheries and Forestry must be advised of the transport route to the AA site.

3) After the animals arrive at an Australian airport, they must be transferred in their transport containers onto vehicles, along with personnel and equipment, and proceed directly to the AA site.

4) All biosecurity risk material (e.g. bedding, feed, water and waste material) remaining at the airport must be sealed in bags and disposed of as biosecurity waste under the supervision of the Department of Agriculture, Fisheries and Forestry.

5) All other equipment used during transport that has been in contact with the animal (including the outside of the crate) must be cleaned and disinfected under supervision of the Department of Agriculture, Fisheries and Forestry before leaving the airport.

4.4.5 Post-arrival quarantine requirements

Post-arrival quarantine

The minimum post-arrival quarantine period of 30 days applies. Any variation from the post-arrival quarantine requirements must be specifically authorised by the Department of Agriculture, Fisheries and Forestry.

Location

The AA site must be located within a secure part of a zoo, wildlife park or research institute approved under relevant Australian State or Territory legislation to hold the species being imported, separated from public access areas and where it is under regular supervision by a registered veterinarian.

Facilities

The post-arrival quarantine facility must meet the Department of Agriculture, Fisheries and Forestry requirements of a class 7.9 AA site.

Operation

1) The AA site must be approved by the Department of Agriculture, Fisheries and Forestry before entry of an animal into the AA.
2) All post-arrival quarantine operations and procedures must follow those outlined for an AA class 7.9 facility and also include:
    a) A registered veterinarian must inspect the AA site within 72 hours before entry of any animal to ensure it has been cleaned and disinfectant has been applied to their satisfaction.
    b) The post-arrival quarantine period will commence from the time of entry into the facility of the last animal.
    c) Vehicles for transporting animals must not leave the AA site until thoroughly cleaned and disinfected.
    d) If any animal dies during post-arrival quarantine, the Department of Agriculture, Fisheries and Forestry must be notified as soon as possible and no later than 48 hours later, and the animal must undergo a post-mortem investigation by a registered veterinarian to determine the cause of death.
    e) The Department of Agriculture, Fisheries and Forestry must be advised as soon as possible, and no later than 48 hours later, of any disease incident and its outcome.
    f) Animals and goods subject to biosecurity control must not leave the AA site during post-arrival quarantine without the permission of the Department of Agriculture, Fisheries and Forestry.
    g) At the satisfactory completion of post-arrival quarantine, the animals will be released from biosecurity control into premises approved by the appropriate State or Territory governments for the holding of zoo hippos.
3) The following post-arrival risk management measures apply as appropriate:
    a) New World screwworm and Old World screwworm (where Option 2 has been selected to meet Australia's import conditions):
        i) Within 72 hours of arrival all hippos in the consignment were examined by the veterinarian in charge of post-arrival quarantine and found to be visibly free from infested wounds.

            Note: examined in this context means that all surfaces of the animal(s) have been examined, including skin folds and the oral cavity. This may require sedation or anaesthesia.

        ii) If infestation is found, the wound must be immediately treated with an officially approved oily larvicide at the recommended dose, and the Department of Agriculture, Fisheries and Forestry must be immediately contacted for further advice.

4.5 Biosecurity measures for imports of zoo hippo semen from approved countries

4.5.1 Documentation

Each consignment of zoo hippo semen must travel with an original international veterinary certificate that conforms to Article 5.10.3 of the WOAH Terrestrial Code, signed by an Official Veterinarian of the country of export.

An Official Veterinarian means a veterinarian authorised by the Veterinary Authority of the country of export to perform certain official tasks associated with animal health and/or public health, and inspections of commodities and, when appropriate, to certify in conformity with the certification procedures in chapter 5.2 of the WOAH Terrestrial Code.

The veterinary certificate must:

1) Be written in English and a language understood by the Official Veterinarian of the country of export.
2) Meet the requirements of the certification – zoo hippo semen section of this review (section 4.5.2) and state that all the pre-export requirements have been met (including pre-collection, collection and post-collection requirements).
3) Include the name and address of the zoological or wildlife park of origin.
4) Include the name and address of the exporter and importer.
5) Identify the import permit against which it was issued.
6) Provide the name and species for each semen donor.
7) Provide the herd or stud book number for each semen donor.
8) Provide unique identification for each zoo hippo (International Organization for Standardization (ISO) microchip number) including description, species, sex and age.
9) Provide the date(s) of each semen collection for each donor.
10) Provide the date of entry that donor entered the collection centre or resident herd.
11) Provide the number of straws in the consignment for each donor and contain the means to verify the identification of the semen straws with the identification details of the donor.
12) Include the original laboratory reports along with dates of sampling for any tests required, the type of test used and the test results. This information must be contained in a table against donor information.
13) Provide the dates of isolation of the semen donor from other animals that did not meet the same biosecurity conditions.

The Official Veterinarian must:

1) Sign, date and stamp (with the stamp of the Veterinary Authority) each page of the veterinary certificate and all attached documents (e.g. laboratory reports) that form part of the veterinary health certification.

2) Endorse each page of copies of supporting documents with date, signature and Official Veterinarian stamp.

3) Record their name, signature and contact details on the veterinary certificate.

4.5.2 Certification

The following must be met, and be certified to as such by the Official Veterinarian:

1) General:
    a) The semen was not removed from containers for further processing or aggregation unless previously arranged with the Department of Agriculture, Fisheries and Forestry.
    b) All semen collected for export to Australia must meet all of the risk management measures specified in this section.
    c) In cases where testing of semen is applied, at least one sample from every ejaculate must be assessed, unless otherwise directed by the department. Where multiple samples are tested, all must return the required result.
2) Semen collection and processing:
    a) The semen was hygienically collected, handled and processed:
        i) At a registered zoo or wildlife park in the exporting country that meets the general risk measures for zoo semen as detailed at the start of chapter 4.
        ii) Using disinfected or sterilised implements.
        iii) Using products of animal origin, including additives or a diluent, that were obtained from sources which present no animal health risk or were treated prior to use such that the risk is managed, in accordance with requirements for bovine, small ruminant and porcine semen in the WOAH Terrestrial Code (Article 4.7.7.1).
3) Diagnostic testing:
    a) The samples for diagnostic testing were collected by an Official Veterinarian or a veterinarian authorised by the Official Veterinarian.
    b) Tests for disease were carried out at a laboratory approved by the competent authority.
    c) The tests were conducted in accordance with the current WOAH Manual for Diagnostic Tests and Vaccines for Terrestrial Animals or were approved by the department.
    d) All disease testing results are tabulated, including donor identification, dates of sampling for test, type of tests used, test results and are verified by the Official Veterinarian.
4) The Official Veterinarian:
    a) Ensured all samples for testing were taken either by the Official Veterinarian or a veterinarian authorised by the Official Veterinarian.
    b) Ensured that the donors were tested in accordance with all requirements.
    c) Recorded the required details for each donor on the table attached to the veterinary certificate.
    d) Ensured the hygienic collection, handling and processing of the semen.

e) Verified the permanent identification of the semen straws with the identification details of the donor and the date of collection or a code from which this information could be determined.

5) Disease freedom and risk management – general:

a) The donor animal was not under quarantine restriction for the 90 days immediately prior to each semen collection, on the day(s) of each semen collection and for the thirty (30) days immediately after each semen collection.

b) Donors showed no clinical signs of infectious or contagious disease on the day(s) of each semen collection and for thirty (30) days before and after each collection day(s).

c) The donor animal must be resident in an approved, licensed or registered zoo or wildlife park in the exporting country since birth or for at least 12 months immediately before collection.

i) The herd of origin of the donor animal (including any mammals sharing the same enclosure) has remained closed during the previous 12 months.

Note: A closed herd in this context means that new mammals were not introduced to the collection (including mammals that were part of the collection for a time and were removed for a period prior to reintroduction).

6) Specific risk management measures for the following diseases have been met:

a) Brucellosis (*B. abortus* and *B. melitensis*):

i) For 12 months immediately before each semen collection the donor did not reside on any premises where clinical, epidemiological or other evidence of brucellosis (*B. abortus* and *B. melitensis*) has occurred in any species during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

b) *Mycobacterium tuberculosis* complex:

i) Option 1:

A For 12 months immediately before each semen collection the donor animal did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

AND

B The donor animal received 2 separate skin tests for tuberculosis in the 210 days prior to each semen collection, with negative results. The tests were performed a minimum of 90 days apart from each other and one was performed in the 30 days immediately before each semen collection. Each test was either a TST or CTST. Each test was read 72 hours post-inoculation.

Note: where multiple semen collections were performed and the testing timeframes for each overlap, all skin tests for tuberculosis must be performed at least 90 days apart.

OR

ii) Option 2:

A For 12 months immediately before each semen collection the donor animal did not reside on any premises where clinical, epidemiological or other evidence of infection with *Mycobacterium tuberculosis* complex has occurred during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

AND

B The donor animal was tested for tuberculosis using 2 different tests, performed during the 30 days immediately before each semen collection, with both tests returning negative results. One test was either a TST or CTST and the test was read 72 hours post-inoculation. The second test was a serological test performed on a blood sample taken during this period using the TB STAT-PAK or another serological test approved by the department.

Note: where multiple semen collections were performed and the testing timeframes for each overlap, all skin tests for tuberculosis must be performed at least 90 days apart.

(The veterinary certificate must indicate the option that applies).

c) Rift Valley fever:

i) For 90 days immediately prior to each semen collection the donor animal was continuously resident in a country where no clinical, epidemiological or other evidence of Rift Valley fever has occurred in any species during the 5 years prior to each semen collection and the disease is compulsorily notifiable.

6) Storage and transport:

a) From the time of chilling or freezing until export, the reproductive material in the consignment was:

i) Kept in sealed sterile containers (e.g. straws, ampoules or vials) and code marked in line with the international standards of the International Committee for Animal Recording (ICAR).

ii) Stored and transported EITHER:

A Only with other zoo hippo semen collected for export to Australia, or of equivalent health status;

OR

B With other export certified germplasm eligible for export to Australia;

and all germplasm containers were intact and there were no damaged or broken straws, ampoules or vials in the shipping container.

iii) Kept in a secure place within an approved centre or laboratory and under the supervision of the Official Veterinarian.

iv) Stored and transported in storage or shipping containers containing only new, unused liquid nitrogen.

v) Stored for at least 30 days.

7) Shipping containers (liquid nitrogen shippers or tanks):

   a) EITHER:

      i) The shipping container was new.

      OR

      ii) Immediately prior to loading, the shipping container was emptied and inspected and any loose straws removed. The shipping container, including all surfaces in contact with the straws, ampoules or vials was then disinfected with one of the following disinfectants: 2% available chlorine (e.g. chlorine bleach), 2% Virkon or irradiated at 50 kGy.

   b) The veterinary certificate must indicate the option that applies. For used shipping containers, the date of disinfection, the disinfectant used and its active chemical must be recorded on the certificate.

8) Official government seals:

   a) Under the supervision of an Official Veterinarian prior to export to Australia:

      i) The identity of the semen was checked prior to being placed into new, unused liquid nitrogen in a shipping container for export that was new or disinfected as specified in clause 7.a.

      ii) The containers (e.g. straws, ampoules or vials) for reproductive material in the consignment were checked and confirmed as being sealed.

   b) Only zoo hippo semen that met Australian import conditions was included in the shipping container.

   c) An official government seal was applied by an Official Veterinarian to the shipping container and the number or mark on the seal recorded on the veterinary certificate.

4.6 Review of processes

4.6.1 Review of policy

The Department of Agriculture, Fisheries and Forestry can review the import policy after the first year of trade or when the disease or phytosanitary status in approved countries may have changed, or if new information about a disease becomes available that may impact the biosecurity risk.

Glossary

Term	Definition
AA	approved arrangement
ALOP	Appropriate level of protection
anergy	Absence of the normal immune response to a particular antigen or allergen
appropriate level of protection (ALOP) for Australia	The *Biosecurity Act 2015* defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero.
approved arrangement site	An approved arrangement site audited and approved by the department, where post-arrival quarantine occurs.
approved countries	For the purpose of this review, approved countries are Austria, Belgium, Canada, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Japan, Luxembourg, Netherlands, New Zealand, Portugal, Singapore, Spain, Sweden, the United Kingdom, and the United States
artiodactyl	Any member of the mammalian order Artiodactyla
Australian territory	Australian territory as referenced in the *Biosecurity Act 2015* refers to Australia, Christmas Island and Cocos (Keeling) Islands.
AUSVETPLAN	Australian Veterinary Emergency Plan
BA	Biosecurity advice
BHV-1	*Varicellovirus bovinealpha1* (Bovine alphaherpesvirus-1)
biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
biosecurity measures	The *Biosecurity Act 2015* defines biosecurity measures as measures to manage any of the following: biosecurity risk, the risk of contagion of a listed human disease, the risk of listed human diseases entering, emerging, establishing themselves or spreading in Australian territory, and biosecurity emergencies and human biosecurity emergencies.
biosecurity risk	The *Biosecurity Act 2015* refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
BoDV	*Orthobornavirus bornaense* (Borna disease virus)
BTEC	Brucellosis and Tuberculosis Eradication Campaign
CATT	Card agglutination test
CMI	cell mediated immunity
CNS	Central Nervous System
CTST	Comparative tuberculin skin test
DAFF	Department of Agriculture, Fisheries and Forestry
DNA	deoxyribonucleic acid
EADRA	Emergency animal disease response agreement
ELISA	enzyme-linked immunosorbent assay
endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
FMD (FMDV)	Foot-and-mouth disease (Foot-and-mouth disease virus)

Term	Definition
goods	The *Biosecurity Act 2015* defines goods as an animal, a plant (whether moveable or not), a sample or specimen of a disease agent, a pest, mail or any other article, substance or thing (including, but not limited to, any kind of moveable property).
Hippos	Hippopotamus. May represent either species of extant hippopotamus: *Hippopotamus amphibius* (common hippopotamus) and *Choeropsis liberiensis* (syn. *Hexaprotodon liberiensis*) (pygmy hippopotamus).
HS	Haemorrhagic septicaemia
host	An organism that harbours a parasite, mutual partner, or commensal partner, typically providing nourishment and shelter.
IBR	Infectious bovine rhinotracheitis
IgG	Immunoglobulin G
IgM	Immunoglobulin M
import permit	Official document authorising a person to bring or import particular goods into Australian territory in accordance with specified import requirements.
IPV	Infectious pustular vulvovaginitis
MTBC	*Mycobacterium tuberculosis* complex
non-regulated risk analysis	Refers to the process for conducting a risk analysis that is not regulated under legislation (*Biosecurity import risk analysis guidelines 2016*).
Official Veterinarian	A veterinarian authorised by the Veterinary Authority of the country of export to perform certain official tasks associated with animal health and/or public
OIE	Previous name for the World Organisation for Animal Health
pathogen	A biological agent that can cause disease to its host.
PCR	polymerase chain reaction
PEQ	Pre-export quarantine
PPD	Purified protein derivative
PRNT	plaque-reduction neutralisation test
qPCR	Quantitative polymerase chain reaction
quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing or treatment.
RABV	*Lyssavirus rabies* (rabies virus)
restricted risk	Risk estimate with phytosanitary measure(s) applied.
risk analysis	Refers to the technical or scientific process for assessing the level of biosecurity risk associated with the goods, or the class of goods, and if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or class of goods to a level that achieves the ALOP for Australia.
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
RVF (RVFV)	Rift Valley fever (*Phlebovirus riftense* (Rift Valley fever virus))
SFTS	severe fever with thrombocytopenia syndrome
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
stakeholders	Government agencies, individuals, community or industry groups or organisations, in Australia or overseas, including the proponent or applicant for a specific proposal, that have an interest in the policy issues.
surveillance	An official process that collects and analyses information related to animal health.

Term	Definition
TST	Tuberculin skin test
unrestricted risk	Unrestricted risk estimates apply in the absence of risk mitigation measures.
vector	An organism that does not cause disease itself, but which causes infection by conveying pathogens from one host to another.
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WOAH Terrestrial Code	WOAH Terrestrial Animal Health Code 2023
WOAH Manual	WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2023
WTO	World Trade Organization

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