Australian Government
Department of Agriculture, Water and the Environment

Review of risks associated with glanders (*Burkholderia mallei*) in horses and horse semen

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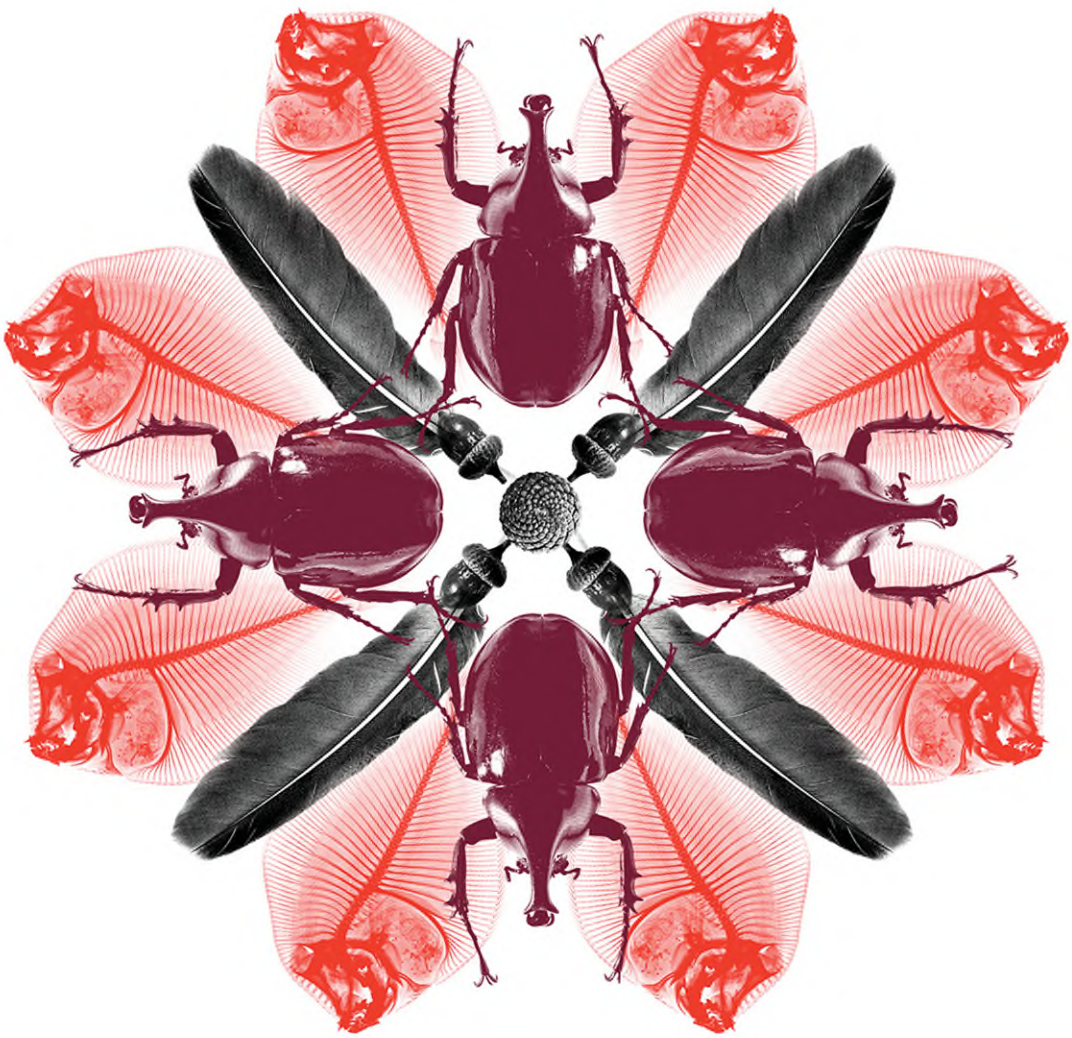

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Contents

Summary

The Department of Agriculture, Water and the Environment has reviewed the level of biosecurity risk in relation to glanders (*Burkholderia mallei*) to determine if it has changed since the completion of the *Import risk analysis report for horses from approved countries: Final report (*the Horse IRA), released in March 2010 and the *Import risk analysis report for horses from approved countries: Final policy review (*the Horse Review), released in August 2013. These reports considered the biosecurity risks for Australia associated with the importation of live horses from approved countries. Glanders was not present in approved countries and therefore Australia adopted risk management measures in accordance with the World Organisation of Animal Health (OIE) recommendations. 'Horse' refers to horses, donkeys and mules unless otherwise specified.

This policy review takes into account new and relevant peer-reviewed scientific information, advice from international scientific experts, and relevant changes in industry practices and operational practicalities.

This policy review recommends that the import conditions for importation of horses and horse semen to Australia from approved countries be standardised for glanders and continue to align with the OIE recommendations for importation from countries free from glanders. This policy review replaces the former glanders chapters in the Horse IRA and the Horse Review.

Glanders

Background

To protect Australia's unique biosecurity status, the Department of Agriculture, Water and the Environment imposes strict import conditions that must be met before horses can be imported into Australia. The last 60 days of residence must be spent in a country approved to export horses directly to Australia. Currently, horses must reside in a glanders free country for 180 days prior to export to Australia. Countries must be free from glanders for at least three years in order to export horses to Australia or provide sufficient evidence of country freedom in accordance with the OIE Code. These conditions are in accordance with OIE Code recommendations that horses reside in a glanders free country or zone, in which there have been no cases of glanders during the past three years, for six months immediately prior to export (OIE, 2021).

Glanders is a highly contagious bacterial disease of horses, donkeys and mules (Dvorak and Spickler, 2008; Hungerford, 2007). The causative agent of glanders is *Burkholderia mallei* (formerly *Pseudomonas mallei*), a small gram negative, non-sporulating bacillus (Hungerford, 2007). *B. mallei* is closely related to *B. pseudomallei*, the causative agent of melioidosis (pseudoglanders, Whitmore disease) in humans and animals (Spickler, 2018). Glanders is a World Organisation for Animal Health (OIE) listed disease which is exotic to Australia and nationally notifiable. Glanders is a zoonotic disease and is considered a bioterrorism agent by the United States Centers for Disease Control and Prevention (CDC, 2017).

After an initial spike in cases at the time of identification of the bacterial aetiology of glanders in 1882, the incidence of disease dropped globally (OIE Technical disease cards, 2020). This is likely due to the introduction of eradication policies (OIE Technical disease cards, 2020). Glanders is considered a re-emerging disease in India and the Middle East due to a steady increase in the number of cases after years of minimal to no case detections (Khan et al, 2013; Anderson, 2013).

Glanders has been reported in carnivores that have consumed infected horse meat (Hungerford, 2007; Khaki et al, 2012). Rare cases have been reported in camels, goats and sheep (Dvorak and Spickler, 2008; Wernery et al, 2011). Dvorak and Spickler (2008) found that a variety of mammals could be infected experimentally. However, cattle, pigs, rats and birds are relatively resistant to experimental infection (Dvorak and Spickler, 2008).

In horses the disease can manifest in acute, chronic and latent forms. The acute form results in septicaemia and death within few days (Hungerford, 2007). The chronic form results in ulcerating, purulent nodules developing in the respiratory tract, skin and lymph nodes (Hungerford, 2007). Chronic illness can last for months before death or recovery to become a carrier (Hungerford, 2007). Latent infections are difficult to detect with current diagnostic tests (Kettle and Wernery, 2016).

There has been one reported occurrence of glanders in Australia in 1891 (Geering et al, 1995). This incident involved circus horses imported from the United States into a quarantine facility in Sydney (Geering et al. 1995). All infected horses and their contacts were euthanased, and the remaining horses exported back to the United States (Ellis, 2020).

Technical information

Agent properties

B. mallei is a gram negative, non-motile and non-sporulating bacillus of the family *Burkholderiaceae* (OIE Technical disease cards, 2020). Unlike other members of the *Burholderiaceae* family, it is a facultative intracellular obligate bacteria that relies on host transmission for dispersal (Timoney, 2014). *B. mallei* has a polysaccharide capsule to enhance environmental survival and virulence (Timoney, 2014).

B. mallei can survive outside of host species under specific environmental conditions found in tropical environments (OIE Technical disease cards, 2020). *B. mallei* is susceptible to intense heat, direct sunlight and disinfectants (Timoney, 2014). Direct sunlight inactivates *B. mallei* within 24 hours, and it is destroyed when exposed to temperatures over 55˚C for more than 10 minutes (OIE Technical disease cards, 2020). However, Dvorak and Spickler (2008) found that the bacteria could remain viable in water at room temperature (20˚C to 25˚C) for up to one month. Some studies have found that the bacteria could remain viable in contaminated stables for up to six weeks in humid or wet conditions out of direct sunlight (OIE Technical disease cards, 2020; Verma et al, 2014). *Burkholderia pseudomallei* is present in northern Australia and a small area of Western Australia (near Perth). Conditions in these areas may also be conducive for *B. mallei* to be sustained within the environment. *B. mallei* is susceptible to most common disinfectants such as iodine, benzalkonium chloride (1/2000), sodium hypochlorite (500 ppm available chlorine), 70% ethanol and 2% glutaraldehyde (OIE Technical disease cards, 2020).

There is a lack of information available on the survival of *B. mallei* in refrigerated or freezer conditions.

Distribution

Once prevalent worldwide, glanders has been eradicated from many areas of the world including Canada, Western Europe and the United States (Dvorak and Spickler 2008; Derbyshire, 2002). However, the disease persists in many parts of Asia, North Africa, South America and the Middle East (Kettle and Wernery, 2016). Between 1996 and 2021, the OIE received reports of cases of glanders in Afghanistan, Bahrain, Belarus, Bolivia, Brazil, China, Eritrea, Ethiopia, Germany, India, Iran, Iraq, Kuwait, Lebanon, Mongolia, Nepal, the Philippines, Russia and Turkey (OIE Code, 2020; Wernery et al, 2011; Singha et al, 2020; Sholz et al, 2014). The illegal movement of horses across borders has been linked to glanders outbreaks, highlighting the importance of border control measures. In 2021, Nepal recorded its first outbreak of glanders. Evidence indicates that this was due to the illegal movement of infected horses entering Nepal from India’s Uttar Pradesh province (ProMed, 2021; OIE WAHIS report, 2021).

Glanders has previously been reported in two countries approved to export horses to Australia. The United Arab Emirates reported a case of glanders in 2004 and Germany reported one case in 2006 and one in 2014 (ProMed, 2004; OIE WAHIS report, 2015). In the United Arab Emirates case, the disease was identified in an imported horse during post-import quarantine and the horse did not enter the general horse population. In the 2014 German case, the horse was in the general horse population when it tested positive for glanders during routine pre-export screening for another country. This led to Australia suspending the import of live horses and their semen from Germany until the occurrence was investigated and freedom from disease demonstrated. The incident was resolved and the department now recognises Germany as glanders free. There have been no other reports of glanders in any other approved countries (OIE WAHIS report, 2020).

The department has been asked by industry about the glanders health status of Mauritius, the Republic of South Africa and South American countries. These countries have not been assessed for glanders status by the department. As a result, horses from these regions must undertake six months residency in a country considered glanders free by the department. Due to a lack of transport routes and current import requirements, it would not currently be possible to export horses directly from any of these regions to Australia.

Mauritius

There is insufficient evidence to indicate that Mauritius is free from glanders. Although the country has never reported a case of glanders to the OIE, there is a lack of published information about horse diseases in Mauritius. An audit of horse health systems in Mauritius was conducted by the European Union in 2008 and identified '… operational and technical shortcomings, related to the export procedure of horses to the European Union, and systemic deficiencies related to the animal health controls and rules for import into the country …' (European Commission audit report of animal health in Mauritius, 2009). Major export markets, such as the European Union and the United States, do not recognise Mauritius as glanders free and require imported horses to undergo testing. As a result, Australia would need to conduct a detailed assessment about the horse health system and border controls in Mauritius.

Republic of South Africa

The last reported case of glanders in the Republic of South Africa was in 1945, but glanders remains endemic in northern Africa (OIE WAHIS report, 2020). A European Union audit conducted in 2013 identified a number of shortcomings in the Republic of South Africa's management and surveillance for horse diseases. It stated that '… surveillance of African horse sickness and other horse diseases is [further] affected by the unclear organisation of the laboratory network and significant shortcomings observed in the national laboratory …' and weak disease surveillance (European Commission audit report of animal health in the Republic of South Africa, 2013). African horse sickness, another disease of significant biosecurity concern to Australia, is present in the Republic of South Africa and limits the market access for horses from the Republic of South Africa. The United States requires additional quarantine time and testing procedures for African horse sickness and glanders for horses being imported from the Republic of South Africa.

South America

Glanders is endemic in many countries in South America (Kettle & Wernery, 2016). In addition, not all South American countries routinely report to the OIE. Bolivia reported 23 cases of glanders in horses to the OIE in 2001. Brazil reported more than 20 new cases of glanders to the OIE in 2019 (OIE WAHIS report, 2021). Rolim et al (2018) identified that the environmental conditions in South America were appropriate to support *B. pseudomallei* survival. These conditions may also be conducive to supporting the survival of *B. mallei* in the environment; although *B. mallei* is not as resistant to environmental conditions as *B. pseudomallei*. The European Union and United States accept horses from certain areas of South America but do not recognise these areas as glanders free and require imported horses to undergo testing for glanders. Australia has not assessed any South American countries for exporting horses or their reproductive material.

Epidemiology

B. mallei is readily transmitted by respiratory secretions, and skin exudates that can contain large numbers of infective bacteria (CFSPH, 2019). Transmission commonly occurs via direct ingestion, inhalation or skin contamination of abraded skin or mucus membranes (Dvorak & Spickler, 2008). Indirect transmission can occur by fomites such as contaminated food, water, general husbandry equipment, breeding equipment or people (Dvorak & Spickler, 2008). Vertical and venereal transmission have been suggested but not demonstrated (Khan et al, 2012; Gregory & Waag, 2007). Other potential transmission pathways have not been ruled out. Al-Ani et al, (1998) reported severe orchitis in two stallions infected with glanders but did not determine if *B. mallei* was present in the semen of the affected horses. Such localised inflammation may be an extension of the cutaneous form of glanders, contracted via direct contact with contaminated bedding (Al-Ani et al, 1998).

Chronically or latently infected animals with minimal signs of disease may serve as a pathogen reservoir and are often responsible for the maintenance and spread of disease (Dvorak and Spickler 2008). Chronic and subclinically infected cases can shed bacteria continuously or intermittently (OIE, 2009).

Infection is not influenced by age, breed and sex (Khan et al, 2012). Overcrowding, close contact with infected animals and poor hygiene practices are believed to increase the likelihood of transmission (Khan et al, 2012).

Big cats and other carnivores have acquired *B. mallei* infection from eating infected horse meat (Dvorak and Spickler, 2008; Khaki et al, 2012). Khaki et al (2012) reported a case of lions and a tiger contracting glanders at Tehran Zoo, Iran after eating contaminated horse meat. A similar case was also reported in Turkey (Khaki et al, 2012). Small ruminants such as goats and sheep may be infected if in direct contact with infected horses (Dvorak and Spickler, 2008; Malik et al, 2012).

Glanders infections in humans are rare (Ulrich et al, 2005). In humans, glanders is an occupational disease that affects individuals in close contact with infected animals such as farmers, trainers, veterinarians and laboratory personnel (Saqib et al, 2012). The only reported human case of glanders since 1949 occurred in 2000 when a laboratory worker in the United States did not wear appropriate personal protective equipment to handle a glanderous sample (Srinivasan et al, 2001). The individual recovered after long term antibiotic treatment (Srinivasan et al, 2001). Human glanders has a 95% case fatality rate in untreated infections and a 50% case fatality rate in aggressively treated individuals (Saqib et al, 2012). Person-to-person transmission of glanders is also a rare event that has been primarily associated with direct contact with exudates from skin lesions, respiratory secretions or sputum of an infected individual (Verma et al, 2014).

Pathogenesis

Mucus membranes of the eye, skin, respiratory and gastrointestinal tract are the preferred tissues for *B. mallei* to enter a host (Verma et al, 2014). *B. mallei* uses the host's lymphatic system and bloodstream to migrate to predilection sites such as the lungs (Verma et al, 2014).

B. mallei, as a facultative intracellular pathogen, enters and replicates in epithelial and phagocytic cells. This is critical to its success as a pathogen. Once inside the host cell, *B. mallei* secretes proteins to disrupt the vacuolar membrane, escapes into the host cell cytosol and then move within the cell using actin-based motility (Gaylov et al, 2010). Once inside the cytosol, the bacteria moves into adjacent cells via membrane fusion creating multi-nucleated giant cells (Gaylov et al, 2010). This intracellular lifestyle and the formation of multinucleated giant cells help *B. mallei* avoid the host immune response.

The pathogenesis and host immune response to *B. pseudomallei* is better understood than its response to *B. mallei*. An immune response is not always triggered by infection with *Burkholderia* spp. Acute disease in response to *B. pseudomallei* has been proposed to be due to hyperinflammation following a breakdown in immune regulation (Gan, 2005). Chronic infections in response to *B. pseudomallei* are thought to occur when the extent of the infection is not identified by the host immune system, resulting in a progressive onset of clinical signs (Gan, 2005). Latent infection with *B. pseudomallei* may result from the organism not being recognised by the host immune system (Gan, 2005). The mechanisms by which *B. mallei* causes dysregulation of the host immune system are not well defined and are thought to be similar to *B. pseudomallei.*

Diagnosis

Clinical signs

Horses, donkeys and mules can develop acute, chronic or latent forms of glanders. The chronic form is often further classified as cutaneous, nasal or pulmonary depending on organs involved in the infection and subsequent clinical signs.

Acute glanders is more frequently diagnosed in donkeys and mules than in horses (Khan et al, 2012; Mota et al, 2010). This form of the disease is characterised by marked pyrexia, coughing, inspiratory dyspnoea, watery unilateral or bilateral nasal discharge and deep, rapidly spreading ulcers on the nasal mucosa (Khan et al, 2012). As the disease progresses, the nasal discharge becomes a thick mucopurulent or haemorrhagic discharge which causes severe respiratory distress (Khan et al, 2012). Submaxillary lymph nodes may be swollen and painful, and facial lymphatic vessels thickened (Khan et al, 2012). Death usually occurs within 7-10 days of the onset of clinical signs due to bronchopneumonia and septicaemia (Kettle and Wernery, 2016).

The chronic form of glanders more commonly affects horses rather than donkeys and mules (Khan et al, 2012, Spickler, 2018). It is insidious in onset and clinical signs may include coughing, malaise, intermittent pyrexia and a chronic purulent nasal discharge (usually unilateral). Other signs may include ulcers and nodules on the nasal mucosa, cutaneous lesions that rupture and ulcerate, chronic enlargement and induration of the lymphatics and lymph nodes, painful oedema of the legs and swollen joints. Chronic infection can develop over weeks to months (Dvorak and Spickler 2008; Spickler 2018). It is slowly progressive and often fatal, although animals may survive for years (Spickler 2018).

Chronic cutaneous glanders is also known as ‘farcy’ and is typified by crater-like ulcerating lesions around the muzzle and hindlimbs (Spickler, 2018). In some cases, lesions can be found on other areas of the body. Ulcers are often associated with lymphatic vessels and result in enlarged lymphatics known as ‘farcy chords’, and may produce a purulent discharge.

Chronic nasal glanders is characterised by unilateral or bilateral nasal discharge with associated crusting around the nostrils (Arun et al, 1999; Spickler 2018). The nasal discharge is purulent, or haemorrhagic due to the ulcerating lesions in the nasal cavity. Inflammation and the healing of ulcers can lead to stellate (star-shaped) white scars on the nasal septum (Arun et al, 1999). Associated lymphadenopathy may be present (Spickler, 2018).

Chronic pulmonary glanders results from the progression of nasal glanders to the lower respiratory tract (Kettle and Wernery, 2016). Ulcers can be present in the trachea, lungs and in some cases the liver and spleen (Kettle and Wernery, 2016; Spickler, 2018).

The latent form of glanders occurs infrequently and is generally only seen in horses (Khan et al, 2012). Clinical signs, if present, include low grade, intermittent fever, intermittent nasal discharge and laboured breathing. Post-mortem lesions can be found in the lungs. These animals can remain lifelong carriers and spread the disease via respiratory secretions (Khan et al, 2012; Dvorak and Spickler, 2008).

Pathology

Glanders does not have a pathognomonic pathology to allow for a definitive diagnosis via pathology. Typically nodules and / or ulcers are found in many tissues, such as the upper respiratory tract, lungs, pleura, liver and spleen (Spickler 2018; Mota et al, 2010). Lymph nodes may be enlarged with nodules, congestion or fibrosis. Farcy cords may be present. Orchitis has also been reported in infected stallions (Spickler 2018).

Cutaneous glanders is accompanied by histopathology indicating vasculitis and haemorrhage. Foci of pyogranulomatous inflammation in lymph nodes are also commonly present (Mota et al, 2010).

Nasal glanders is associated with severe diffuse purulent inflammation, and breakdown of the nasal epithelium and septum (Mota et al, 2010). Haemorrhage, vasculitis and abscessation progressing to granulomatous lesions are also present in nasal glanders as in cutaneous glanders (Mota et al, 2010).

Pulmonary glanders is characterised by granulomatous, or pyogranulomatous, lesions in the lungs. The lesions consist of a central area of caseous necrosis and surrounded by inflammatory cells such as macrophages, giant cells, lymphocytes and plasmocytes (Mota et al, 2010). Areas of oedema, focal haemorrhage and intra-alveolar fibrin deposits have also been noted in lung tissue of horses with pulmonary glanders (Mota et al, 2010).

Testing

To avoid introducing infected animals into glanders-free horse populations, a validated test with high sensitivity should be considered for horses prior to movement. A variety of tests are available for the diagnosis of glanders in horses. Bacterial culture is considered the gold standard diagnostic test. However, the complement fixation test (CFT) is the recommended test to confirm freedom from infection for the international movement of horses (OIE Manual, 2020). A number of other serological tests have been developed to diagnose glanders in horses. All current tests have limitations that may lead to false negative results. The OIE Manual recommends diagnosis of glanders via direct agent demonstration, complemented by CFT and another test of equal or higher sensitivity and higher specificity than the CFT for confirmation of positive test results (OIE Manual, 2020).

Handling of samples must be done under strict biosecurity controls to prevent human infection (OIE Manual, 2020).

Bacterial culture

Isolation and identification of *B. mallei* from the culture of samples from lesions or exudates is considered the gold standard for definitive diagnosis of glanders. Where possible, samples should be collected from uncontaminated closed lesions from suspect horses. The organism can also be grown from lymph node samples and respiratory exudates, such as nasal discharges. Samples should be incubated at 37˚C on a culture medium such as glycerol agar or sheep blood agar for a minimum of 72 hours (OIE Manual, 2020). However, sheep blood agar is readily overgrown by other bacteria which may reduce the sensitivity of the test (CFSPH, 2018; OIE Manual, 2020). Once cultured, the bacteria are identified via biochemical reaction or by polymerase chain reaction (PCR) (OIE Manual, 2020). A limitation of culture is that it has a low sensitivity. It can be hard to isolate and culture *B. mallei* if the concentration of bacteria in tissues and exudates is low, such as early in the disease or in subclinical carriers (CFSPH, 2018). In addition, the presence of other organisms in samples may hinder isolation of *B. mallei* due to bacterial overgrowth.

Serological testing

<u>Mallein test</u>

The mallein test is an allergic hypersensitivity test which is inexpensive and easy to apply to large groups of animals. It is often used in countries where glanders is endemic. Mallein, a purified protein fraction of *B. mallei* is injected into the skin of the lower eyelid of a suspect infected horse (OIE Manual, 2020). The protein derivative can also be administered as eye drops or subcutaneously to the middle of the neck but these versions of the test are less sensitive. The result of the test is interpreted 48 hours later. Positive reactions are characterised by oedematous swelling of the lower eyelid. A purulent conjunctival discharge, fever, photosensitivity and lacrimation may also be present. A negative reaction is characterised by no change or minor swelling.

The mallein test has a specificity of nearly 100% but a low sensitivity of 75.7% (Naureen et al, 2007; Filho et al, 2012). However, false positives can occur due to infection with *B. pseudomallei* or inadequate purification of the mallein antigen (Pal et al, 2012; Filho et al, 2012). In addition, the tests can cause transient seroconversion in healthy uninfected animals (Hagebock et al., 1993). The high level of false negatives from this test makes it unsuitable for use when clearing horses for international movement. Although, its very low rate of false positives mean that it may be used in eradication programs (OIE Manual, 2020). The OIE Manual (2020) recommends that the mallein test may be used in remote endemic areas where sample transport and storage for other tests is not practicable. However, the test is not commonly used to diagnose glanders due to welfare implications.

<u>Strauss reaction</u>

The Strauss reaction is another method used to diagnose glanders. This test involves injecting a serum sample from a suspect *B. mallei* infected animal intraperitoneally into a laboratory guinea pig (Elschner et al, 2009). Guinea pigs are highly susceptible to *B. mallei* infection (Elschner et al, 2009). A positive result is characterised by acute orchitis in the guinea pig (Elschner et al, 2009). The Strauss reaction is a highly subjective test and has animal welfare concerns, making it unsuitable for screening animals for international trade.

Complement fixation test (CFT)

The CFT is recommended by the OIE for confirmation of individual animal freedom from glanders prior to international movement (OIE Manual, 2020). The test has been validated in horses, mules and camels but not donkeys (OIE Manual, 2020). Variable efficacy has been reported for use of the CFT to detect acute, chronic and inapparent infections (Verma et al, 2014). The CFT cannot reliably detect latent infections in horses (Kettle & Wernery, 2016).

The sensitivity and specificity of the CFT is affected by factors such as the type of antigen used, its preparation and the production facility (OIE Manual, 2020; Eschner et al, 2019). While the OIE Manual includes a procedure for antigen preparation and commercial CFT antigens are also available (OIE Manual, 2020), antigens used in the CFT are often not standardised or may be crudely prepared. The incubation used during CFT can also affect the quality of results. Abreu et al (2020) identified that temperature and duration of incubation impact CFT, with cold incubation leading to greater complement fixation and increased sensitivity. The sensitivity of the CFT ranges from 91.4% to 98%, whilst a specificity of 96.4% to 100% has been reported (Naureen et al; 2007; Pal et al, 2012, Sprague et al 2009, Elschner et al, 2019).

False negative results can occur in old, pregnant or emaciated horses (Elschner et al, 2011; Neubauer et al, 2005). False positive results can also occur through cross reactions with *B. pseudomallei* in serological tests like the CFT due to the close phylogenic relationship with *B. mallei* (Elschner et al, 2019; Wernery 2019).

Enzyme linked immunosorbent assay (ELISA)

Several ELISA tests have been developed commercially to diagnose glanders in horses. These tests have not been fully validated due to the lack of true positive sera samples to complete a large scale study of diagnostic tests (Elschner et al, 2019; Elschner et al, 2021). ELISA tests have limited application and are not suitable for screening prior to the international movement of horses (OIE Manual, 2020; Khan et al, 2012). Like CFT, these serological tests are not sensitive enough to differentiate between *B. mallei* and *B. pseudomallei,* with antibodies attaching to common epitopes of antigens (Verma et al, 2014). The sensitivity and specificity of different ELISA tests range from 85% to 92.5% and 99.5% to 100% respectively (Shakibamehr et al, 2021, Elschner et al, 2019; Sprague et al 2009).

Molecular testing

Polymerase chain reaction (PCR)

Due to the close genetic relationship between *B. mallei* and *B. pseudomallei*, development of molecular tests has been difficult. However, PCR assays have been developed to identify infected animals and differentiate between *B. mallei* and *B. pseudomallei* (Ulrich, 2006; Abreu, 2020; Lowe 2016). Two assays are described in the OIE Manual, but neither have been fully validated or studied in different laboratories (OIE Manual, 2020). The specific genetic evolution of *B. mallei* means that its genome may be unstable during passaging in the laboratory, rendering the test results invalid if not regularly monitored (Ulrich et al., 2006). The sensitivity of the available molecular assays is not well described for clinical samples, thus a negative result cannot be used to rule out a glanders infection (OIE Manual, 2020). The samples recommended for PCR testing are tissue samples from skin, lung, mucous membrane of the nasal conchae and septae, liver or spleen, that have been inactivated and preserved in formalin (OIE Manual, 2020). Serum is not a recommended sample for PCR testing. To enhance test applicability, it is recommended that PCR be run in conjunction with other tests (OIE Manual, 2020).

Treatment

Antibiotics are not recommended for the treatment of infected horses, donkeys or mules. *B. mallei* is resistant to many antibiotics and requires long term administration of a combination of antibiotics. Ineffective treatment with antibiotics may also result in the development of a chronic or latent infection. A 12 week treatment protocol consisting of parenteral administration of enrofloxacin and trimethoprim/sulfadiazine, followed by the oral administration of doxycycline was implemented during a confined glanders outbreak in 23 horses in Lahore, Pakistan (Saqib et al, 2012). Antibiotic treatment did not lead to recrudescence over the 360 day follow-up period suggesting that long term courses of antibiotics may be effective (Saqib et al, 2012). However, this treatment regimen is expensive, and additional studies are required with larger sample sizes and longer term follow-up to determine if this antibiotic protocol is effective. Treatment of infected horses outside of endemic areas is usually not recommended due to the zoonotic potential.

Control

Vaccination

There are currently no commercial vaccines available to control *B. mallei* infection in animals or humans. There have been no published studies evaluating a vaccine for horses. Heat-killed *B. mallei* vaccines have been shown to confer limited protection against infection in a mouse model (Whitlock et al, 2008). Ulrich et al (2005) examined the ability of two live attenuated vaccines to induce immunity in mice exposed to aerosolised *B. mallei*. Partial immunity to aerosolised *B. mallei* was seen with one of the vaccines administered to mice (Ulrich et al 2005). Despite this, *B. mallei* was still detected in the splenic cells of all mice which survived the low dose *B. mallei* suggesting the vaccine allowed the mice to survive a usually lethal challenge but not clear the infection (Ulrich et al, 2005). This implies that vaccinated mice could be infective and transmit glanders.

Eradication

Stamping out procedures are common in glanders control programs. The Indian government published the '2016 Action Plan for Control and Containment of Glanders in Horses' and the '2019 National Action Plan for Control and Eradication of Glanders in India' which require all horses suspected to have glanders to undergo testing and be euthanased to prevent spread of the disease (Indian Department of Animal Husbandry, Dairying and Fisheries, 2016; Indian Department of Animal Husbandry and Dairying; 2019). In preparation for the 2016 Olympics, the Brazilian Ministry of Agriculture, Livestock and Supply updated the National Horse Health Program to include glanders. This program required the compulsory euthanasia of all glanders positive horses detected in Brazil with no owner compensation (Fonesca-Rodriguez et al, 2019). Testing and euthanasia of glanders positive horses remains in place in Brazil. In 2018, Indonesia detected 10 glanders positive horses when conducting sampling to establish a horse disease free zone for the 18th Asian Games (ProMed, 2018). All 10 horses were euthanased (ProMed, 2018).

In Australia, glanders is a Category 2 disease in the Emergency Animal Disease Response Agreement (EADRA). This means that the cost of disease control would be shared 80% by government and 20% by industry (Animal Health Australia, 2018). In the event of a glanders outbreak in Australia, Australia's policy is to identify and destroy infected animals, quarantine infected premises and disinfect or destroy all possible fomites (Animal Health Australia, 2018). This policy would be supported with movement controls, surveillance and public awareness campaigns (Animal Health Australia, 2018).

Current biosecurity measures

Australia has import conditions for horses and horse semen. Currently, glanders is not present in any country that is approved to export horses or horse semen to Australia. Australia's current biosecurity measures are consistent with the OIE Code recommendations and require certification of country freedom from disease. Australia's relevant requirements are:

Live horse clauses

- *'For 180 days immediately before export, or since birth if under six months of age, the horse was continuously resident and free of quarantine restriction in a country where no clinical, epidemiological or other evidence of glanders occurred during the previous three years before export and the disease is compulsorily notifiable.'*
- *'all horses in the pre-export quarantine (PEQ) facility remained free from evidence of infectious or contagious disease, and had no contact with horses not of equivalent health status'*

Semen clauses

- *'During the 2 months immediately prior to the first collection of semen for this consignment, the stallion was continuously resident in the approved country, where glanders is compulsorily notifiable and where glanders has not been reported during the 2 years immediately prior to, or during, the collection of semen for this consignment'*
- *'The donor stallion was examined by an approved veterinarian* on each day of collection of semen for this consignment and was clinically healthy'* *An approved veterinarian is a veterinarian authorised by the government veterinary administration to supervise the semen collection centre.

Risk assessment

Risk assessment is defined in the OIE Code as '… the evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard'.

Chapter 2.1 of the OIE Code provides recommendations for conducting import risk analyses, describing the risk assessment steps in Article 2.1.4 as entry, exposure and consequence assessments and their integration into a risk estimation, producing overall outcome of the risks associated with the hazards identified at the outset. In this review the hazard identified is glanders.

This assessment was conducted using a qualitative approach. The likelihood that an event will occur was evaluated and reported qualitatively, using qualitative likelihood descriptors for the release and exposure assessment, and the outbreak scenario in Table 1 in Appendix 1.

This risk assessment for glanders followed the method outlined in Section 3.2 Risk Assessment of the Horse IRA. Relevant tables for this risk assessment are included in Appendix 1.

Entry assessment

The entry assessment estimates the likelihood that glanders would be present in live horses and horse semen imported into Australia from any country.

The following factors were taken into account when determining the likelihood of glanders entering Australia in an imported horse and horse semen:

- Glanders is an OIE listed disease.
- Once prevalent worldwide, glanders has been eradicated from many countries, including Canada, Western Europe and the United States (Dvorak and Spickler 2008; Derbyshire, 2002).
- Since 1996, glanders outbreaks have occurred in Afghanistan, Bahrain, Belarus, Bolivia, Brazil, China, Eritrea, Ethiopia, Germany, India, Indonesia, Iran, Iraq, Kuwait, Lebanon, Mongolia, Nepal, the Philippines, Russia and Turkey (OIE Code, 2019; Wernery et al, 2011; Singha et al, 2020; Sholz et al, 2014).
- Endemic disease persists in many parts of Asia, North Africa, South America and the Middle East (Kettle and Wernery, 2016).
- Glanders is considered a re-emerging disease in some countries.
- Acute glanders occurs frequently in donkeys and mules (Khan et al, 2012). Chronic glanders occurs more commonly in horses (Khan et al, 2012; Mota et al, 2010).
- Horses can become inapparent carriers due to latent infections, which can still readily transmit the disease without displaying clinical signs of illness (Dvorak & Spickler, 2008; Khan et al, 2012).
- Evidence of venereal transmission of glanders is unclear.
- It is unclear if *B. mallei* would survive in fresh or frozen semen or embryos.
- Tests available to diagnose glanders have varying levels of sensitivity and specificity. False negatives occur to varying degrees with all available tests.
- There is no vaccine available against glanders.
- Culture has limitations making it inappropriate as a test option for clearing horses for international trade.
- CFT is the only test recommended for diagnosis of glanders in horses exported internationally (OIE Code, 2019).
    - The sensitivity of the CFT ranges from 91.4% to 98%, whilst a specificity from 96.4% to 100% has been reported (Naureen et al; 2007; Pal et al, 2012, Sprague et al 2009, Elschner et al, 2019).
    - False negative results can occur in old, pregnant or emaciated horses (Elschner et al, 2011; Neubauer et al, 2005).
- Serological tests are not sensitive enough to differentiate between *B. mallei* and *B. pseudomallei,* with antibodies attaching to common epitopes of antigens (Verma et al, 2014).
    - The sensitivity and specificity of different ELISA tests ranges from 85% to 92.5% and 99.5% to 100% respectively (Shakibamehr et al, 2021, Elschner et al, 2019; Sprague et al 2009).
- Cases of glanders have been detected in post-arrival quarantine in other countries (excluding Australia) despite adherence to OIE recommendations for testing.

Based on these considerations, the likelihood of entry of glanders in an imported horse was estimated to be **moderate**, and the likelihood of entry in imported horse semen was **low**.

Exposure assessment

The exposure assessment estimates the likelihood that a susceptible animal in Australia will be exposed to glanders introduced via an imported horse or horse semen. It takes into account the exposure groups most likely to be affected as well as the possible pathways by which exposure of these groups could occur. For glanders the exposure group considered is all horses (including feral horses). For imported horses, the most likely exposure pathways are via direct contact and fomites. For horse semen, the most likely exposure pathways are insemination and fomites.

The following factors were taken into account when determining the likelihood of susceptible animals being exposed to glanders via an imported horse or horse semen:

- Glanders is a highly infectious disease that is spread via direct contact, aerosolisation of infected secretions and fomites.
- *B. mallei* is readily transmitted by respiratory secretions, and skin exudates that can contain large numbers of infective bacteria (CFSPH, 2019).
- Transmission commonly occurs via direct ingestion, inhalation or skin contamination of abraded skin or mucus membranes (Dvorak & Spickler, 2008).
- Indirect transmission can occur by fomites such as contaminated food, water, general husbandry equipment, breeding equipment or people (Dvorak & Spickler, 2008).
- Chronically or latently infected animals with minimal signs of disease may serve as a pathogen reservoir (Dvorak and Spickler 2008).
- Chronic and subclinically infected cases can shed bacteria continuously or intermittently (OIE, 2009).
- Rare cases of glanders have been reported in camels, goats and sheep (Dvorak and Spickler, 2008; Wernery et al, 2011).
- Cattle, pigs, rats and birds are relatively resistant to experimental infection (Dvorak and Spickler, 2008).
- Glanders is an occupational disease that affects individuals in close contact with infected animals such as farmers, trainers, veterinarians and laboratory personnel (Saqib et al, 2012).
- There is no information about the potential effects of glanders on marsupials.
- Overcrowding, close contact with infected animals and poor hygiene practices are believed to increase the likelihood of transmission (Khan et al, 2012).
- Evidence of venereal transmission of glanders is unclear.
- Indirect transmission may occur by contamination of husbandry and breeding equipment.
- It is unclear if *B. mallei* would survive and be able to be transmitted by fresh or frozen semen or embryos.
- While *B. mallei* is a facultative intracellular obligate bacteria, relying on host transmission for dispersal, it can survive outside of the host species under specific environmental conditions found in tropical environments (OIE Technical disease cards, 2020).
- *B. mallei* is susceptible to intense heat, direct sunlight and disinfectants (Timoney, 2014).

- Direct sunlight inactivates *B. mallei* within 24 hours, and it is destroyed at temperatures over 55˚C for more than 10 minutes (OIE Technical disease cards, 2020).
- Dvorak and Spickler (2008) found the bacteria could remain viable in room temperature water (20˚C to 25˚C) for up to one month.
- Some studies have found that the bacteria could remain viable in contaminated stables for up to six weeks in humid or wet conditions out of direct sunlight (OIE Technical disease cards, 2020; Verma et al, 2014).
- *B. mallei* is susceptible to most common disinfectants such as iodine, benzalkonium chloride (1/2000), sodium hypochlorite (500ppm available chlorine), 70% ethanol and 2% glutaraldehyde (OIE Technical disease cards, 2020).
- Big cats and other carnivores have acquired *B. mallei* infection from eating infected horse meat (Dvorak and Spickler, 2008; Khaki et al, 2012).
- Australia imports large quantities of horse semen (both fresh and frozen) each year which are widely used and distributed all around the country.

Based on these considerations, the likelihood of susceptible animals being exposed to glanders via an imported horse was estimated to be **moderate**, and the likelihood of exposure to glanders from horse semen was **high**.

Estimation of the likelihood of entry and exposure

Using the matrix in Table 2 in Appendix 1, the overall likelihood of entry and exposure is estimated by combining the likelihood of entry and the corresponding likelihood of exposure.

For horses, with the likelihood of entry estimated to be moderate and combined with the likelihood of exposure estimated to be moderate, the likelihood of entry and exposure for glanders was estimated to be **low**.

For horse semen, with the likelihood of entry estimated to be low and combined with the likelihood of exposure estimated to be high, the likelihood of entry and exposure for glanders was estimated to be **low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with disease agent entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the disease agent for the most likely outbreak scenario, and determining the direct and indirect effects (health, environmental and socioeconomic) should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible animals has occurred, following importation of an infected horse or horse semen, a number of possible outbreak scenarios could follow, ranging from no spread to establishment of widespread disease.

The most likely outbreak scenario was determined by the extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to glanders is considered to be a widespread outbreak whereby glanders establishes in directly exposed populations of susceptible animals (horses) and spreads within multiple states/territories through direct contact, insemination with contaminated semen, and fomite transmission.

The following factors were considered relevant to an estimate of the likelihood of establishment and/or spread associated with exposure of susceptible animals to glanders.

- Glanders is a highly infectious disease that is spread via direct contact, aerosolisation of infected secretions and fomites.
- Glanders is a facultative intracellular obligate bacteria that relies on host transmission for dispersal.
- Rare cases of glanders have been reported in camels, goats and sheep (Dvorak and Spickler, 2008; Wernery et al, 2011).
- Cattle, pigs, rats and birds are relatively resistant to experimental infection (Dvorak and Spickler, 2008).
- *B. mallei* is readily transmitted by respiratory secretions, and skin exudates that can contain large numbers of infective bacteria (CFSPH, 2019).
- Transmission commonly occurs via direct ingestion, inhalation or skin contamination of abraded skin or mucus membranes (Dvorak & Spickler, 2008).
- Indirect transmission can occur by fomites such as contaminated food, water, general husbandry equipment, breeding equipment or people (Dvorak & Spickler, 2008).
- Chronically or latently infected animals with minimal signs of disease may serve as a pathogen reservoir (Dvorak and Spickler 2008).
- Chronic and subclinically infected cases can shed bacteria continuously or intermittently (OIE, 2009).
- *B. mallei* can survive outside of the host species under specific environmental conditions found in tropical environments (OIE Technical disease cards, 2020).
- *B. pseudomallei* (melioidosis) is present in northern Australia and a pocket of Western Australia. Conditions in this area may be suitable for *B. mallei* to be sustained within the environment for short periods of time (up to one month).
- *B. mallei* is susceptible to intense heat, direct sunlight and disinfectants (Timoney, 2014).
- Evidence of venereal transmission of glanders is unclear.
- It is unclear if *B. mallei* would survive in fresh or frozen semen or embryos.
- Tests available to diagnose glanders have varying levels of sensitivity and specificity. False negatives occur to varying degrees with all available tests.
- False positive results can occur due to the use of crude antigen triggering cross-reactions with non-specific antibodies in serum samples (Sprague et al, 2009).
- There is no reliable treatment for glanders in animals. Antibiotic treatment of glanders is a prolonged, expensive process with a very high rate of failure in horses (Saqib et al, 2012; Kettle & Werney, 2016).
- There is no vaccine available for glanders in horses (Ulrich et al, 2005).
- Eradication of glanders from Australia’s feral horse population may be difficult if the disease were to establish in Australia.
- The requirements for movement of horses between Australian states and territories is inconsistent. Some areas require significantly more documentation and registration prior to movement being permitted. These inconsistencies could lead to impaired traceability.

Based on these considerations for the identified outbreak scenario, the likelihood of establishment and/or spread of glanders was estimated to be **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and / or spread of glanders is the determination of the effects (health, environmental and socioeconomic) resulting from the outbreak scenario. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria. Further details on the method for determining the effects resulting from the outbreak scenario can be found in Appendix 1 and the Horse IRA.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of glanders for each criterion. The magnitude of effects and geographic levels are described in Tables 3 and 4 respectively in Appendix 1. The method for combining effects is outlined in Table 5 in Appendix 1.

Direct effects

The effect on the life or health (including production effects) of susceptible animals.

- In horses the disease can manifest in acute, chronic and latent forms.
- The acute form results in septicaemia and death within few days (Hungerford, 2007).
- The chronic form results in ulcerating, purulent nodules developing in the respiratory tract, skin and lymph nodes (Hungerford, 2007).
- Chronic illness can last for months before death or recovery to become a carrier (Hungerford, 2007).
- Glanders is a zoonosis.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be significant at the regional level. The effect on the national economy or the Australian community as a whole and not just on directly affected parties, corresponds to minor at the state level (national effect score of D in Table 5 in Appendix 1).

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- There is no evidence of glanders in marsupials and other wildlife. It is unlikely to affect these animals.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be unlikely to be discernible at all levels (national effect score of A in Table 5 in Appendix 1).

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- The disease is exotic to Australia and is nationally notifiable.

- If glanders was identified in Australia, the Australia's policy is to identify and destroy infected animals, quarantine infected premises and disinfect or destroy all possible fomites AHA, 2018).
- Glanders is classified as a Category 2 disease in the EADRA (AHA, 2018). This means that 80% of the costs of disease control will be paid by governments and 20% by the relative industries.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be significant at the state level. The effect on the national economy or the Australian community as a whole and not just on directly affected parties, corresponds to minor at the national level (national effect score of E in Table 5 in Appendix 1).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries suppling inputs to, or using outputs from, directly affected industries

- As a result of the detection of glanders, movement restrictions would be imposed on exposed horses and potentially infected fomites.
- Horse racing and other equestrian events may be prohibited for varying periods in some affected jurisdictions.
- Following the detection of glanders in one jurisdiction of Australia, other jurisdictions may close their borders to all horses until the extent of the outbreak was determined.
- Industries supporting horse activities such as stockfeed manufacturers, veterinarians, farriers and saddlers would also be affected.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be significant at the state level. The effect on the national economy or the Australian community as a whole and not just on directly affected parties, corresponds to minor at the national level (national effect score of E in Table 5 in Appendix 1).

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- The Australian horse industry contributes approximately $6.3 billion to gross domestic product (Gordon, 2001).
- An outbreak of glanders in Australia would have a severe impact on the horse industry and horse export trade.
- Horse exports would likely be disrupted for a significant period of time for several markets which require country freedom.
- Eradication of glanders from Australia's feral horse population may be difficult if the disease were to establish in Australia.
- Re-establishing Australia's freedom from glanders, in line with OIE Code standards would be a lengthy and costly process.
- If glanders were to become established, renegotiation of trade conditions would be necessary with those markets that would accept testing.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be significant at the state level. The effect on the national economy or the Australian community as a whole and not just on directly affected parties, corresponds to minor at the national level (national effect score of E in Table 5 in Appendix 1).

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- Glanders is not considered to lead to any indirect effects on the environment.

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be unlikely to be discernible at all levels (national effect score of A in Table 5 in Appendix 1).

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- Disruption of horse events would have significant social consequences for people involved.
- Where horses were important to the local economy, the economic viability of communities within affected regions may be compromised due to the effect on associated industries.
- The Australian response to an outbreak would involve euthanasia of infected horses and destruction of contaminated equipment. These response measures are likely to lead to major disruption to social functioning for horse owners and those employed in the industry.
- Studies completed in the United Kingdom following the foot and mouth disease outbreak of 2001, identified a significant, long standing social impact associated with the loss of stock (Deaville et al, 2003). This included mental health issues, physical health issues and relationship breakdowns (Deaville et al, 2003).

Based on these considerations, the effect of the establishment and / or spread of glanders in Australia for this criterion was estimated to be significant at the state level. The effect on the national economy or the Australian community as a whole and not just on directly affected parties, corresponds to minor at the national level (national effect score of E in Table 5 in Appendix 1).

Estimation of the likely consequences

The measure of effect (A-G) obtained for each direct and indirect criterion was combined to give the overall effect of a disease agent. The rules outlined in Table 6 in Appendix 1 were devised for the combination of direct and indirect effects.

Based on these rules described, where the effect of a disease with respect to one or more criteria is E, the overall effect associated with the outbreak scenario is considered to be **moderate**.

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and / or spread for the scenario using Table 7 in Appendix 1 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread (high) was combined with the estimate of the overall effect of establishment and/or spread (moderate), to result in a **moderate** likely consequences.

Risk estimation

Risk estimation is the integration of the likelihood of entry and exposure and the likely consequences of establishment and/or spread to derive the risk associated with the entry, exposure, establishment and/or spread of glanders introduced by the importation of horse or horse semen into Australia. Using Table 8 in Appendix 1, the likelihood of entry and exposure (low) is combined with the likely consequences of establishment and/or spread (moderate), resulting in a risk estimation of low.

The unrestricted risk associated with glanders is determined to be **low**. The unrestricted risk exceeds Australia's ALOP and therefore risk management is deemed necessary.

Commodity	Likelihood of entry	Likelihood of exposure	Likelihood of entry and exposure	Likelihood of establishment and/or spread	Overall effect of establishment and/or spread	Likely consequences	Unrestricted risk
Live horses	MOD	MOD	LOW	HIGH	MOD	MOD	LOW
Horse semen	LOW	HIGH	LOW				LOW

Figure 1: Summary of risk assessment.

Conclusion

Risk management measures are required to achieve Australia's ALOP with regard to the risk of glanders for the importation of live horses and horse semen. The department considered risk management options including country or zone freedom, premises status, testing and treatments that could be applied to reduce the likelihood of entry and/or exposure for glanders to achieve Australia's ALOP. Based on the preceding information, the department concluded that, other than country freedom, no single risk management option reduced the unrestricted risk sufficiently to achieve Australia's ALOP for the importation of live horses or their semen.

1. Live horses

Glanders is not currently present in approved countries and is not present in Australia. In Australia glanders is a nationally notifiable disease and biosecurity measures are in place. Based on the preceding information, risk management measures continue to be warranted (Figure 1). The department has determined that the current biosecurity measures are appropriate risk management for glanders and reduce the likelihood of entry to achieve Australia's ALOP.

These measures are:

- After due enquiry, for six months (180 days) immediately before export, or since birth if under six months of age, the horse has been continuously resident and free of quarantine restriction in [insert approved country] or other country where no clinical, epidemiological or other evidence of glanders occurred during the previous three years before export; and the disease is compulsorily notifiable.

- The horse has been examined by the official veterinarian within 24 hours before leaving the PEQ facility for the port of export and has been found to be free from evidence of infectious or contagious disease.

2. Semen

As with live horses, glanders is not currently present in countries approved to export horse semen to Australia. Based on the preceding information, risk management measures continue to be warranted. The department has determined that the current biosecurity measures are appropriate risk management for glanders and reduce the likelihood of entry to achieve Australia's ALOP.

These measures are:

- During the two months immediately prior to the first collection of semen for this consignment, the stallion was continuously resident in [insert approved country], where glanders is compulsorily notifiable and where glanders was not reported during the three years immediately prior to, or during, the collection of semen for this consignment.
- The donor stallion was examined by an approved veterinarian on each day of collection of semen for this consignment and was clinically healthy.

Appendix 1

Risk assessment tables

Table 1: Nomenclature for qualitative likelihoods

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

Table 2: Matrix for combining likelihood of entry and exposure

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

Table 3: Nomenclature for magnitude of effect

Effect	Descriptive definition
Highly significant	The effect is extremely serious and irreversible and likely to disturb either economic viability of the intrinsic value of the criterion.
Significant	The effect is serious and substantive but reversible and unlikely to disturb either economic viability or the intrinsic value of the criterion.
Minor significance	The effect is recognisable but minor and reversible.
Unlikely to be discernible	The effect is not unusually distinguishable from normal day to day variation in the criterion.

Table 4: Definition of geographic levels

Geographic levels	Description
Local	an aggregate of households or enterprises (a rural community, a town or a local government area).
District or region	a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').
State or territory	a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).
National	Australia wide (Australian mainland states and territories and Tasmania).

Table 5: Assessment of direct or indirect effects on a national scale

National effect score	National	State or territory	District or region	Local
G	**Highly significant**			
F	**Significant**			
E	**Minor**	Greater than 'minor' at state or territory level equals at least 'minor' at national level		
D	Unlikely to be discernible	**Minor**	Greater than 'minor' at district or region level equals at least 'minor' at state or territory level	
C	–	Unlikely to be discernible	**Minor**	Greater than 'minor' at local level equals at least 'minor' at district or region level
B	–	–	Unlikely to be discernible	**Minor**
A	–	–	–	**Unlikely to be discernible**
		Geographical level		

Table 6: Rules used for combining direct and indirect effects

Rule	Effect scores for each direct and indirect criteria	Overall effect
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate

Rule	Effect scores for each direct and indirect criteria	Overall effect
4	One or more criteria have an impact of ‘D’; or all criteria have an impact of ‘C’.	Low
5	One or more criteria have an impact of ‘C’; or all criteria have an impact of ‘B’.	Very Low
6	One or more but not all criteria have an impact of ‘B’; or all criteria have an effect of ‘A’.	Negligible

Table 7: Matrix for determining likely consequences: combining the likelihood and overall effect of establishment and/or spread.

Likelihood of establishment and/or spread	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

Overall effect of establishment and spread

Table 8: Risk estimation matrix

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

Appendix 2

Criteria for assessing country freedom from glanders

The unrestricted glanders biosecurity risk to Australia associated with the importation of live horses and horse semen exceeds Australia's ALOP and warrants biosecurity measures (Figure 1). Consequently, the recommended import conditions for the export of horses from non-approved countries to Australia, require the horse/s to have resided in glanders free countries for six months (180 days) prior to export. The final 60 days must be spent in a country approved to export horses to Australia. All countries approved to export horses to Australia are glanders free (for a minimum of three years).

This difference in residency requirements has led to questions about the glanders status of a number of countries which have not been assessed by the department for the importation of horses. For a country to be considered glanders free by Australia, the department would need to undertake an assessment to ensure that the glanders biosecurity risk meets Australia's appropriate level of protection.

The department takes an evidence based, scientific approach to such assessment of a country's glanders status for the import of live horses and their reproductive material into Australia. This assessment may consider:

- the animal health status of the country
- the effectiveness of veterinary services and other relevant certifying authorities
- competent authority controls over animal health, including relevant legislation, quarantine policies and practices
- surveillance practices including import controls for horses
- the standard of reporting to the OIE of notifiable disease outbreaks
- effectiveness of veterinary laboratory services, including compliance with relevant international standards
- effectiveness of systems for control over certification/documentation of products intended for export to Australia
- border controls and health status of neighbouring countries.

After a country has been assessed by the department as glanders free, this may change if there is a disease incursion or significant changes in the competent authority's controls and oversight. In these cases, the department may no longer consider a country to be glanders free for the purposes of the importation of live horses and semen into Australia.

Glossary

Term or abbreviation	Definition
ALOP	Appropriate level of protection
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.
AUSVETPLAN	Australian Veterinary Emergency Plan
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.
CFT	Complement fixation test
EADRA	Emergency animal disease response agreement
ELISA	enzyme-linked immunosorbent assay
IRA	Import risk analysis
OIE	World Organisation for Animal Health
OIE Code	OIE Terrestrial Animal Health Code
OIE Manual	OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
PCR	polymerase chain reaction
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.

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