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Quantitative risk assessment for the introduction of lumpy skin disease virus into Australia via non-regulated pathways

Final report

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Abbreviations and acronyms

Abbreviation	Definition
ABS	Australian Bureau of Statistics
AHAW	Animal Health and Welfare
BTV	bluetongue virus
CI	credible interval
CMIP	Coupled Model Intercomparison Project
the Department	Department of Agriculture, Fisheries and Forestry
DAFF	Department of Agriculture, Fisheries and Forestry
DNA	deoxyribose nucleic acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FAOSTAT	FAO database on food and agriculture statistics
FMDV	foot and mouth disease virus
GDAS	Global Data Assimilation System meteorological model for use in HYSPLIT
HYSPLIT	Hybrid Single-Particle Lagrangian Integrated Trajectory atmospheric dispersion model
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organization for Standardization
JEV	Japanese encephalitis virus
qPCR	quantitative polymerase chain reaction
LSD	lumpy skin disease
LSDV	lumpy skin disease virus
MARS	Maritime Arrivals Reporting System
NAQS	Northern Australia Quarantine Strategy
NCEP-NCAR	a meteorological model used in HYSPLIT
NOAA	National Oceanic and Atmospheric Administration
NT	Northern Territory
PERT	Program Evaluation Review Technique
PNG	Papua New Guinea
QGIS	quantum geographic information system
QLD	Queensland
QRA	quantitative risk assessment

Abbreviation	Definition
R_0	basic reproduction number
Ro-Ro	roll-on, roll-off
SA2	statistical area level 2
SA4	statistical area level 4
SEJ	structured expert judgement
SSP	shared socioeconomic pathway
TAPPAS	Tool for Assessing Pest and Pathogen Aerial Spread
$TCID_{50}$	50% tissue culture infectious dose
TSIS	Torres Strait Information System
US/USA	United States of America
WA	Western Australia
WOAH	World Organisation for Animal Health (formerly the Office International des Épizooties (OIE))

Executive summary

Introduction

Lumpy skin disease (LSD) is a highly contagious disease of cattle and water buffalo caused by the capripoxvirus lumpy skin disease virus (LSDV). Since 2021, LSDV has spread rapidly through Southeast Asia; it was detected in Indonesia in 2022, heightening concerns of onward spread to Australia (World Organisation for Animal Health, 2022). Spread is thought to be primarily driven by mechanical transmission through biting/blood-sucking insects (Chihota et al., 2001; Tuppurainen and Oura, 2012; Sprygin et al., 2019; Namazi and Tafti, 2021). Live animal movements into Australia are strictly regulated; however, arthropod movement continues to pose an incursion risk for LSDV and other vector-borne diseases. While several risk mitigation measures have already been, and continue to be, implemented for LSDV, formal risk assessment can further target these activities, ensuring that available resources are allocated most efficiently.

In a recent structured expert judgement (SEJ) exercise, specialists estimated there was a 28% chance of an LSD outbreak (via any pathway) occurring in Australia in the next five years (Centre of Excellence for Biosecurity Risk Analysis, 2022). SEJ exercises are not based on modelling but use an internationally recognised process to make evaluative judgements on a range of complex and uncertain systems that otherwise may not be possible. Rapid SEJ exercises are one tool that has been used to broadly characterise the threat Australia faces, and if a material threat is indicated, inform further activities including more specific risk assessments. Thus, Ausvet Pty Ltd was commissioned to conduct a risk assessment on non-regulated pathways (including leakage from regulated pathways) for LSDV entry and exposure in Australia. Additional objectives of this project were to identify information gaps, to determine the relative importance of these gaps to prioritise future research and to develop robust qualitative and quantitative risk assessment (QRA) methodologies for LSDV.

Using a qualitative risk assessment framework, we previously estimated the probability of LSDV incursion via four non-regulated pathways to be negligible but noted that this assessment did not account for the volume of vector movements into Australia, which may change the overall results of the assessment (Zalcman, Hall and Cowled, 2022). Critically, even a negligible risk can become significant when multiplied by a large entry volume. Furthermore, we identified several limitations to our qualitative analysis that made it prudent to additionally undertake a QRA where we could incorporate uncertainty and variability. Here, we report our findings from this quantitative analysis.

Methodology

The objective of this risk assessment was to answer the risk question: 'Assuming a situation where LSDV is endemic throughout Southeast Asia and Papua New Guinea (PNG), how many LSDV incursions into Australia per year are estimated through the four specified non-regulated pathways?'

We consulted with the Department during the contracting stage of this work and during project inception to clarify pathways for inclusion and conducted a review of the scientific literature to ensure that our pathways were clearly defined and plausible for the transmission of LSDV. The four selected pathways were:

- windborne dispersal of arthropod vectors

- commercial vessels carrying hitchhiker arthropod vectors (excluding returning live export vessels)
- returning live export vessels carrying hitchhiker arthropod vectors
- Torres Strait Treaty movements carrying hitchhiker arthropod vectors (Figure 1).

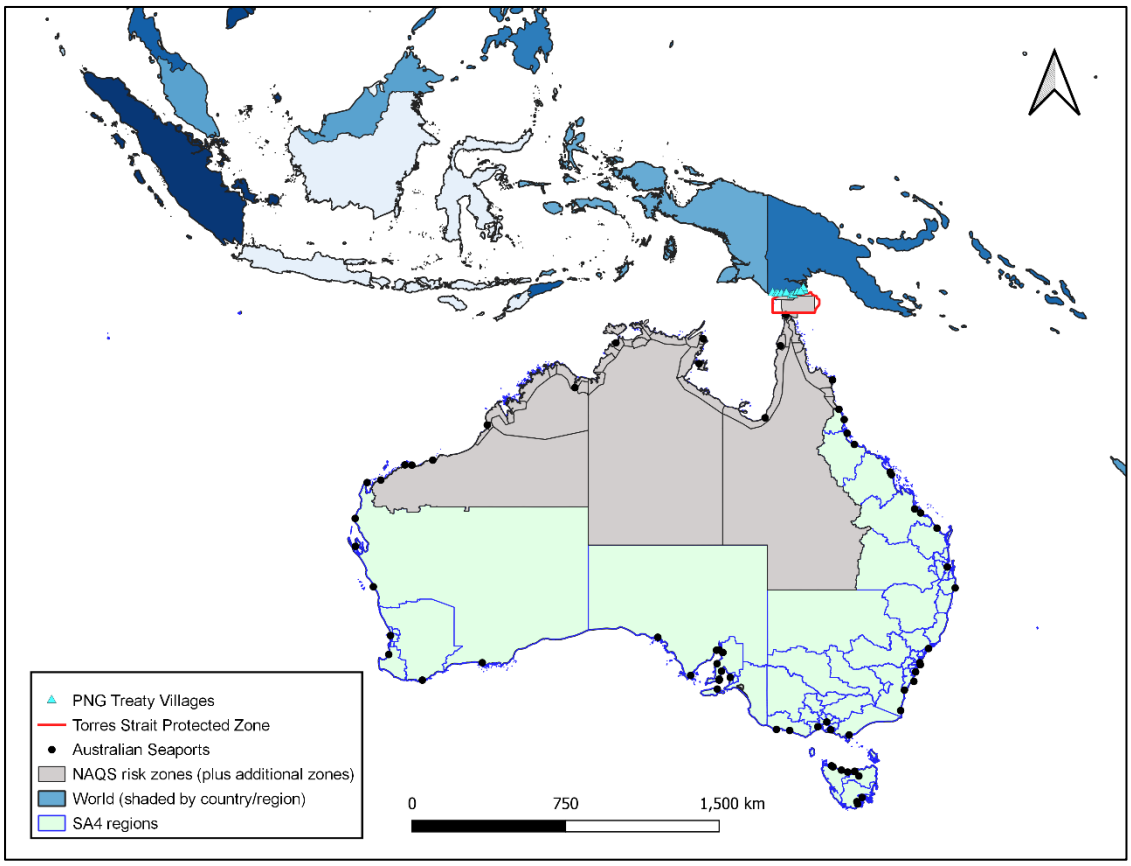

Figure 1 Overview map showing relevant features for this analysis

Origin countries are coloured in shades of blue, with Indonesia subdivided into eastern, central and western subregions. Scalebar accuracy may be limited due to use of a geographic coordinate system.

Non-commercial vessels were assumed to arrive in insufficient volumes to pose a significant threat. The illegal importation of infected hides was considered within the Torres Strait Treaty movements; however, information from the Torres Strait Information System revealed that no hides have been detected since inspections commenced in 2018, making LSDV entry via infected hides extremely unlikely.

We defined an incursion as clinical LSDV in a single Australian bovine, regardless of whether this animal transmitted infection onwards to other susceptible bovines. We conducted analyses under three scenarios:

1. At least 30–50 insects are necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors are necessary for transmission
3. A single insect is sufficient for transmission

It is not known precisely how many insects are necessary to transmit a sufficient dose of LSDV to initiate an infection in a bovine; however, based on published experimental transmission studies to date, the minimum number of infectious insects demonstrated thus far is 36 horseflies (*Haematopota* spp.) (Sohier et al., 2019), 50 *Aedes* mosquitoes (Chihota et al., 2001) or >200 *Stomoxys* stable flies (Sohier et al., 2019; Issimov et al., 2020). Transmission from *Culicoides* midges to bovines has not yet been demonstrated experimentally. Recent unpublished studies from the Pirbright Institute again found no evidence of transmission via *Culicoides nubeculosus* midges; however, it was found that as few as 14 *Aedes* mosquitoes

could transmit LSDV to recipient cattle (P. Beard, pers. comm.)[1]. Some experts believe that a single insect is insufficient to initiate an LSDV infection in a bovine. Sprygin et al. (2019) stated that:

> Because vector transmission is considered to be of a mechanical nature and the numbers [*sic*] of infective viruses on insects' mouthparts is likely to be low, in the absence of other supporting factors, air currents would need to transfer hundreds of contaminated vectors onto a single susceptible animal to induce full clinical disease.

The minimum infectious dose for LSDV has been estimated at $>10^1$ 50% tissue culture infectious dose ($TCID_{50}$) (Carn and Kitching, 1995b), and it was calculated that an individual *Stomoxys calcitrans* could only transfer $10^{-0.8}$ $TCID_{50}$ (Sohier et al., 2019). Observed infection rates from vectors in laboratory studies have thus far all used batches of insects to achieve LSDV transmission; for example, even when batches of 50–200 *Stomoxys calcitrans* were fed on infectious bovines at peak infectiousness and subsequently re-fed within 1 hour on highly susceptible naïve cattle over several consecutive days, LSDV transmission only occurred in 30% of acceptor animals (Sohier et al., 2019). Taken together, the findings from multiple experimental transmission studies currently suggest that multiple vectors are necessary to initiate a bovine infection (Carn and Kitching, 1995a; Chihota et al., 2001, 2003; Sprygin et al., 2019; Issimov et al., 2020). However, we cannot definitively state that transmission from a single insect is impossible.

Critically, unlike bluetongue virus (BTV), there is no robust evidence for biological transmission of LSDV in arthropods. This view is widely accepted in the literature (Chihota et al., 2001, 2003; Tuppurainen et al., 2013b; Lubinga et al., 2015; Sohier et al., 2019; Sprygin et al., 2019; Issimov et al., 2020; Paslaru et al., 2021). Notably, there is no evidence for biological transmission for any vertebrate poxviruses, although many are known to be transmitted mechanically by arthropod vectors (Foil and Gorham, 2000). The detection of virus beyond the mouthparts does not in and of itself demonstrate biological transmission. However, again, we cannot conclusively state that biological transmission does not occur.

Within each pathway we assessed the incursion risk independently for multiple combinations of origin country, arrival destination and vector category. Origin countries were selected based on spatial analyses. Indonesia was subdivided into western, central and eastern subregions for improved geographic resolution due to the large east-west distance of this country. Arrival destinations were the Northern Australian Quarantine Strategy (NAQS) risk zones plus additional inland regions of northern Australia for the windborne dispersal pathway, Australian seaports for the shipping pathways, or the NAQS risk zones in northern Queensland (Q1a, Q1b or Q2) for the Torres Strait Treaty movements pathway. We classified vectors into three broad categories: midges (*Ceratopogonidae*), mosquitoes (*Culicidae*) and heavy fliers (including stable flies, tabanids and other brachyceran flies).

For each of the four pathways we developed scenario trees that outline the conditional series of events (or nodes) necessary for incursion of LSDV into Australia. Quantitative data were sought from the peer-reviewed literature, the grey literature and from expert opinion to assign probability distributions to each node in our model within each pathway (Table 1). Due to the complexity of combinations, desire for reproducibility and the stochastic nature of our analysis, we conducted the risk assessment within the R statistical computing environment. Atmospheric dispersion modelling was performed using HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory model) from the United States National Oceanic and Atmospheric Administration to investigate the probability of vectors dispersing to Australia from countries to our north.

[1] Interim results that have generously been made available ahead of publication.

Table 1 Summary of model parameterisation

Node	Data source
Windborne dispersal of arthropod vectors	
En_1 Number of bovines at origin	(Gilbert et al., 2018b, 2018a; FAO, 2022)
En_2 Bovines infected with LSDV	
Seroprevalence ×	(Berg et al., 2015)
Duration of infectiousness /	(Weiss, 1968; Carn and Kitching, 1995; Tuppurainen, Venter and Coetzer, 2005; Osuagwuh et al., 2007; Babiuk et al., 2008; Annandale et al., 2010; Berg et al., 2015; Sohier et al., 2019; Sanz-Bernardo et al., 2021)
Duration of immunity	(Berg et al., 2015)
En_3 Number of vectors biting each bovine per infectious day	(Gubbins, 2019)
En_4 Bovine-to-vector transmission	(Chihota et al., 2001, 2003; Gubbins, 2019; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021)
En_5 Vector transported to Australia by wind	
Vector enters wind stream ×	Extrapolation from qualitative opinion
Winds suitable for dispersal to Australia ×	HYSPLIT atmospheric dispersion modelling
Vector deposited on the Australian continent	HYSPLIT atmospheric dispersion modelling
En_6 Vector survives dispersal and remains infectious	
Vector survives dispersal ×	Extrapolation from qualitative opinion
Vector remains infectious	(Chihota et al., 2001; Issimov et al., 2021; Paslaru et al., 2022)
Ex_A Susceptible bovine present	(Australian Bureau of Statistics, 2021a) and NAQS officer, pers. comm.
Ex_B Vector(s) bite bovine	
Vector(s) present	Extrapolation from qualitative opinion
Vector biting rate	Expert opinion
Ex_C Bovine is infected	(Weiss, 1968; Chihota et al., 2001, 2003; Magori-Cohen et al., 2012; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021)
Commercial vessels carrying hitchhiker arthropod vectors (including returning live export vessels)	
En_1 Number of bovines at origin	(Gilbert et al., 2018b, 2018a; United States Department of Agriculture, 2021; FAO, 2022)
En_2 Bovines infected with LSDV	As detailed for the windborne dispersal pathway En_2
En_3 Number of vectors biting each bovine per infectious day	As detailed for the windborne dispersal pathway En_3
En_4 Bovine-to-vector transmission	As detailed for the windborne dispersal pathway En_4

Node	Data source
En_5 Vector flies to seaport	Extrapolation from qualitative opinion and (Central Intelligence Agency, 2020; US Census Bureau, 2021)
En_6 Vector lands on vessel	Extrapolation from qualitative opinion
En_7 Vessel travels to Australian seaport	
Commercial vessels	
Vessel travels to Australian waters ×	(Gaulier and Zignago, 2010; United States Census Bureau, 2022; Observatory of Economic Complexity, no date)
Vessel travels to specific Australian seaport	(Bureau of Infrastructure and Transport Research Economics, 2021)
Returning live export vessels	(Australian Government Department of Agriculture, Water and the Environment, 2022)
En_8 Vector survives transport and remains infectious	
Vector survives transport ×	(Border Management Group, 2003)
Vector remains infectious	As detailed for the windborne dispersal pathway En_6
En_9 Vector disembarks without detection	
Commercial vessels	Extrapolation from qualitative opinion
Returning live export vessels	Extrapolation from qualitative opinion
En_{10} Environmental conditions suitable for vector activity at arrival destination	Bureau of Meteorology and (Bailey and Meifert, 1973; Murray, 1987a; Reinhold, Lazzari and Lahondère, 2018)
Ex_A Susceptible bovine present	(Australian Bureau of Statistics, 2021a)
Ex_B Vector(s) bite bovine	As detailed for the windborne dispersal pathway Ex_B
Ex_C Bovine is infected	As detailed for the windborne dispersal pathway Ex_C
Torres Strait Treaty movements carrying hitchhiker arthropod vectors	
En_1 Number of bovines at origin	As detailed for the windborne dispersal pathway En_1
En_2 Bovines infected with LSDV	As detailed for the windborne dispersal pathway En_2
En_3 Number of vectors biting each bovine per infectious day	As detailed for the windborne dispersal pathway En_3
En_4 Bovine-to-vector transmission	As detailed for the windborne dispersal pathway En_4
En_5 Vector reaches vessel	Extrapolation from qualitative opinion and (Central Intelligence Agency, 2020; US Census Bureau, 2021)
En_6 Vector survives transport	Extrapolation from qualitative opinion
En_7 Vector disembarks without detection	Extrapolation from qualitative opinion
Ex_A Susceptible bovine present	(Australian Bureau of Statistics, 2021a)
Ex_B Vector(s) bite bovine	As detailed for the windborne dispersal pathway Ex_B
Ex_C Bovine is infected	As detailed for the windborne dispersal pathway Ex_C

The final incursion risk was estimated using two-dimensional Monte Carlo simulation with 1,000 uncertainty and 1,000 variability iterations. Sensitivity analysis was conducted to determine which nodes our models were most sensitive to. Additionally, we re-parameterised our models based on possible future climate change outcomes to investigate the potential impacts of climate change on LSDV incursion risk.

Results

We assessed 5,532 independent combinations of origin country, arrival destination and vector category across the four selected pathways: 516 combinations for the windborne dispersal pathway, 3,285 combinations for the commercial shipping (excluding returning live export vessels) pathway, 1,722 combinations for the returning live export vessels pathway and 9 combinations for the Torres Strait Treaty movements pathway.

While developing our models it became clear that robust data were not available for most nodes, leading to many of the parameters being highly uncertain. Thus, our models generated extremely wide credible intervals (CI) for the estimated risk of LSDV incursion into Australia for all pathways except the Torres Strait Treaty movement, for which the incursion risk was deemed to be zero**. It is critical to emphasize that undue attention should not be focused on the actual number of incursions calculated in this assessment, because of these highly uncertain parameters.** Indeed, the utility of this assessment is principally in identifying the comparative risks between different origin countries, arrival destinations and vector categories to better prioritise surveillance and mitigation efforts. Furthermore, our assessment identifies key parameters that must be better understood to accurately estimate the true risk of LSDV incursion into Australia.

Noting the uncertainty of many parameters, the estimated median risk of LSDV incursion into Australia via our three scenarios was (Figure 2):

1. **One incursion every 14,652 years if at least 30–50 vectors feeding on a single bovine are necessary for LSDV transmission (or 7×10^{-5} entries per year with 95% CI of 2×10^{-7} to 0.004 per year)**
2. **One incursion every 286 years if multiple, fewer vectors (i.e. 3–5) are necessary (median: 0.003 entries per year, 95% CI 9×10^{-6} to 0.22 per year)**
3. **One incursion every 5–6 years if a single insect is sufficient (median: 0.18 entries per year, 95% CI 6×10^{-4} to 8 per year)**

It is not known precisely how many insects are necessary to transmit a sufficient dose of LSDV to initiate an infection in a bovine and we cannot rule out that a single insect isn't capable of LSDV transmission under the right circumstances; however, based on experimental transmission studies, the minimum number of infectious insects required for successful LSDV transmission thus far is 14 *Aedes* mosquitoes (P. Beard, pers. comm.)[2]. Our findings suggest that individual PCR-positive insects will likely arrive in Australia and may be detected during surveillance activities, but this prevalence may not reflect the real risk of an outbreak if multiple infected insects must feed on a single bovine after arrival to initiate infection. Likewise, nucleic acid of foot and mouth disease virus (FMDV) and African swine fever virus are frequently detected in seized pork products; however, this does not necessarily equate with infectious virus or sufficient viral loads to initiate an infection but demonstrates that there is a viable pathway for virus to enter.

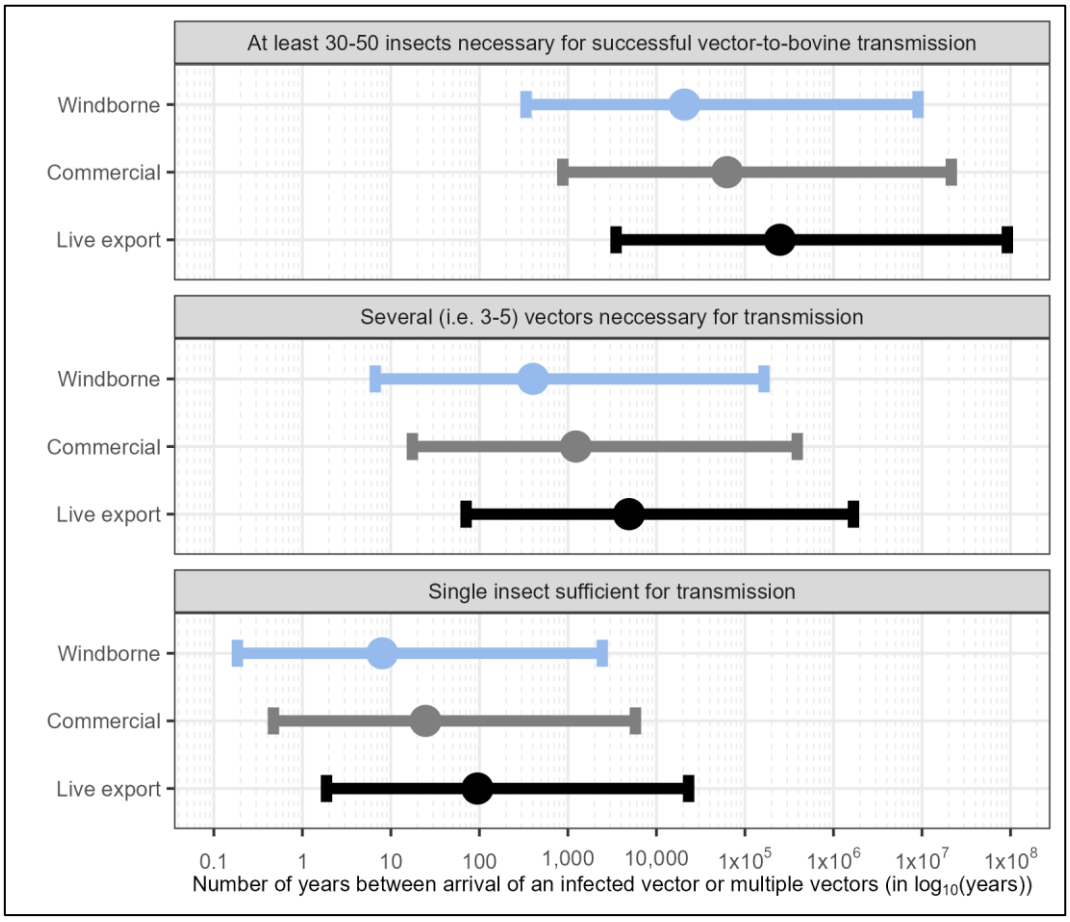

Figure 2 Estimated years between LSDV incursions into Australia via windborne dispersal, commercial shipping and returning live export vessels

The median estimate is represented by the central dot, i.e. one incursion every 20,000 years. Bars represent 95% credible intervals. The Torres Strait Treaty movements pathway is not shown since the probability of entry was estimated as 0 with no uncertainty.

[2] Interim results that have generously been made available ahead of publication.

Assuming the intermediate scenario that several vectors (i.e. 3–5 insects) are necessary to initiate infection, **the highest risk incursion pathway according to our model was through windborne dispersal**, with a median of 0.002 incursions of LSDV into Australia every year, or 1 incursion every 403 years. We estimated a median of 8×10^{-4} incursions per year, or 1 incursion every 1,229 years, via commercial shipping (excluding returning live export vessels) and a median of 2×10^{-4} incursions per year, or 1 incursion every 4,899 years, via returning live export ships. The lower risk associated with returning live export ships is due to the additional decontamination procedures in place for these vessels.

For all pathways, midges were assessed as the vector category of highest risk, followed by heavy fliers and then mosquitoes. This was driven primarily by the larger number of midges estimated to feed on bovines relative to the other vectors, noting that this was a highly uncertain parameter; thus, this should be interpreted cautiously. Importantly, transmission from *Culicoides* midges to bovines has not yet been demonstrated experimentally and recent work from the Pirbright Institute again was not able to find evidence of LSDV transmission from *Culicoides*, even under ideal laboratory conditions (P. Beard, pers. comm.)[3]. Thus, our estimated risk of LSDV incursion via midges may, in fact, be considerably overestimated. Critically, our atmospheric dispersion modelling (HYSPLIT) did not differentiate between the three vector categories: midges, mosquitoes and heavy fliers. HYSPLIT is designed to model dispersion of gases and fine atmospheric particles such as volcanic ash and dust storms and does not incorporate self-directed flight or movement of insects within a wind stream. HYSPLIT has previously been used to model long-distance aerial dispersion of midges (García-Lastra et al., 2012; Eagles et al., 2013, 2014; Durr, Graham and van Klinken, 2017; Aguilar-Vega, Fernández-Carrión and Sánchez-Vizcaíno, 2019), mosquitoes (Huestis et al., 2019; EFSA Panel on Animal Health and Welfare et al., 2020) and non-specific vector species (Klausner, Fattal and Klement, 2017) in separate studies; it has never been used to compare different vectors. Furthermore, it is challenging to incorporate insect-specific parameters with the limited empirical data available; for example, variation in survival/deposition rates between different vector categories is poorly understood, particularly in extreme conditions such as tropical cyclone events. Notably, stable flies are reportedly restricted to within 60 metres of the ground, even during wind-assisted dispersal, limiting long-range aerial spread (Showler and Osbrink, 2015). Thus, our estimated risk of LSDV incursion via heavy fliers may be overestimated. We assume that the capacity of an insect to travel long distances is affected by their size, weight, overall robustness, innate behaviour and other factors that HYSPLIT does not account for. Incorporation of these features would require a substantial modelling effort with development of new simulation modelling approaches.

Our model showed that windborne dispersal was possible from central and eastern Indonesia, Timor-Leste and PNG from October to May, although the highest risk period was December to April, in agreement with previous studies (Eagles et al., 2014)**.** This assumed that flights of up to 48 hours' duration were possible (i.e. insects could survive for up to 48 hours), noting that this is longer than other estimates reported in the literature. Again, our atmospheric dispersion modelling did not differentiate between vector categories, as discussed above. **Our model identified the Tiwi Islands and regions around the Van Diemen Gulf and Cobourg Peninsula east of Darwin (NAQS risk zones N6, N8a, N8b, N7, N5 and N4) as having the highest risk of incursion via a windborne dispersal event**, suggesting that these risk zones should be prioritised for enhanced surveillance activities, particularly during the high-risk months of December to April (Figure 3). These risk zones had the highest cumulative probability of the proportion of days suitable for windborne dispersal and the

[3] Interim results that have generously been made available ahead of publication.

probability of particles arriving from central Indonesia, and thus, since the probability of incursion from central Indonesia was relatively high due to the high bovine numbers in that region, these corresponding arrival destinations were also high.

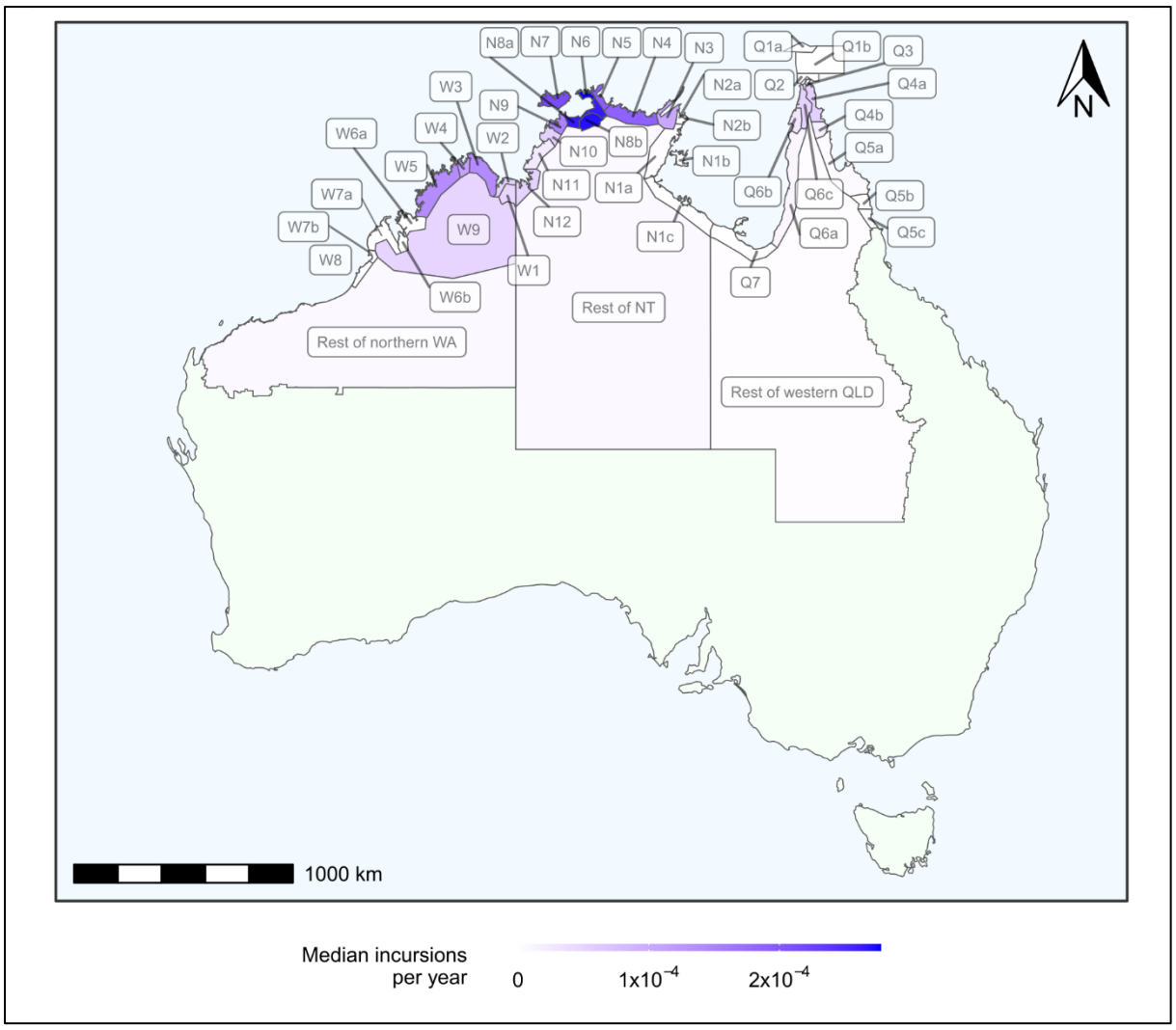

Figure 3 Estimated median LSDV incursions per year by NAQS risk zone via windborne dispersal of several (i.e. 3–5) vectors, aggregated by vector species and origin country

LSDV incursion risk via commercial shipping (excluding returning live export vessels) was assessed as highest from those countries with the highest bovine populations; however, this did not account for the distribution of bovines within those countries. Critically, our commercial shipping model was highly sensitive to the probability of a vector dispersing to a seaport and the probability of a vector landing on a ship. Both parameters are highly uncertain and would depend on the proximity of bovines to seaports. Therefore, this finding should be interpreted cautiously. Unsurprisingly, the higher volume Australian seaports, such as Port Hedland, Gladstone, Dampier, Hay Point and Port Walcott, were assessed as having the highest risk of LSDV incursion via commercial shipping (Figure 4). These findings reinforce the importance of current hitchhiker pest mitigation strategies, such as fumigation, inspections and the use of on-board insectocutors.

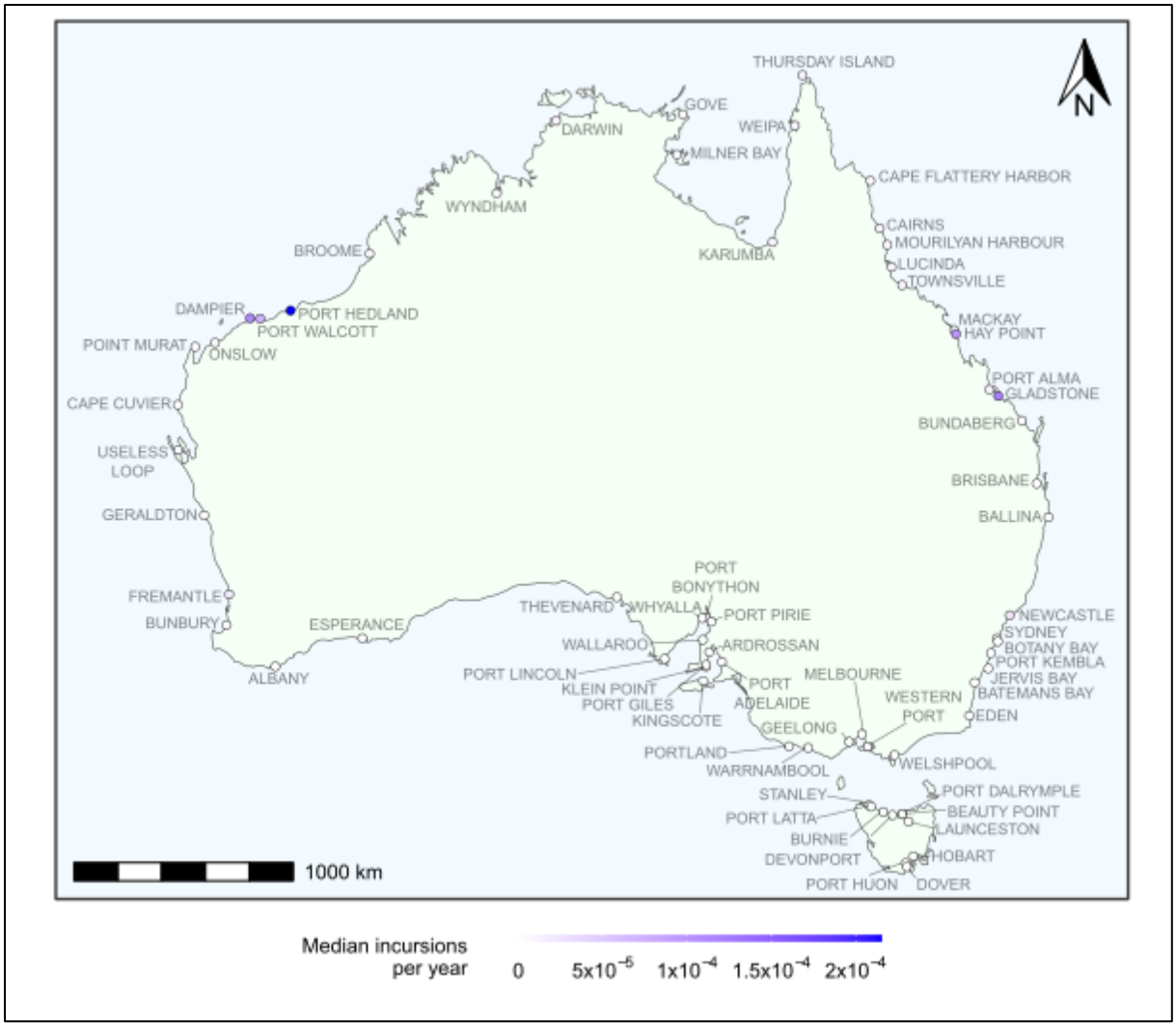

Figure 4 **Estimated median LSDV incursions per year by arrival seaport via arrival of several (i.e. 3–5) hitchhiker vectors on commercial ships (excluding returning live export vessels), aggregated by vector species and origin country**

For returning live export vessels, notably the central Indonesia to Darwin route was assessed as the highest risk for LSDV incursion, although overall the estimated risk from returning live export vessels was considerably lower than other pathways (Figure 5). Like the commercial shipping pathway, this was predominantly driven by the large number of bovines in central Indonesia relative to other live export trade partners and must be interpreted cautiously. Darwin was a higher risk arrival destination because a relatively large proportion of arriving vessels return from regions with high bovine numbers.

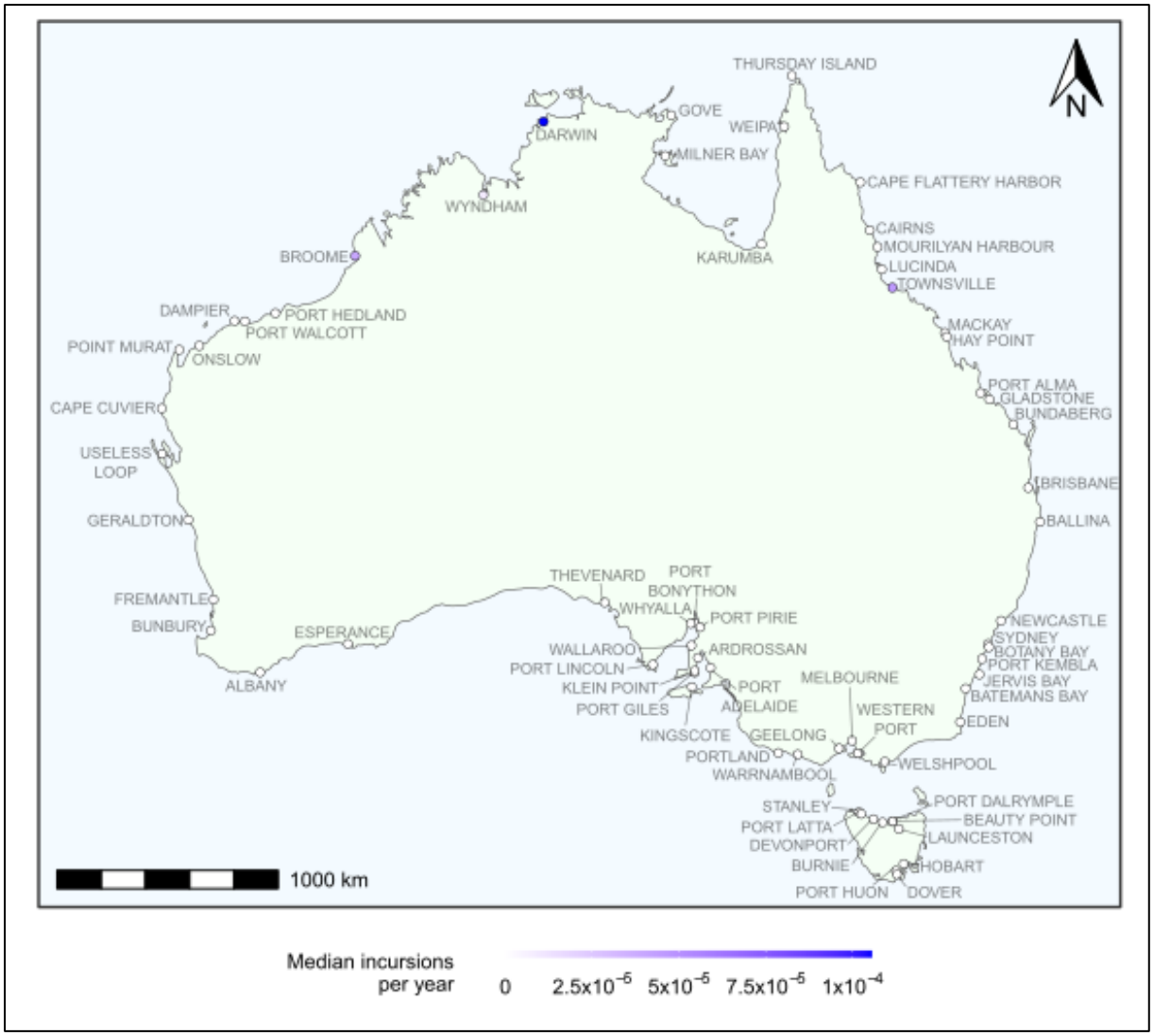

Figure 5 Estimated median LSDV incursions per year by arrival seaport via arrival of several (i.e. 3–5) hitchhiker vectors on returning live export vessels, aggregated by vector species and origin country

Our analyses suggest an increasing risk of LSDV incursion through the impacts of climate change, with a trend towards more days with suitable meteorological conditions for windborne dispersal in recent years compared to the 1980s and an expanded geographical range of optimal environmental conditions for vector activity across Australia.

Discussion

LSDV incursion into Australia via the four non-regulated pathways assessed appears to be unlikely under our intermediate scenario where several vectors (i.e. 3–5) are necessary for vector-to-bovine transmission, noting the wide CIs of our assessment. However, this risk increases substantially if a single insect is sufficient for transmission. The risk becomes negligible if many infectious vectors (i.e. 30–50) are necessary for LSDV transmission. Importantly, unlike viruses such as BTV that undergo biological replication within the insect host, evidence from the peer-reviewed scientific literature suggests that **it is improbable that the introduction of a single infectious vector would result in LSDV establishing in Australia** (Yeruham et al., 1995; Chihota et al., 2003; Kahana-Sutin et al., 2017; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). **Our models were**

very sensitive to the number of infectious vectors necessary to transmit a minimum infective dose of LSDV and work is urgently required to address this significant gap in our understanding of LSDV biology.

A recent SEJ exercise estimated the probability of LSDV incursion in the next five years (from any pathway) to be 28% (Centre of Excellence for Biosecurity Risk Analysis, 2022). Notably, there was substantial diversity of opinion amongst the experts, with many considering the risk much lower and others considering the risk to be higher (range of 4–56%, denoting the 10th and 90th percentiles). SEJ exercises are not based on modelling but use an internationally recognised process to make evaluative judgements on a range of complex and uncertain systems that otherwise may not be possible. Rapid SEJ exercises are one tool that has been used to broadly characterise the threat Australia faces, and if a material threat is indicated, inform further activities including more specific risk assessments. (DAFF officer, pers. comm.).

One of the primary outputs of this analysis was assessment of the comparative risks between different origin countries, arrival destinations and vector categories to better prioritise surveillance and mitigation efforts. **We identified the Tiwi Islands and the region extending east of Darwin up to and including the Cobourg Peninsula to be at higher risk of windborne incursion of vectors.** This has utility beyond LSDV, for example for arboviruses, other mechanically vectored animal, plant and human pathogens and for incursion of exotic flying insects of biosecurity importance. We additionally identified shipping pathways, with their existing mitigation efforts, as less likely pathways for LSDV incursion than windborne dispersal.

A key finding of this assessment was the extreme uncertainty associated with many of the parameter values used in the analysis, highlighting some of our knowledge gaps around this pathogen. Our models were particularly sensitive to the number of vectors feeding on a bovine over the course of an infection, the probability of a vector acquiring LSDV after feeding on an infectious bovine (particularly in the context of *Bos indicus* cattle), the probability of a bovine acquiring LSDV after being bitten by a vector and the probability of multiple vectors arriving (under the scenarios where multiple vectors were necessary). Additionally, the windborne dispersal model was highly sensitive to the probability of a vector being transported to Australia by wind and surviving windborne dispersal. Both shipping pathways were highly sensitive to the probability of a vector dispersing to a seaport, landing on a ship and disembarking without detection. Therefore, these represent key future research priorities for LSDV in the Australian context.

Our models are subject to considerable limitations and assumptions, which are discussed in detail in the main body of the report and are summarised below:

- We assumed that LSDV was endemic in all neighbouring countries for the purposes of this analysis. Ausvet is working closely on LSDV and FMDV in Indonesia, and our understanding is that LSDV currently remains confined to Sumatra and, more recently, Java; therefore, at this stage the LSDV incursion risks are overestimated in our assessment.
- We assessed origin locations at a country-level geographical resolution, while parameters such as livestock density in the origin country, LSDV incidence, vector abundance, the likelihood of vector dispersal to seaports, the likelihood of a ship travelling to Australia and the likelihood of windborne dispersal will vary considerably between regions within a country.
- We didn't consider temporal factors in our analysis. Vector abundance, especially, varies both on diurnal and seasonal scales, leading to a temporal LSDV incursion risk that we did not assess.
- We elected to group vectors into three categories: midges, mosquitoes and heavy fliers. Importantly, there is considerable species variation within these categories; however, given the lack of species-specific vector information in the literature, particularly with respect to relevant

zoophilic vector species and their relationship to LSDV, further breakdown by vector category is unlikely to be useful in the absence of additional data.

- The atmospheric dispersion model that we used to investigate long-distance windborne spread is designed to model particles and does not adequately represent many of the specific considerations necessary to model insect dispersal at fine scales, such as the ability to undertake self-directed flight or insect survival during dispersal. This would require development of a purpose-built, complex simulation model that was beyond the scale of this project.
- Many of the experimental studies on which we parameterised our model had limited representativeness in the context of our assessment. Noting that these laboratory studies are challenging to undertake and were conducted admirably given the technical limitations, these data are not ideal for our purposes. For example, these studies use highly susceptible *Bos taurus* breeds and typically detect LSDV using quantitative polymerase chain reaction, which will over-estimate viral infectivity. Sample sizes are small, leading to high uncertainty in their findings.
- We considered an incursion of LSDV to be the clinical infection of a single Australian bovine. This doesn't consider whether onward transmission occurred from this animal or whether this infection was detected. Simulation modelling using the Australian Animal Disease Spread Modelling framework with modifications for LSDV may shed additional light on the likelihood of onward transmission and outbreak size resulting from an incursion.
- We had limited information on the distribution and abundance of bovines across northern Australia, particularly in relation to feral animals. NAQS aerial survey data were used to estimate feral bovine numbers; however, these numbers were not corrected for sampling/perception bias and obtaining accurate estimates of free-ranging wildlife is challenging.

Conclusion

Based on our QRA, the probability of LSDV incursion into Australia via the four pathways assessed appears to be very low, under the intermediate scenario where several vectors are necessary for successful LSDV transmission. This is especially true in the current context where the disease burden in neighbouring countries is low. The risk increases substantially if a single vector is sufficient for transmission, noting that this has not been achieved experimentally. The risk becomes negligible if many vectors (i.e. greater than 30–50) are necessary for vector-to-bovine transmission, as the current literature suggests.

It is important to note that the parameters used in this assessment are subject to considerable uncertainty and will likely change over time as new data become available and as circumstances change globally. This may change the results of our assessment. The model is readily repeatable as it is coded in R and can be easily updated as new evidence is published.

Arguably, the most prominent gap in our understanding of LSDV transmission is the number of vectors necessary to initiate an infection. We strongly suggest that future research is directed towards examining the role of single insects in LSDV transmission. Furthermore, our atmospheric dispersion modelling did not distinguish between vector categories in this assessment. The development of a near-real-time atmospheric dispersion model with a user-friendly interface that can model insect-specific parameters is strongly recommended. This could be used to further assess windborne incursion risks and to target vector surveillance efforts to regions and times when meteorological conditions are suitable for long-distance windborne dispersal of vectors.

Current mitigation measures, such as insecticide treatments of incoming vessels, contribute to the effective management of the LSDV incursion risk and must be maintained and adapted as the disease situation changes in potential origin countries.

1 Introduction

1.1 Lumpy skin disease virus

Lumpy skin disease (LSD) is a highly contagious disease of cattle and water buffalo caused by lumpy skin disease virus (LSDV), a double-stranded DNA virus in the family *Poxviridae*, genus *Capripoxvirus* (Tuppurainen et al., 2017; Issimov et al., 2020). Clinically, the disease is characterised by generalised nodular skin lesions, fever, lymph node enlargement, a drop in milk production and poor reproductive performance (Khan et al., 2021). Affected farmers experience severe economic losses through a sharp decline in milk yield, reduced milk quality, hide damage, reduction in body weight, abortion, infertility and, in rare cases, death of animals (Babiuk et al., 2008a). Typically, morbidity in diseased herds is low to moderate, ranging from 5–20%; however, rarely it can approach 100% (Woods, 1988; World Organisation for Animal Health, 2010; Tuppurainen and Oura, 2012).

LSD was first reported in Zambia in 1929 and spread quickly within Africa (Morris, 1930; Khan et al., 2021). The virus was largely contained to the African continent until 1984 when it moved into the Middle East (House et al., 1990; Davies, 1991; Tuppurainen and Oura, 2012). In 1989 the virus spread to Israel and, over the following years, continued to spread across contiguous countries throughout the Middle East and central Asia, moving into Iran and into other central Asian countries (Yeruham et al., 1995; European Food Safety AHAW Panel, 2016). In 2015, the disease first emerged in Europe, spreading to several countries where it was effectively controlled through mass vaccination using live-attenuated vaccines (European Food Safety, 2018). Russia is the only country on the European continent to report outbreaks of LSD since 2017 (World Organisation for Animal Health, 2022).

Recently, LSDV has impacted Asia. China reported its first outbreak in August 2019, with the virus later spreading to Taiwan via unknown pathways (Gupta et al., 2020; Lu et al., 2021). In southern Asia, Bangladesh reported the disease in July and September of 2019 (Gupta et al., 2020). India first reported the disease in August 2019 (Gupta et al., 2020; Kumar et al., 2021). Nepal experienced their first outbreak of LSD in June 2020 (Acharya and Subedi, 2020). Sri Lanka and Bhutan then reported outbreaks in September 2020 (Azeem et al., 2022). An outbreak of LSDV also occurred in feral cattle in Hong Kong in October 2020 (Flannery et al., 2021).

In Southeast Asia, the disease was reported for the first time in 2021 in Laos, Thailand, Cambodia, Vietnam and Malaysia (World Organisation for Animal Health, 2022). Indonesia subsequently reported their first outbreak on 2 March, 2022 (World Organisation for Animal Health, 2022). In most cases, anecdotal reports preceded official reports, a typical occurrence in the region (Smith et al., 2015). The Indonesian outbreak has thus far been restricted to Sumatra and Java, to the authors' knowledge. Presumably, LSDV spread across the narrow Malacca Strait from Malaysia to Indonesia via windborne dispersal of vectors and/or movement of infected animals. Although the key drivers of cattle movements in this region are typically from east to west, from rural areas to central locations like Jakarta on Java Island (M. Patching, Boralis group, pers. comm.), spread of other notable livestock diseases (such as classical swine fever) has been observed from west to east (Sawford, 2015). Singapore also reported the disease for the first time in 2022 (World Organisation for Animal Health, 2022), despite being considered to be at negligible risk of incursion with low uncertainty, based on a qualitative risk assessment conducted by the Food and Agriculture Organization (FAO) in 2020 (Roche et al., 2020).

Since the earliest outbreaks of LSD in Africa, the long-distance dispersal of LSDV has been associated with movement of infected cattle along roads and railways and on foot (Ince, Cakir and Dereli, 2016; Sprygin et al., 2018, 2019). For example, it is speculated that the introduction of LSDV into Turkey in

2013 was associated with trade in unvaccinated animals exacerbated by conflict in the area and displacement of refugees (Sprygin et al., 2019). However, LSDV outbreaks in endemic regions typically coincide with the onset of the rainy season, suggesting that short-distance spread is facilitated by arthropod vectors (Weiss, 1968; Tuppurainen and Oura, 2012; Mercier et al., 2018). It is now understood that transmission between animals is predominantly mechanical through arthropods, although other routes such as via contaminated feed and water and via semen of diseased animals have also been reported (Annandale et al., 2014; Sprygin et al., 2019; Namazi and Tafti, 2021). There is also limited evidence of intrauterine transmission (Rouby and Aboulsoud, 2016). Importantly, LSD outbreaks do occur even in the absence of significant vector activity (Carn and Kitching, 1995a; Magori-Cohen et al., 2012; Sprygin et al., 2019). Experimentally, intravenous inoculation of LSDV led to considerably higher rates of generalised disease than did intradermal inoculation, suggesting that inoculation into the bloodstream, for example via haematophagy, is likely required to successfully initiate an infection (Carn and Kitching, 1995a). However, the highest viral loads are found in the nodules of clinical bovines, greatly exceeding the viral loads in the blood (Carn and Kitching, 1995a). Insect transmission from lesions is considerably more efficient than experimental inoculation, suggesting that insects actively enhance pathogenesis in the host, although the mechanism is not yet known (P. Beard, pers. comms.).

There has been much work on identifying specific insect species capable of vectoring LSDV. The current scientific consensus is that the virus can be acquired by most, if not all, haematophagous insects; successful transmission will then depend on biting behaviour (e.g. time to re-feeding, pain of bite, host preference), the duration of the gonotrophic cycle, vector abundance and host availability, among other factors (Kahana-Sutin et al., 2017). When assessing the risk of LSDV entering and establishing in the United Kingdom, Horigan et al. (2018) observed that:

> Whilst the competency of vectors in the [United Kingdom] is currently unknown, the fact that the disease has moved steadily up from southern Africa through many different climatic zones involving potentially many different vectors suggests that it is also likely to be transmitted by vectors present in the [United Kingdom].

Since this was written, the disease has also spread through Asia, further demonstrating that, in a practical sense, most countries (including Australia) are likely to have vector species capable of transmitting the disease; thus, the identification of individual vector species may be largely irrelevant.

Interrupted feeders that regularly parasitise cattle that are found in high abundance are the best candidates for transmitting LSDV (Berg et al., 2015). Stable flies, mosquitoes, midges, tabanids and ticks have all been identified as potential vectors.

Flies are insects of the order *Diptera*, characterised by having two pairs of wings: one for flight and one as a mechanosensory organ. The term 'fly' in entomology refers specifically to around 125,000 species of dipterans, or true flies. Technically, mosquitoes and midges are all part of the order *Diptera* and are therefore considered flies. In our analysis we define 'heavy fliers' as the brachyceran flies such as stable flies (*Stomoxys calcitrans*), house-flies (*Musca*), blowflies (*Calliphoridae*), march/horse flies (*Tabanidae*), soldier flies (*Stratiomyidae*) and louse flies (*Hippoboscidae*).

Mosquitoes are insects within the family *Culicidae*. There are approximately 3,500 species of mosquitoes, several of which transmit pathogens such as Zika virus, *Plasmodium* (which causes malaria) and dengue virus. Mosquitoes live in most parts of the world and are particularly attracted to areas with standing water, because the larval and pupal stages require water with little or no flow to survive (Centers for Disease Control and Prevention, 2022). Mosquitoes have long been suspected of playing a role in transmission of LSDV (Sprygin et al., 2019).

While several families of dipterans are commonly referred to as midges, of most relevance to LSDV transmission is the family *Ceratopogonidae*. This family contains more than 5,000 species, including *Culicoides*

spp., and are distributed worldwide on every continent except Antarctica (Boorman, 1993). *Culicoides* species are known vectors of diseases such as African horse sickness virus, bluetongue virus (BTV) and epizootic haemorrhagic disease virus, and can be dispersed long distances by wind (Burgin et al., 2013; Eagles et al., 2014; Paslaru et al., 2022). *Culicoides* midges have been implicated in LSDV transmission, although transmission of LSDV from *Culicoides* to bovines has never been demonstrated experimentally (Chihota et al., 2003; Eagles et al., 2014; Şevik and Doğan, 2017; Sanz-Bernardo et al., 2021). Recent unpublished work from the Pirbright Institute again found no evidence for transmission of LSDV by *Culicoides* midges, even under ideal laboratory conditions (P. Beard, pers. comm.)[4].

There are multiple examples of long-distance windborne dispersal of arthropod vectors, most notably *Culicoides* (Sellers, 1980; Murray, 1987b, 1987b; Murray and Kirkland, 1995; Mellor, Boorman and Baylis, 2000; Ducheyne et al., 2007; Eagles et al., 2014; Durr, Graham and van Klinken, 2017). Many of these long-distance dispersal events have been associated with disease introductions. For example, bovine ephemeral fever is thought to have spread from Korea to Japan via wind and wind was shown to assist in the spread of BTV in Europe (Shirakawa, Ishibashi and Ogawa, 1994; Hendrickx et al., 2008). Eagles et al. (2014) reported the detection of several exotic *Culicoides* species associated with isolates of novel BTV from the Top End of the Northern Territory. These specimens, collected as part of an active arbovirus surveillance program, were used to assess the plausibility of dispersal from neighbouring countries. They determined that windborne dispersal of *Culicoides* into Australia was possible from as far west as Lombok, Indonesia, and as far east as southern Papua New Guinea (PNG), based on a 20-hour dispersal window. The detection of two exotic *Culicoides* species from Douglas Daly shows that dispersal beyond the Northern Australia Quarantine Strategy (NAQS) risk zones is possible. However, no origin source outside Australia could be identified for this incursion, raising the possibility of spread from an initial undetected incursion site within Australia (Eagles et al., 2014). A meta-analysis of mosquito flight distances revealed reports of windblown dispersal between 600 and 800 km for several *Culex* and A*edes* species (Verdonschot and Besse-Lototskaya, 2014). Ritchie and Rochester et al. (2001) proposed that Japanese encephalitis virus (JEV) was introduced to Australia by windblown *Culex* mosquitoes. Reports on wind dispersal of heavy fliers are scarce, but *Stomoxys calcitrans* was detected 225 km away from the release site in one study (Hogsette and Ruff, 1985). Notably, BTV and JEV are biologically transmitted, with viral replication occurring within their arthropod hosts. In contrast, there is no robust evidence for biological transmission of LSDV. Additionally, transmission from *Culicoides* midges to bovines has not yet been demonstrated experimentally.

The potential for wind-assisted spread of LSDV through long-distance dispersal of vectors has been proposed, especially following the LSD outbreaks that occurred in Israel in 1989 and 2006 (Yeruham et al., 1995; Magori-Cohen et al., 2012; Klausner, Fattal and Klement, 2017). In both outbreaks, Egypt was suffering from an exceptionally severe epizootic of LSD at a similar time and no other outbreaks were identified in the broader geographic region (Klausner, Fattal and Klement, 2017). Most cattle herds in Israel are closed and the borders are tightly controlled due to conflict, so introduction of the disease through movement of infected cattle was considered highly unlikely (Klausner, Fattal and Klement, 2017). This led the authors to suggest that these Israeli outbreaks occurred as a result of long-distance windborne spread of vectors (Klausner, Fattal and Klement, 2017).

In addition to windborne dispersal of arthropod vectors, another potential incursion pathway for vector-borne diseases is the presence of hitchhiker arthropods on vessels and aircraft (Border Management

[4] Interim results that have generously been made available ahead of publication.

Group, 2003; Oliveira et al., 2018). These pests and pathogens may travel opportunistically on ships and aircraft or in shipping containers or non-containerised cargo such as cars, tyres or machinery (Inspector-General of Biosecurity, 2018). Aircraft were not considered in our assessment because of the low volume of air freight compared to sea freight; for example, from 2017 to 2021 less than 0.5% of the imported trade volume was as air freight (National Freight Data Hub, 2022).

Commercial vessel biosecurity risks are managed through the Maritime Arrivals Reporting System (MARS) and detailed risk mitigation measures are in place. All commercial vessels arriving in Australia must submit details of their biosecurity status and last port of call pre-arrival (Inspector-General of Biosecurity, 2018). The Department operates a vessel compliance scheme through MARS to facilitate risk-based, targeted inspections; each ship is inspected unless a risk-based assessment exempts that voyage.

Returning live export vessels are managed separately. Pre-arrival, livestock ships are thoroughly cleaned, are disinfected with soda ash, and receive two insecticide treatments, although the effectiveness of these treatments may not be uniform across decks (DAFF officer, pers. comm.). While treatments are ineffective against larvae, pupae and eggs, transstadial and transovarial transmission of LSDV have only been reported for ixodid ticks (Tuppurainen et al., 2011). Insectocutors are located on board for the primary purpose of screwworm fly (*Chrysomya bezziana*) surveillance and preferentially attract heavy fliers. These are turned on once in Australian waters and remain on in port (NAQS officer, pers. comm.). Additionally, every returning livestock vessel is inspected on arrival at its first port.

These risk management regimes are well-targeted, effective and efficient and undoubtedly limit entry of substantial biosecurity risk material, pests and diseases. However, the challenges posed to Australia by hitchhiker pests are likely to increase in the future due to increased global trade (Inspector-General of Biosecurity, 2018).

1.2 Potential impacts of climate change

Because of the critical role of protein-feeding arthropods in LSDV transmission, it is no surprise that the distribution and spread of LSDV are heavily influenced by environmental conditions and landscape (Tuppurainen et al., 2013b; Abera et al., 2015; Lubinga et al., 2015; Alkhamis and VanderWaal, 2016). Therefore, it is reasonable to consider the impacts of climate change on the probability of LSDV incursion into Australia. Climate change through human-induced greenhouse gas emissions has resulted in marked warming since the mid-20th century (IPCC, 2021). Australia has already experienced increasing temperatures, shifting rainfall patterns and rising oceans, presenting considerable challenges to humans, animals and the environment (Moise et al., 2015). Much attention has been focused on the impacts of climate change on vector-borne diseases, although not necessarily specifically in the Australian context and not with a focus on LSDV (reviewed in Rocklöv and Dubrow, 2020). In the context of human vector-borne diseases, a major threat to Australia is the introduction and establishment, or range expansion, of important vector species such as *Aedes albopictus* or *Aedes aegypti*, respectively (Hall et al., 2021). Additionally, altered environmental conditions are likely to affect vector survival and vector behaviour (Beebe et al., 2013; Ali, Carlile and Giasuddin, 2020). Furthermore, host susceptibility to infection may be impacted due to exacerbation of physiological stressors. Considerations of the impacts of climate change on LSDV are similar.

Warmer climates are generally conducive to insect survival and development, with optimal temperatures for mosquitoes, midges and heavy fliers typically between 20 and 25°C (Gilles, David and Duvallet, 2005; Hendrickx et al., 2008; Venter, Boikanyo and de Beer, 2019; Rocklöv and Dubrow, 2020; Tugwell et al., 2021). Substantial future warming is projected with very high confidence for northern Australia, including the Torres Strait. Mean warming is predicted to increase by 0.5–1.3ºC above 1986–2005 levels, and

maximum and minimum temperatures are also projected to increase (Suppiah et al., 2011; Moise et al., 2015). Northern Australia is expected to experience an increase in the frequency of hot days and a longer duration of warm spells (Moise et al., 2015). Therefore, increased temperatures may inhibit LSDV vector survival to some extent across far northern Australia, particularly during the monsoon season (December to April), which is the high-risk period for windborne dispersal. However, increased temperatures through central and southern Australia may facilitate range expansion of vectors, may increase the abundance of vector species capable of transmitting LSDV and may increase the proportion of the year suitable for vector activity and disease transmission.

Through the 20th century there was a trend towards slightly increased rainfall, particularly across the north-western regions (Moise et al., 2015). However, natural variability, including prolonged periods of drought as well as above-average rainfall, driven in part by the El Niño Southern Oscillation, has dominated these longer-term trends. Natural climate variability is expected to continue to be the major driver of annual mean rainfall in northern Australia, although substantial changes to wet-season rainfall cannot be ruled out (based on conflicting results from different models) (Moise et al., 2015). The Torres Strait is perhaps projected to experience less change in rainfall than other parts of northern Australia (Suppiah et al., 2011). The intensity of heavy rainfall events is projected to increase, with high confidence, while changes to drought frequency are less clear (Moise et al., 2015). Changes in relative humidity are projected to be minimal (Suppiah et al., 2011; Moise et al., 2015). Moderate to heavy rainfall is known to impede flight of *Culicoides* (Burgin et al., 2013; Eagles et al., 2014). However, vector abundance is widely known to be associated with wet areas, with LSDV outbreaks typically coinciding with the onset of the rainy season in endemic regions (Tuppurainen and Oura, 2012). Overall, rainfall changes due specifically to climate change are unlikely to be major drivers of LSDV dynamics in northern Australia.

Only small changes in mean surface wind speed (i.e. –4 to +3% for northern Australia and –1.12 to 2.47% for the Torres Strait) are projected for northern Australia under near-future climate models (Suppiah et al., 2011; Moise et al., 2015). Tropical cyclones are projected, with medium confidence, to increase in intensity (by 2–11%) but are anticipated to occur less frequently (Suppiah et al., 2011; Moise et al., 2015). While these cyclone events may be more likely to transport vectors from our near neighbours, strong winds are known to inhibit flight activity and reduce insect survival, particularly for fragile species like mosquitoes (Hendrickx et al., 2008; Agren et al., 2010; Boyle et al., 2012; Sanders et al., 2012; Sedda et al., 2012; Chen et al., 2020). More information on vector survival under these extreme conditions is required to better understand how an increased frequency of tropical cyclone events may affect LSDV incursion.

Climate change may also directly impact international shipping through an increased frequency and intensity of extreme weather events. This could lead to damage to ships, cargo and ports, changes in shipping routes, and perhaps higher fuel consumption by ships due to changes in wind velocity and patterns (Kuhn and Beaufoy, 2009). All of these are likely to lead to increased travel times, thereby reducing the probability of importing live and infectious vectors in the context of LSDV transmission.

1.3 Introduction to risk analysis

Risk analysis is an established scientific discipline underpinned by an extensive theorical framework. In animal health, the key principles of risk analysis largely come from import risk analysis. However, these principles can be applied to other types of risk analysis.

Risk analysis has four related components: risk communication, hazard identification, risk assessment and risk management (Figure 6).

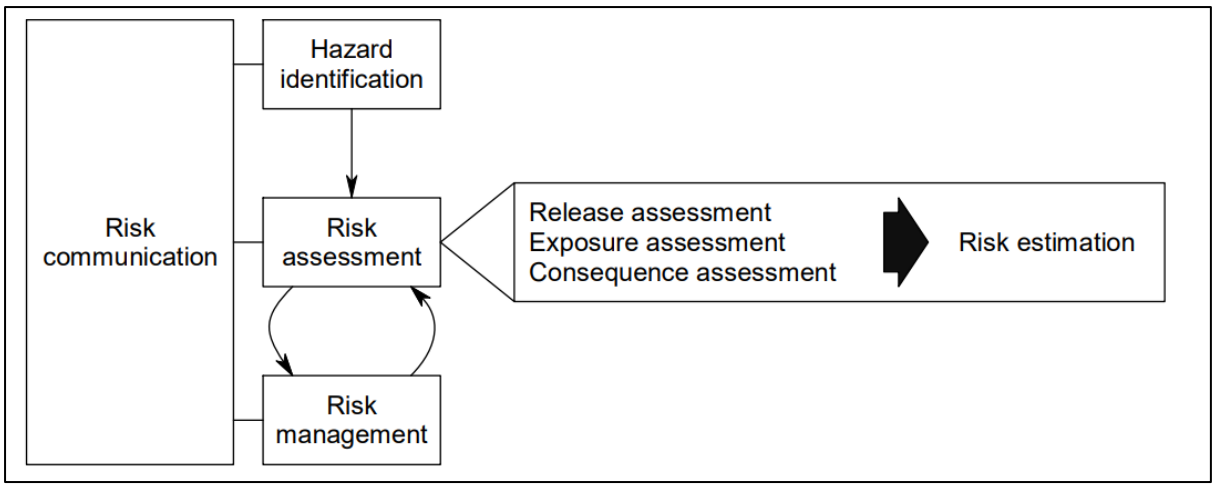

Figure 6 Import risk analysis components and risk assessment steps[5]

While hazard identification, risk assessment and risk management occur sequentially, risk communication should occur throughout the risk analysis process. This project concentrates specifically on the risk assessment component of the hazard: LSDV entry into Australia via four non-regulated pathways.

Risk assessment also has several components: release assessment, exposure assessment and consequence assessment. Release assessment is also referred to as entry assessment, while exposure assessment is sometimes termed establishment assessment. Together, these components enable the final step of risk assessment: risk estimation.

Entry (release) assessment consists of describing the pathways necessary for the introduction of a hazard or pathogen into a new environment. In the case of LSDV, this would involve describing the sequence of steps necessary for LSDV to enter Australia.

Exposure (establishment) assessment consists of describing the pathways necessary for exposure of animals to the hazard or pathogen after entry and the establishment of the hazard in the importing country. In the case of LSDV, exposure assessment may describe how a vector, fomite or infected animal carrying LSDV that has entered Australia could infect a susceptible bovine.

Consequence assessment involves evaluating the consequences of entry and exposure to the hazard or pathogen. Consequences can be direct (such as production losses) or indirect (such as the cost associated with a control or elimination program). For our assessment we have assumed, without conducting formal economic analyses, that introduction of LSD would have extreme consequences for Australia's bovine industries.

Risk estimation produces an overall measure of the risk being assessed, considering entry, exposure and consequence assessment (Australian Government Department of Agriculture and Water Resources, 2016). In qualitative risk assessment, the likelihood of an event occurring and the magnitude of the consequences, and therefore the final risk estimation, are expressed using qualitative descriptors such as 'high', 'moderate' or 'low'. Qualitative risk assessments are relatively simple to understand and easy to implement. Qualitative risk assessments are often sufficient but can be followed by a quantitative

[5] From RP Subasinghe, SE McGladdery and BJ Hill, 'Surveillance and zoning for aquatic animal diseases', *FAO fisheries technical paper 451*, Food and Agriculture Organization of the United Nations, 2004, accessed 9 September 2022.

assessment where further detailed insights are required (Murray and World Organisation for Animal Health, 2010). We previously conducted a qualitative risk assessment on the incursion of LSDV into Australia via the same four non-regulated pathways assessed here. In that previous assessment, we estimated the probability of LSDV incursion through the four pathways to be negligible but noted that this assessment did not account for the volume of vector movements into Australia, which may change the overall results of the assessment (Zalcman, Hall and Cowled, 2022). From those findings, it was considered worthwhile to undertake a quantitative risk assessment (QRA) to further estimate the probability of LSDV incursion and incorporate uncertainty into the assessment.

In a QRA, the likelihood of occurrence is expressed in numerical terms, such as 'one disease introduction every 100 years'. QRA requires quantitative data and can provide more detailed insights in circumstances where there are high levels of complexity and uncertainty. However, numerical results are often mistakenly considered to be highly precise, when in fact the quality of the underlying data may substantially limit their precision. Thus, results of QRAs should be interpreted with caution and the uncertainty of the outputs must be considered (Murray and World Organisation for Animal Health, 2010). Nevertheless, quantitative assessments encourage a more rigorous approach by forcing assessors to outline specific probability distributions at each individual step throughout the entry and exposure pathways. For both qualitative assessments and QRAs it is important to be transparent and to fully describe the underlying assumptions and data sources used for the analysis (Murray and World Organisation for Animal Health, 2010).

QRAs can be conducted using deterministic approaches or stochastic/probabilistic approaches (Vose, 2000). In a deterministic approach, a single point estimate is made for each parameter and the final risk estimate is derived by combining these individual values. However, biological parameters are rarely represented by a single fixed value. In a stochastic approach, each parameter is represented by a probability distribution, which spans the range of possible values and weights each value by the probability of its occurrence (Vose, 2000). Probability distributions incorporate both the uncertainty of a parameter, which is due to a lack of precise knowledge, and variability, the intrinsic variation in a parameter due to chance. For example, the number of vectors that will feed on an individual bovine in a year has not been robustly measured, making this an uncertain parameter. This parameter will also be variable, because vectors will preferentially feed on some hosts over others due to factors such as host size and genetic factors like excretion of volatile compounds. Parameters are combined by randomly sampling from each input distribution over thousands of iterations and combining these samples in a final risk calculation to generate a distribution of the overall risk, incorporating both variability and uncertainty. The ability to robustly model uncertainty is a major advantage of QRAs. For example, quantitative analyses provide additional insights such as where uncertainty can be reduced through further research and investigation. Again, it is essential to consider both the level of uncertainty and variability when interpreting the final risk estimation.

In our assessment, we have limited QRA to entry and exposure assessment. We focus on deriving quantitative likelihood estimates for the entry of LSDV into Australia and exposure of Australian bovids to LSDV via four non-regulated pathways.

1.4 Purpose of this risk assessment

Originally, this project had two broad objectives: 1) to develop a general risk assessment framework for disease incursion, and 2) to use this framework to conduct a qualitative risk assessment for the entry of LSD into Australia via non-regulated pathways. It was the intention of the Department of Agriculture, Fisheries and Forestry (the Department, DAFF) that NAQS staff could use the general risk assessment framework to guide ongoing, regular risk assessments to inform their surveillance.

The probability of LSDV entry into Australia will increase as the disease spreads through countries to our north and disease incidence increases. Like most countries, Australia is likely to have vector species capable of transmitting LSDV. Furthermore, Australia has a substantial bovine population and favourable environmental conditions that would facilitate exposure and wider spread. Given the implications for trade, as well as the effects on production, the consequence of entry and exposure is assumed to be extreme.

Notably, bovine densities are relatively low in northern Australia; livestock trade and movement into Australia is stringently controlled and animal health surveillance systems are comparatively strong relative to many countries. Furthermore, a series of events (or steps along a risk pathway) would need to occur sequentially for entry and exposure of LSDV into the Australian bovine population. While several risk mitigation measures have already been, and continue to be, implemented, formal risk assessment allows for targeting of approaches and ensures that the available resources are allocated most efficiently. In particular, risk assessment facilitates the systematic assessment of risk and implementation of risk-based surveillance.

This analysis extends our qualitative risk assessment framework and highlights nodes with high uncertainty as possible areas for future research prioritisation. This assessment is complex and is uniquely designed for the assessment of risk associated with LSDV. It is not intended to be used regularly for other diseases or pathways.

1.5 Risk question

In this assessment we aimed to answer the following question.

"Assuming a situation where LSDV is endemic throughout Southeast Asia and PNG, how many LSDV incursions into Australia per year are estimated through the four specified non-regulated pathways?"

Although the risk question is ultimately based on a future situation, we used current (or the most recently available) data to parameterise our models.

1.6 Definitions and terminology

For the purpose of this report, we use the following definitions for key terms:

Combination in this assessment refers to individual combinations of origin country, arrival destination and vector category within a pathway. For example, the windborne dispersal pathway can be broken down into many individual combinations (e.g. midges from central Indonesia to NAQS risk zone N7 or mosquitoes from Timor-Leste to NAQS risk zone Q6b).

Leakage refers to biosecurity risk material that is not intercepted upon initial inspection at the border.

Monte Carlo simulation involves randomly sampling each probability distribution within the model for many hundreds or thousands of iterations to reproduce the probability density function of the outcome (in this case, the probability of LSDV entry) (Vose, 2000).

Nodes are steps or events within in a pathway that must occur successively for a hazard to enter and establish. In a QRA, each node is parameterised using a probability density function.

Non-regulated pathways refer to pathways for entry and exposure of a hazard that are not heavily controlled through official processes or not controlled at all, such as windborne entry of insects. These are the focus of our assessment.

A **probability density function** defines the range of possible values for a node along the x-axis and the likelihood of those values occurring along the y-axis. These distributions can take various forms, including Normal, discrete, uniform, Program Evaluation and Review Technique (PERT), negative binomial and others (Vose, 2000). Probability density functions allow for incorporation of uncertainty and variability into the model.

Regulated pathways refer to pathways for entry and exposure of a hazard that are heavily controlled through official processes, such as the arrival of passenger aircraft. These are excluded from our assessment.

Risk refers to the likelihood of occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health. This definition is presented in the Terrestrial Animal Health Code of the World Organisation for Animal Health (WOAH). The various components of risk analysis are discussed above.

Scenario in this assessment refers to one of three conditions under which we conducted our assessment:

1. At least 30–50 insects are necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors are necessary for transmission
3. A single insect is sufficient for transmission

A **scenario tree** outlines the conditional series of events (or nodes) necessary for the entry and exposure of a disease.

Uncertainty is the imprecision due to the assessor's lack of knowledge of the parameter (Vose, 2000). This can be reduced through further research and investigation.

Variability is the natural stochasticity (e.g. randomness) in a physical system, due to either chance or individual variation (Vose, 2000).

2 Methodology

2.1 Pathways for inclusion

We consulted with the Department during the contracting stage of this work and during project inception to clarify pathways for inclusion. We presented draft pathways to a group of NAQS staff during a preliminary consultation meeting on 25 March (Appendix 1), from which the final pathways were selected. We also conducted a review of the scientific literature to ensure that our pathways were clearly defined and plausible for the transmission of LSDV. This review was submitted previously as part of this project.

The final four non-regulated pathways for inclusion in the analysis of LSDV entry into Australia were:

- windborne dispersal of arthropod vectors
- commercial vessels (excluding returning live export vessels) carrying hitchhiker arthropod vectors
- returning live export vessels carrying hitchhiker arthropod vectors
- Torres Strait Treaty movements carrying hitchhiker arthropod vectors

Since we did not know the minimum number of vectors necessary to initiate an LSDV infection in a bovine, each pathway was assessed under three separate scenarios:

1. At least 30–50 insects necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors necessary for transmission
3. A single insect is sufficient for transmission

2.2 Vector categories for inclusion

After conducting a review of the scientific literature and consulting with entomologists, we proposed to group LSDV vectors into three broad categories: midges (*Ceratopogonidae*), mosquitoes (*Culicidae*) and heavy fliers (including stable flies, tabanids and other brachyceran flies).

2.3 Origin countries for inclusion

We used spatial analysis and sea route distance modelling to determine origin countries for inclusion for each individual pathway.

For the windborne dispersal pathway, we first consulted the literature to determine the maximum flight durations for midges, mosquitoes and heavy fliers for windborne dispersal. Previous studies have variously reported putative dispersal times of 12 hours, 20 hours and up to 36 hours for *Culicoides* (Sellers, Pedgley and Tucker, 1977; Alba, Casal and Domingo, 2004; Agren et al., 2010; Burgin et al., 2013). Flight durations longer than this are reportedly highly unlikely (Eagles et al., 2013). Importantly, models investigating windborne dispersal of insect vectors have previously identified this as a critical and uncertain parameter (Eagles et al., 2013). As a conservative estimate, we set the maximum flight duration to 48 hours and used the Hybrid Single-Particle Lagrangian Integrated Trajectory Model (HYSPLIT) version 5.2.1 atmospheric dispersion model (Stein et al., 2015) to determine the origin countries from which particles could be dispersed over this flight time.

This model has been used extensively to assess the involvement of windborne arthropods in various infectious disease outbreaks (García-Lastra et al., 2012; Eagles et al., 2013, 2014; Durr, Graham and van Klinken, 2017; Klausner, Fattal and Klement, 2017; Aguilar-Vega, Fernández-Carrión and Sánchez-

Vizcaíno, 2019; Huestis et al., 2019; EFSA Panel on Animal Health and Welfare et al., 2020). HYSPLIT allows for the calculation of both trajectories of a single air parcel or concentration/dispersion plumes of multiple particles (e.g. insects) in either forwards (from a source) or backwards (from an arrival destination) directions for a specified flight duration (Draxler and Hess, 1998). HYSPLIT takes input gridded meteorological data files (*Gridded Climate: NOAA Physical Sciences Laboratory*, no date) and uses these files to integrate the position of an air parcel (for trajectories) or multiple independent particles (for dispersion runs) over time from a given starting location on a given date and time. Various inputs can be adjusted to account for particle-specific parameters. The output can be analysed and visualised using geographic information system tools.

We calculated 48-hour backwards trajectories from the centroids of all arrival destinations (i.e. NAQS risk zones) over the period 1 January 2017 to 31 December 2021 at 6-hourly intervals using the meteorological files from the NCEP-NCAR Reanalysis 1 project (Kalnay et al., 1996) with a 2.5° horizontal resolution.

We implemented HYSPLIT through a custom R script (Hysplit_LSDV_final_NCEP.R, available through Bitbucket) using the packages splitr v0.4.0.9000 (Iannone, 2022), sf v1.0-7 (Pebesma, 2018), tidyverse v1.3.1 (Wickham et al., 2019), lubridate v1.8.0 (Grolemund and Wickham, 2011), scales v1.2.0 (Wickham and Seidel, 2022), readxl v1.4.0 (Wickham and Bryan, 2022) and rgeos v0.5-9 (Bivand and Rundell, 2021). The scripts and accompanying data files are available at the Bitbucket repository https://robynhall@bitbucket.org/robynhall/awe-lsd-21_quantitative.git upon request.

For commercial shipping pathways, we considered all countries within 10 days' travel time as potential origins (see Section 3.6.2 for discussion). Travel times from countries or origin to Australian ports were estimated based on average speeds of 33 km/hr for commercial ships excluding live export vessels and 24 km/hr for live export vessels (Leaper, 2019).We previously used straight line distances to origin countries for the commercial shipping pathways in our qualitative assessment. However, we noted in that report that actual travel durations were considerably longer due to the presence of land masses and other obstacles. Therefore, for our quantitative analysis we estimated shipping distances using a custom R script (get_searoute_data.R) with the packages sf v1.0-8 (Pebesma, 2018), tidyverse v1.3.1 (Wickham et al., 2019), jsonlite v1.8.0 (Ooms, 2014), geosphere v1.5-14 (Hijmans et al., 2021), parallel v4.1.3 (R Core Team, 2021), cowplot v1.1.1 (Wilke, 2020), marmap v1.0.6 (Pante and Simon-Bouhet, 2013), raster v3.5-21 (Hijmans, 2022), rgdal v1.5-32 (Bivand, Keitt and Rowlingson, 2022), gdistance v1.3-6 (van Etten and de Sousa, 2020), seegSDM v0.1-9 (Golding and Shearer, 2019) and smoothr v0.2.2 (Strimas-Mackey, 2021). This script first identifies the closest seaport within each origin country to each Australian seaport (based on the world-administrative-boundaries shapefile (World Food Programme, 2019)), and then calculates the shortest path between these ports while iteratively altering direction to avoid land masses. This excluded countries without seaports. African countries were also excluded, since LSDV has not previously entered Australia from the African continent despite being endemic there for many decades. Seaport details were obtained from the World Port Index (National Geospatial-Intelligence Agency, 2019).

For all pathways, we excluded Australian territories (Norfolk Island, Cocos (Keeling) Islands, Christmas Island, and Heard and McDonald Islands) and Antarctica. We excluded New Zealand because of their current animal disease status, high level of biosecurity controls and historical capacity to manage exotic disease incursions effectively (Davidson, 2002).

We also excluded Pacific Island countries and territories and island nations with a land area of <3,000 km^2, with the exception of Singapore where LSDV has already been detected. Using this criteria, the following regions were excluded: American Samoa, Antarctica, British Indian Ocean Territory, Christmas Island, Cocos (Keeling) Islands, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, French Southern and Antarctic Lands, Guam, Heard and McDonald Islands, Kiribati, Maldives, Marshall

Islands, Mauritius, Nauru, New Caledonia, New Zealand, Niue, Norfolk Island, Northern Mariana Islands, Palau, Réunion, Samoa, Seychelles, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, Wallis and Futuna Islands, and United States Minor Outlying Islands. We assumed the probability of LSDV arriving in island countries of < 3,000 km² to be negligible, partly because we know that ocean borders and relatively low livestock populations have historically kept these nations free of many infectious livestock diseases (Brioudes, 2016).

Hong Kong Special Administrative Region, China mainland, Macau and Taiwan were classified together as 'China and associated autonomous regions'. Indonesia was divided into western, central and eastern regions for improved geographical resolution to enable more nuanced risk assessment (Figure 1). Origin countries were spatially defined using the world-administrative-boundaries shapefile, modified to subdivide Indonesia as described above (World Food Programme, 2019).

2.4 Arrival destinations for inclusion

We considered NAQS risk zones to be the most relevant spatial classification of arrival destinations for the windborne dispersal pathway, based on LSDV control and surveillance activities. Importantly however, NAQS risk zones only extend for a relatively short distance beyond the coastline, whereas vectors may be deposited by wind further inland where the density of susceptible hosts and conditions for vector establishment may be different to those at the coastline. Therefore, we also added three additional arrival destinations ('rest of NT', 'rest of northern WA', 'rest of western QLD') based on modifications of the Statistical Area 4 (SA4) regions 702, 510 and 315, respectively (Australian Bureau of Statistics, 2021b). To derive these additional regions, we calculated the spatial difference between these three SA4s and NAQS risk zones in QGIS (version 3.24.1+Tisler) using the 'Difference' algorithm, based on the shapefiles SA4_2021_AUST_GDA2020 (Australian Bureau of Statistics, 2021b) and NAQS_RiskAreas2015 (NAQS officer, pers. comm.). We then manually aligned the coastline of the two shapefiles.

For the commercial shipping pathways, we obtained a list of the 66 Australian seaports and their point locations from the World Port Index (National Geospatial-Intelligence Agency, 2019).

We considered the NAQS risk zones within the Torres Strait as potential arrival destinations for the Torres Strait Treaty movements pathway (Figure 12).

2.5 Scenario trees and development of the models

Pathways were mapped using scenario trees, where each node of the tree represents an event that must occur to enable the entry and establishment of LSDV in Australia (FAO, 2014). We included three category nodes that, taken together, defined different subpopulations of interest (i.e. the number of vectors biting bovines by origin country) (FAO, 2014).

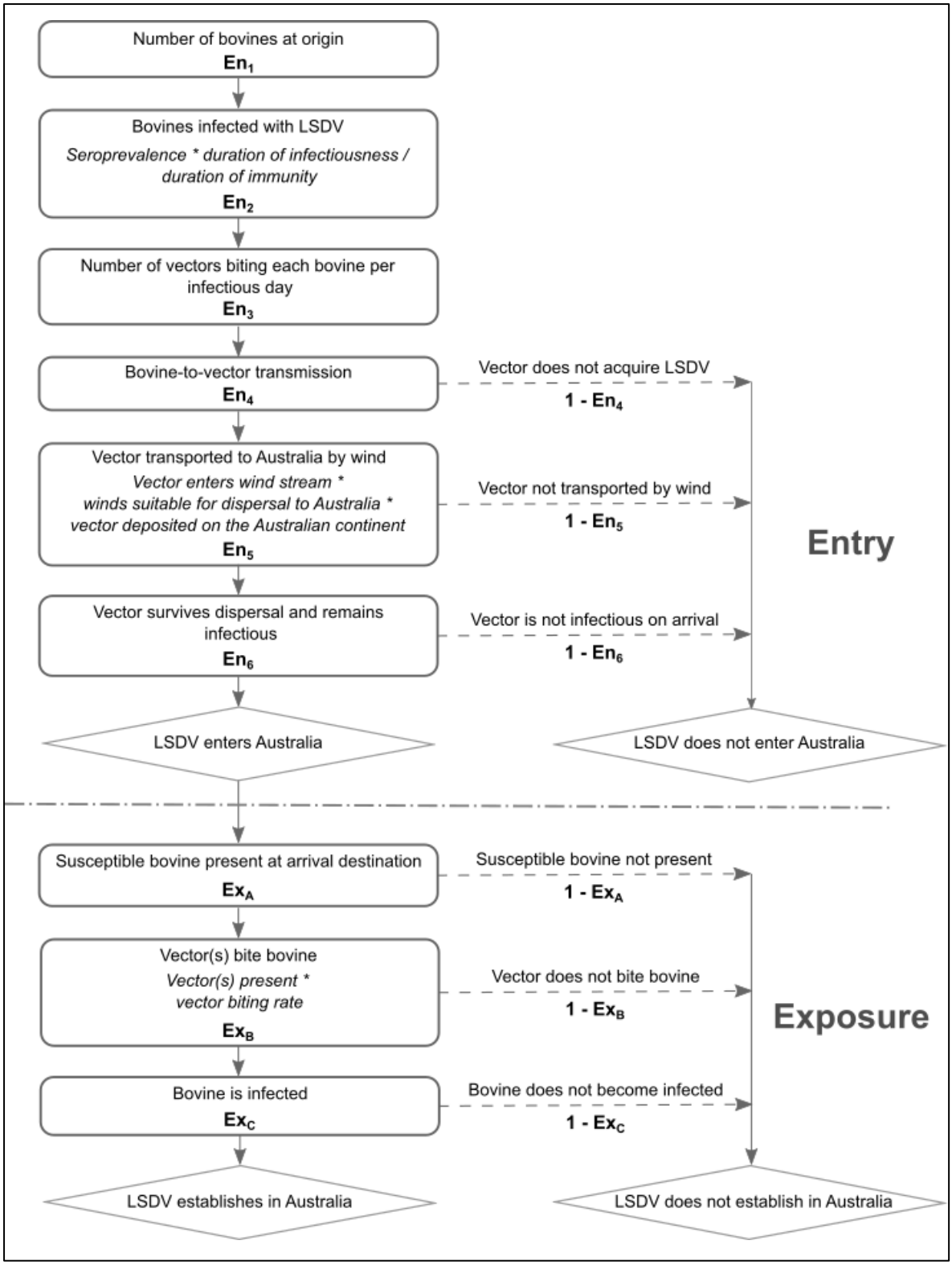

Figure 7 Scenario tree for windborne dispersal of LSDV-carrying arthropods into Australia

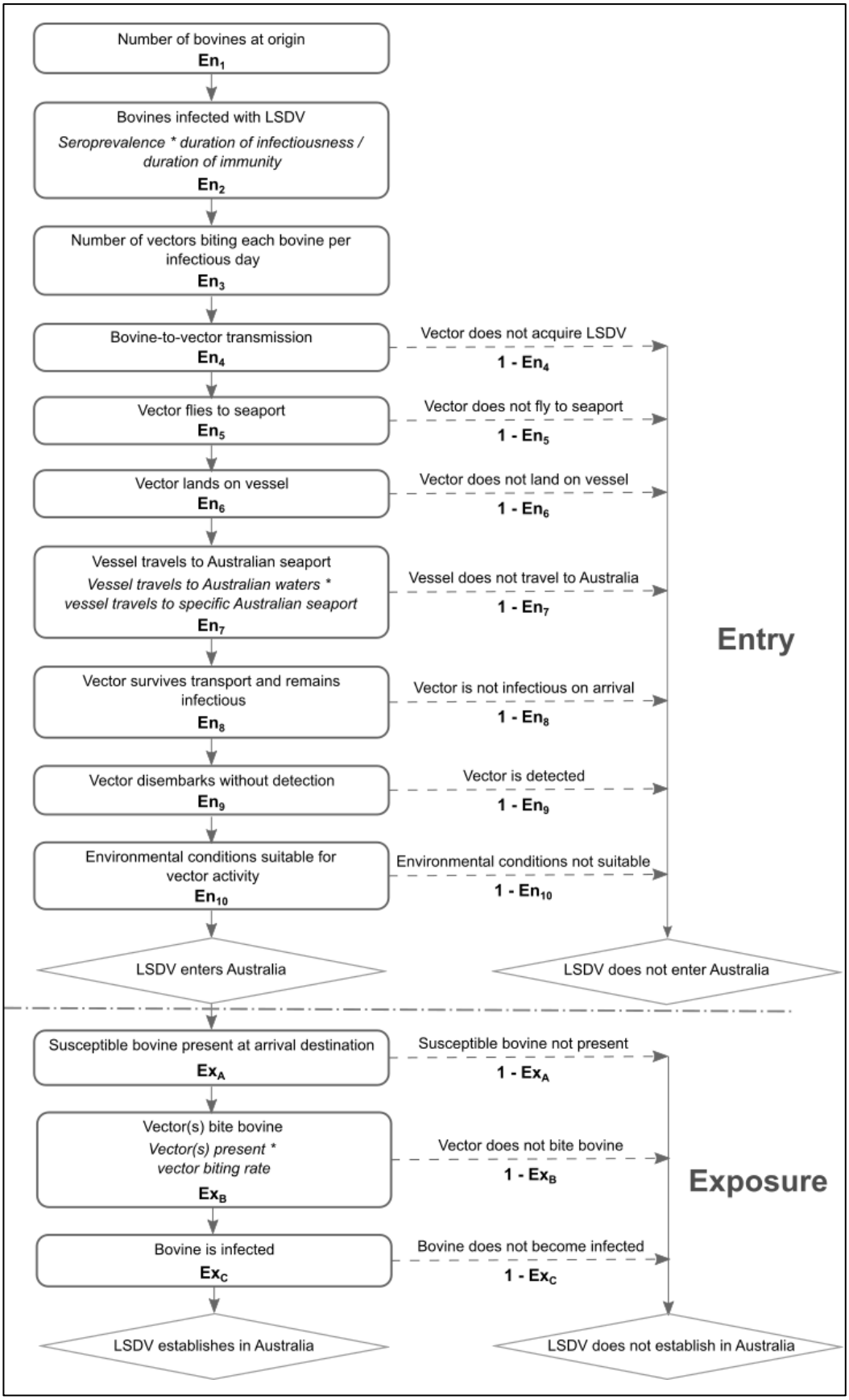

Figure 8 **Scenario tree for transport of LSDV-carrying arthropods via commercial vessels (including returning live export ships)**

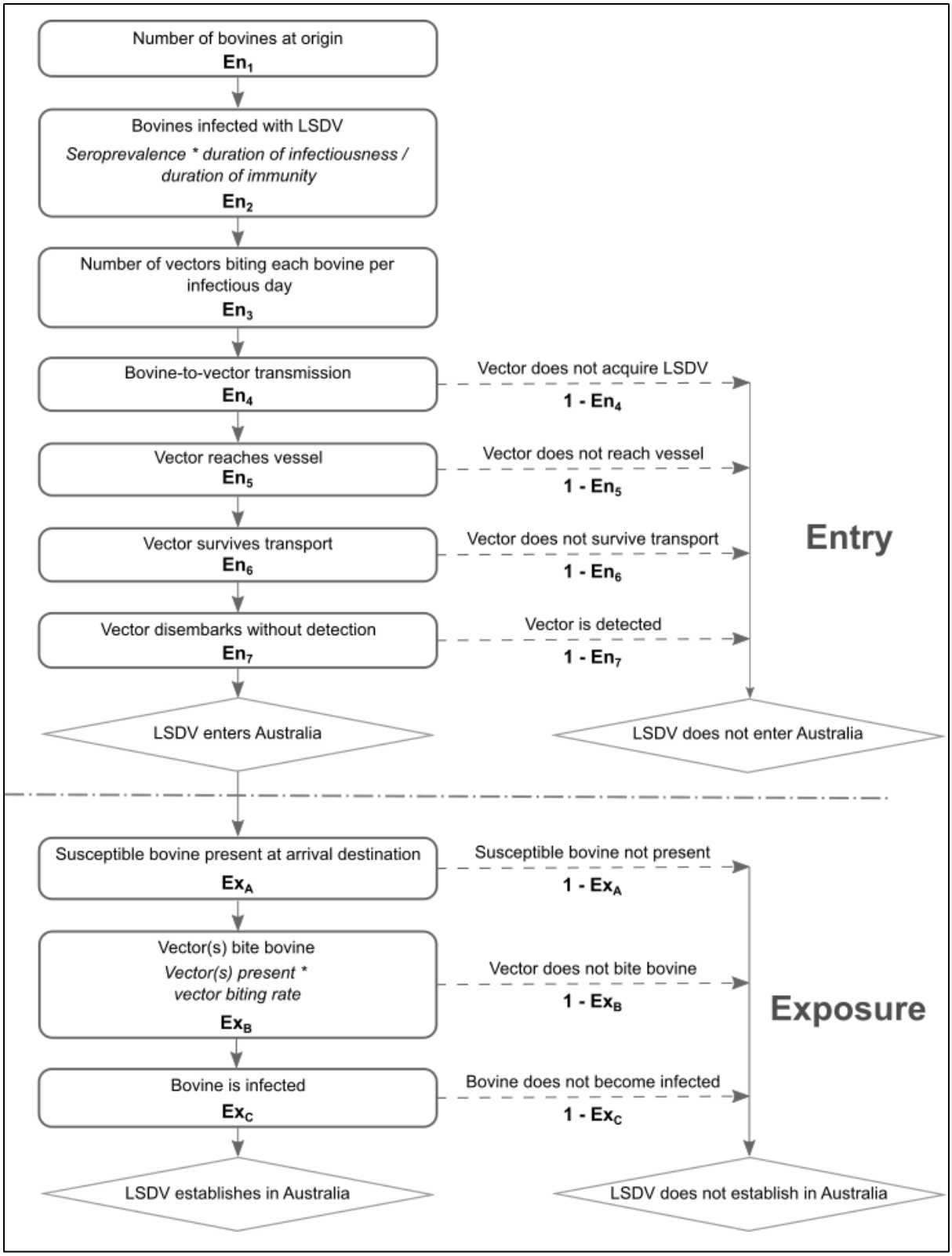

Figure 9 Scenario tree for transport of LSDV-carrying arthropods via Torres Strait Treaty movements

From these scenario trees we formulated an incursion risk model for each pathway.

This can be summarised as $n \times PEn \times PEx$, where n is the number of vectors biting bovines per year, P_{En} is the probability of entry and P_{Ex} is the probability of exposure.

For all pathways, n was calculated as $En1 \times En2 \times En3$, where En_1 is the number of bovines at origin, En_2 is the number of infectious days per bovine per year and En_3 is the number of vectors biting each bovine per infectious day. En_2 was calculated as $\frac{En2.1 \times En2.2}{En2.3}$, where $En_{2.1}$ is the LSDV seroprevalence (or the proportion of bovines infected during their lifetime), $En_{2.2}$ is the duration of infectiousness in days and $En_{2.3}$ is the duration of immunity in years (or the number of years per lifetime).

For all pathways, PEx was calculated as $ExA \times ExB \times ExC$, where Ex_A is the probability of a bovine being present at arrival destination, Ex_B is the probability of vector(s) biting a bovine and Ex_C is the probability of vector-to-bovine transmission. Ex_B was calculated as $ExB.1 \times ExB.2$, where $Ex_{B.2}$, the probability of vector(s) biting bovine, is multiplied by $Ex_{B.1}$, the probability of vectors being present, depending on the number of vectors required in each scenario (i.e. 30–50, 3–5 or 1).

The probability of entry, PEn, was pathway dependent as follows:

For windborne dispersal:

$PEn = En4 \times En5.1 \times En5.2 \times En5.3 \times En6.1 \times En6.2$, where En_4 is the probability of bovine-to-vector transmission, $En_{5.1}$ is the probability of vectors entering high-altitude wind streams, $En_{5.2}$ is the probability of winds being suitable for dispersal to Australia, $En_{5.3}$ is the probability of the vector(s) being deposited on the Australian continent, $En_{6.1}$ is the probability of the vector(s) remaining infectious and $En_{6.2}$ is the probability of the vector(s) surviving long-distance windborne dispersal.

For commercial shipping (including returning live export vessels):

$PEn = En4 \times En5 \times En6 \times En7.1 \times En7.2 \times En8.1 \times En8.2 \times En9 \times En10$, where En_4 is the probability of bovine-to-vector transmission, En_5 is the probability of vector(s) flying to the seaport, En_6 is the probability of vector(s) landing on a vessel, $En_{7.1}$ is the probability of the vessel travelling to Australian waters, $En_{7.2}$ is the probability of the vessel travelling to a specific Australian seaport, $En_{8.1}$ is the probability of vector(s) remaining infectious, $En_{8.2}$ is the probability of vector(s) surviving transport, En_9 is the probability of vector(s) disembarking without detection and En_{10} is the probability of environmental conditions being suitable for vector activity at arrival destination.

For Torres Strait Treaty movements:

$PEn = En4 \times En5 \times En6 \times En7$, where En_4 is the probability of bovine-to-vector transmission, En_5 is the probability of vector(s) reaching a vessel, En_6 is the probability of vector(s) surviving transport and En_7 is the probability of vector(s) disembarking without detection.

2.6 Parameterisation of the models

We consulted the published peer-reviewed scientific literature, grey literature and other publicly available information, such as trade volume data, to estimate a probability distribution for each node. Values varied depending on the country of origin, arrival destination or vector category; for example, livestock numbers varied between origin countries and between arrival destinations, while biting rates varied by vector category.

Expert opinion was sought where data were absent. We consulted three entomologists, Dr Glenn Bellis, Dr Mike Muller and Mr Angus Sly, who provided quantitative estimates of the probability of a vector biting a bovine and/or provided guidance on relevant sources for quantitative parameterisation of other

nodes related to vector biology. We consulted the Biosecurity Operations Division within the Department's Animal Biosecurity branch for information around the likelihood of vectors disembarking from various vessel types without detection. We also consulted with the NAQS Torres Strait and Field Operations team who provided additional information around vessel activity in the Torres Strait.

2.6.1 Windborne dispersal

En_1 Number of bovines at origin

Total cattle and buffalo numbers for each origin country were obtained from FAOSTAT, the global food and agriculture statistics data portal from the Food and Agriculture Organization of the United Nations (FAO, 2022). We used numbers from 2015 to 2020 as inputs for a PERT distribution, taking the 2020 values as the most likely estimate. To parameterise eastern and central Indonesia we used the most recent (2010) Gridded Livestock of the World cattle and buffalo areal-weighted data sets (Gilbert et al., 2018b, 2018a) and calculated the mean number of bovines per km^2 in each Indonesian subdivision using QGIS v3.24.1. We then calculated the ratio of the bovine density between western, eastern and central Indonesia from this data set and calculated the corresponding proportion of total bovine numbers from the FAOSTAT data for Indonesia to ensure comparability of the data source with the other origin countries.

En_2 Bovines infected with LSDV

We estimated the proportion of bovines infected per year in each origin country by dividing the proportion infected at any time in their life by the average lifespan of a bovine (which represents the duration of immunity for LSDV). We then multiplied this by the duration of infectiousness (in days) to estimate the number of infectious cattle days per year by origin country, in a similar approach to that developed by the European Food Safety Authority (EFSA) (Berg et al., 2015). Parameter values were sourced from the peer-reviewed literature. Seroprevalence and the average lifespan of a bovine were modelled as uniform distributions; this was considered more appropriate than use of PERT distributions because we could not robustly assign a 'most likely' value based on the available data. We modelled the duration of infectiousness as a PERT distribution.

En_3 Number of vectors biting each bovine per infectious day

We estimated the number of vectors feeding on a bovine per day based on the vector–host ratios derived by Gubbins et al. (2019). These were modelled as uniform distributions for each vector category. We considered a uniform distribution to be appropriate since there is no clear justification for choosing a 'most likely' value; notably, Gubbins et al. (2019) also used a uniform distribution.

En_4 Bovine-to-vector transmission

Several studies have investigated the proportion of insects that are LSDV-positive after feeding on an infected bovine, with considerable variation in results both within and between studies (Chihota et al., 2001, 2003; Gubbins, 2019; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). We used the minimum and maximum observed values (not the derived 95% confidence intervals) from the literature to define a uniform distribution for each vector category. We considered a uniform distribution to be appropriate since we were not able to justify a 'most likely' value for a PERT distribution based on the limited data available.

En_5 Vector transported to Australia by wind

There are three subcomponents to windborne dispersal over sea (Figure 10). Vectors must:

- enter high-altitude wind streams
- enter at a date and time conducive to transport along an appropriate trajectory
- reach land in the destination country

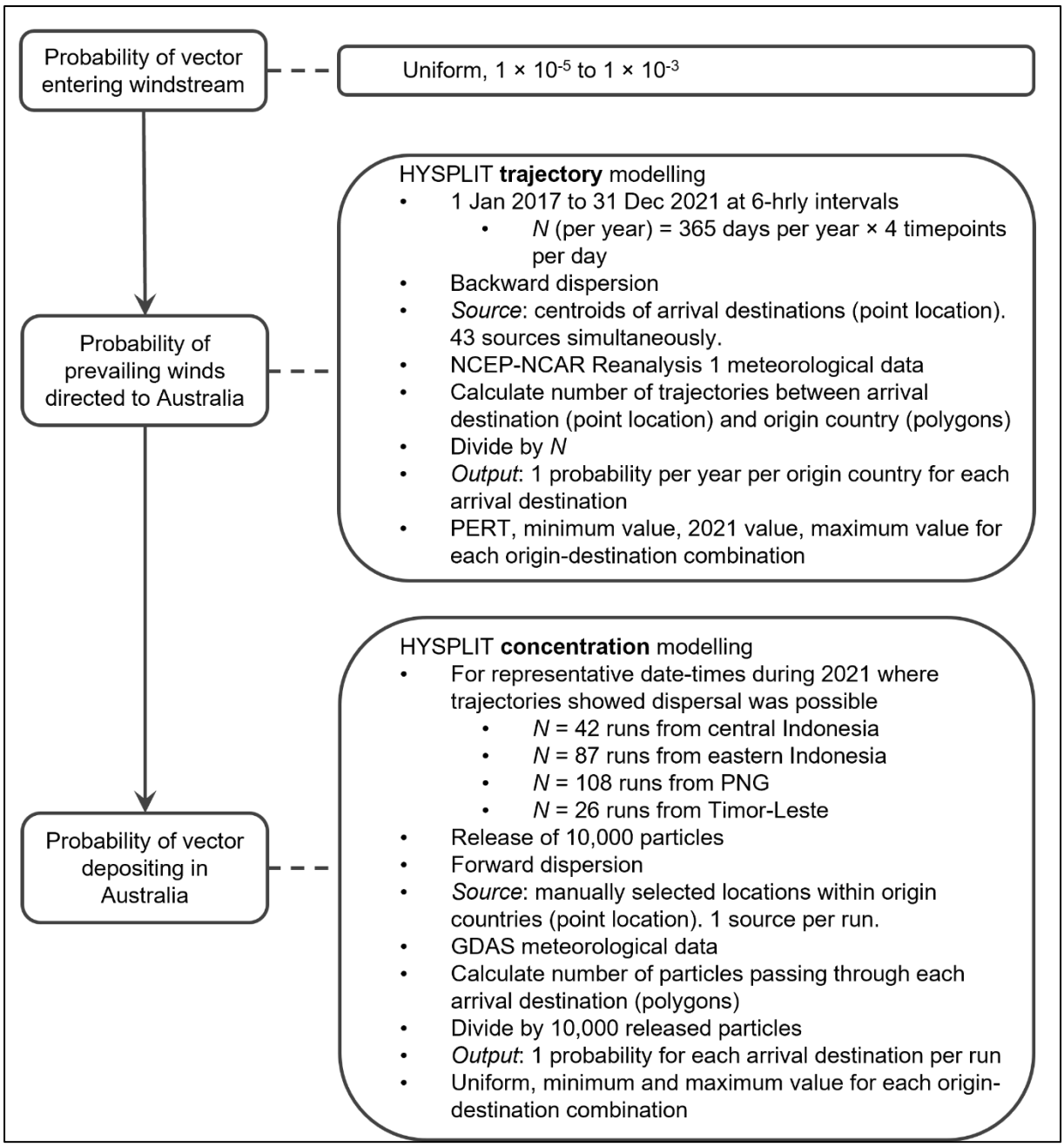

Figure 10 Schematic of parameterisation of En5 for windborne dispersal

No empirical data were available to directly inform the probability of a vector entering a wind stream; thus, we first made a qualitative assessment ('extremely low') and then derived a semi-quantitative estimate according to a previously reported risk assessment methodology (Biosecurity Australia, 2004). We modelled this as a uniform distribution.

To determine the probability of prevailing winds being conducive for vector dispersal to Australia we conducted atmospheric dispersion modelling using HYSPLIT version 5.2.1 (Stein et al., 2015) and the splitr package v0.4.0.9000 (Iannone, 2022) implemented in R version 4.1.3 (R Core Team, 2021), as described for the determination of origin countries (Section 2.3). We calculated 48-hour backwards trajectories from the centroids of all arrival destinations (i.e. NAQS risk zones plus additional areas, n = 43; Figure 13) over the period 1 January 2017 to 31 December 2021 at 6-hourly intervals using

meteorological files from the NCEP-NCAR Reanalysis 1 project (Kalnay et al., 1996). We used NCEP-NCAR meteorological files because these data are available from 1948 onwards; therefore, we could use the same model when investigating the impacts of climate change, ensuring consistency of data across the different time periods (described in Section 2.9). Trajectory parameters followed those described by Durr et al. (2017) and are detailed in Appendix 2. We subsequently converted each output trajectory (43 arrival destinations × 365 days × 5 years × 4 timepoints per day) to a spatial linestring object using the sf package v1.0-7 (Pebesma, 2018). We calculated intersection events between these linestrings and origin country polygons using the modified world-administrative-boundaries shapefile described in Section 2.3 (World Food Programme, 2019). For each combination of destination and origin country, we calculated the proportion of trajectories per year where transport was possible. The minimum value, 2021 value and maximum value for each origin–destination combination over this 5-year period were used as inputs for a PERT distribution (Figure 10).

To estimate the probability of a single particle (i.e. insect) reaching land in Australia, we modelled the forwards dispersion of 10,000 theoretical particles (i.e. sufficient to simulate the proper pollutant distribution) from various source locations within each origin country at times when winds were suitable for dispersal to Australia. For suitable days during 2021 (determined by our trajectory calculations described above), we consulted maps of the trajectories to determine appropriate source coordinates and time points as input parameters for HYSPLIT concentration/dispersion runs. For example, for the 30 January 2021 (shown in Figure 13) simulation we used source coordinates of -9.938 124.055 for central Indonesia, -7.689 131.436 for eastern Indonesia, -8.756 141.499 for PNG and -8.922 126.068 for Timor-Leste. We manually selected source locations because we would have underestimated the dispersal risk if we had used centroids for origin countries; for example, 48-hour trajectories from Australia do not extend to the centroids of central Indonesia, eastern Indonesia or PNG (Figure 13). While we did not perform concentration runs for every possible date and time, we performed multiple runs from each origin country that allowed us to statistically assess the variability in dispersion patterns across a range of dates, times and source locations (n = 42 runs from central Indonesia, 87 runs from eastern Indonesia, 108 runs from PNG and 26 runs from Timor-Leste, reflecting the relative frequency of suitable trajectories to the different locations). Concentration runs were parameterised generally as described by Durr et al. (2017), and further detailed in Appendix 2. For concentration runs we used meteorological data files from the Global Data Assimilation System (GDAS) at 1° resolution (NOAA-Air Resources Laboratory, no date a). This is a higher resolution data set than the NCEP-NCAR Reanalysis 1 data but is only available from 2005 onwards, so was not compatible with our climate change analyses. We implemented HYSPLIT concentration runs through a custom R script (Hysplit_LSDV_final.R) and a Microsoft Windows batch script (hysplit.bat, Appendix 2), using the packages splitr v0.4.0.9000 (Iannone, 2022), sf v1.0-7 (Pebesma, 2018), tidyverse v1.3.1 (Wickham et al., 2019), lubridate v1.8.0 (Grolemund and Wickham, 2011), scales v1.2.0 (Wickham and Seidel, 2022), readxl v1.4.0 (Wickham and Bryan, 2022) and rgeos v0.5-9 (Bivand and Rundell, 2021).

For each concentration run, the resulting particle file was manipulated in R to generate linestring trajectories for each individual particle, from which we calculated the number of particles intersecting with each arrival destination (i.e. NAQS risk zones plus additional regions). Thus, we could calculate the proportion of the original 10,000 released particles that could reach each individual NAQS risk zone. The minimum and maximum proportions for each origin country–arrival destination combination across all runs assessed were used to define a uniform distribution. A uniform distribution was selected because preliminary interrogation of the results showed a poor fit for PERT and other probability distributions.

For some combinations of origin country and arrival destination no suitable days were identified based on trajectory simulations, yet arriving particles were detected based on the concentration runs. For combinations where we encountered this discrepancy, we estimated the proportion of the year suitable

for windborne dispersal by taking the number of concentration runs where particle arrival was detected and dividing by 365 × 4 (since concentration runs were selected based on trajectory data only from 2021 across 4 time points each day). This avoided misassigning a probability of zero to possible dispersion routes. Conversely, there were several scenarios where dispersal was reportedly possible based on trajectory results, yet no particles were detected from concentration runs. In these cases, we first ran additional concentration runs using alternative source coordinates. Where this still failed to identify arriving particles, we set the proportion of suitable days to 0 for the specific origin country–arrival destination combination, considering the concentration runs to be more accurate than the trajectory runs.

En_6 Vector survives dispersal and remains infectious

Informed by the literature, we made a qualitative assessment of vector survival during long-distance windborne dispersal ('extremely low') and converted this to a semi-quantitative probability using a previously reported risk assessment methodology (Biosecurity Australia, 2004). This was modelled as a uniform distribution for all vector categories.

We modelled insect infectiousness as a function of time, deriving input data from the peer-reviewed literature on the proportion of infectious insects at different days post-feeding (Chihota et al., 2001; Issimov et al., 2021; Paslaru et al., 2022). Here, we restricted studies to those that demonstrated infectiousness by virus isolation (i.e. we excluded those studies only reporting quantitative polymerase chain reaction (qPCR) results) because true infectivity is of key importance for this node. Because of the paucity of data, we were not able to generate individual models for each vector category. Thus, data from all suitable studies were combined to generate a single function that was applied to all three vector categories (Equation 1, Appendix 3). Time *t* was determined from the straight line distance between origin country and arrival destination at their closest point using an average wind speed of 3.5 m/s (Suppiah et al., 2011) and rounded up to the nearest day. Straight-line distances were derived in ArcGIS Pro version 2.8.6 using the geoprocessing tool 'Generate Near Table' based on the NAQS_RiskAreas2015 (NAQS officer, pers. comm.) and the modified world-administrative-boundaries shapefiles (World Food Programme, 2019) described above. Distances for the additional arrival destinations ('rest of NT', 'rest of northern WA', 'rest of western QLD') were calculated manually. We calculated the 95% confidence interval for the model and used these as inputs for a PERT distribution (Equation 1). We constrained the maximum value to 1 (i.e. 100% probability of infectiousness).

$$Most\ likely: 63.24 * \frac{t^{-0.57}}{100}$$

$$Minimun: 26.548051 * \frac{t^{-1.14806}}{100}$$

$$Maximum: 99.94074 * \frac{t^{0.01419211}}{100}$$

Equation 1 Model for the probability of a vector being infectious at time *t* (in days)

Ex_A Susceptible bovine present at arrival destination

To estimate whether a bovine would be available to be bitten, we modelled the probability of a bovine being present within 1 km² of an arriving insect vector. We used a negative binomial distribution, where μ was the mean cattle density per km². This distribution is commonly used to model ecological count data while incorporating overdispersion (Lindén and Mäntyniemi, 2011). We used a dispersion parameter of 0.2 based on that previously determined for sheep, as we did not find specific estimates for cattle (Morton and Baird, 1990). While many vector species undertake self-directed flight over distances longer than 1 km (Service, 1997; Whelan, 2010; Verdonschot and Besse-Lototskaya, 2014; Elbers, Koenraadt and

Meiswinkel, 2015; Showler and Osbrink, 2015), we assumed that flight ability may be reduced after experiencing the harsh environmental conditions associated with windborne transport. The probability that *any* bovine would be present was taken as 1 minus the probability of 0 animals being present from the negative binomial distribution.

Cattle numbers or densities are not available based on NAQS risk zones specifically, therefore we extrapolated from SA2 densities. Estimates of cattle numbers at the SA2 level were obtained from the Australian Bureau of Statistics (ABS) 2020–2021 agricultural census (Australian Bureau of Statistics, 2021a). SA2 area polygons were obtained from the SA2_2021_AUST_GDA2020 shapefile (Australian Bureau of Statistics, 2021b). Critically, these estimates do not include feral bovine (feral cattle, buffalo and Banteng) numbers. To account for feral bovines, we obtained data from NAQS field surveys conducted between October 2013 and June 2022 (NAQS officer, pers. comm.). Survey locations were first grouped by NAQS risk zone, then the total feral bovine number across all surveys was calculated for each NAQS risk zone and total numbers were divided by the area of each zone to obtain a feral bovine density estimate. These feral bovine densities obtained from NAQS field surveys were added to the domestic cattle SA2 densities calculated as described above to obtain a total bovine density for each SA2.

Since a NAQS risk zone can comprise several SA2s, cattle density per NAQS risk zone was calculated using a discrete distribution. The values for this distribution were the SA2 densities (after inclusion of feral bovines), and the probabilities were the proportion of each SA2 comprising the NAQS risk zone. For example, if a NAQS risk zone fell entirely within an SA2, then that SA2 density would be selected 100% of the time; but if a NAQS risk zone overlaid three SA2s evenly, then each SA2 density should be selected in 33.3% of iterations. The percentage overlap of SA2s with each NAQS risk zone was calculated using the R script calculate_SA2_NAQS_overlaps.R with the packages sf v1.0-7 (Pebesma, 2018), tidyverse v1.3.1 (Wickham et al., 2019) and readxl v1.4.0 (Wickham and Bryan, 2022). For arrival destinations outside NAQS risk zones (i.e. 'rest of northern WA', 'rest of NT', 'rest of western QLD') we used ABS 2020–2021 SA4 cattle numbers (Australian Bureau of Statistics, 2021a) and calculated the density per km² based on the area of the respective SA4s (Australian Bureau of Statistics, 2021b). SA4 level cattle numbers were not available for the two NT SA4s, thus for the 'rest of NT' we estimated the cattle density at the state level.

Ex_B Vector(s) bite bovine

This node was estimated by multiplying the probability of vectors arriving by the probability of vectors biting a bovine. No data are available to parameterise the probability of multiple vectors arriving simultaneously. However, reports of detections of exotic *Culicoides* only describe single specimens at each sampling occasion (Eagles et al., 2014). Thus, we assumed the probability of at least 30 vectors arriving simultaneously to be extremely low and the probability of 3–5 vectors arriving together to be very low (relative to the probability of a single vector arriving). We derived a semi-quantitative probability estimate for these different scenarios by converting from these qualitative assessments, as described previously, and input these into uniform distributions for each scenario. We considered a uniform distribution to be acceptable because there was no justification for choosing a 'most likely' value for a PERT distribution.

We used expert opinion from two entomologists to estimate the probability of a vector biting an available bovine. The experts provided minimum, maximum and most likely probabilities for each vector category. We generated a PERT distribution for each expert, and these were combined in a discrete distribution with an equal probability for the two experts.

Ex_C Bovine is infected

We estimated the vector-to-bovine transmission rate for LSDV based on the peer-reviewed literature. Notably, LSDV transmission experiments have all used large batches of insects (36–300 insects per batch); thus, these transmission probabilities are representative of a batch of insects, not of individual

insects (Weiss, 1968; Chihota et al., 2001, 2003; Magori-Cohen et al., 2012; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). No data are currently available for transmission rates from individual insects. We modelled the vector-to-bovine transmission rate using a uniform distribution for each vector category, taking the minimum and maximum observed values (not those derived in the 95% confidence interval) from the literature.

2.6.2 Commercial vessels (including returning live export vessels)

En_1 Number of bovines at origin

Livestock numbers in origin countries were derived as described for the windborne dispersal pathway, except for Hawaii. For Hawaii, we obtained an estimate of cattle numbers from the 2021 United States Department of Agriculture state agricultural overview (United States Department of Agriculture, 2021). This was used for both the minimum and maximum value in our probability distribution.

En_2 Bovines infected with LSDV

This was parameterised as detailed for the windborne dispersal pathway.

En_3 Number of vectors biting each bovine per infectious day

This was parameterised as detailed for the windborne dispersal pathway.

En_4 Bovine-to-vector transmission

This was parameterised as detailed for the windborne dispersal pathway.

En_5 Vector flies to seaport

For this parameter, we made a qualitative assessment informed by the literature (i.e. 'very low') and converted this to a semi-quantitative value using a previously reported risk assessment methodology (Biosecurity Australia, 2004). We assumed that bovines will be in closer proximity to ports in less urbanised countries, increasing the likelihood of travelling to a port. Therefore, we assigned a PERT distribution where the minimum and maximum values were derived from this semi-quantitative conversion and the most likely value was derived from the urbanisation index from the Central Intelligence Agencies World Factbook (Central Intelligence Agency, 2020). The urbanisation index for Hawaii for 2010 (the most recent available) was obtained from the United States Census Bureau (US Census Bureau, 2021). The most likely value was scaled between the minimum and maximum based on countries with <=25% urban population, >25–<=50% urban population, >50–<=75% urban population and >75% urban population.

En_6 Vector lands on vessel

We used expert opinion to inform this node (i.e. extremely low) and converted the experts' qualitative assessment to a semi-quantitative value using the previously described risk assessment methodology (Biosecurity Australia, 2004).

En_7 Vessel travels to Australian seaport

Commercial vessels excluding returning live export ships

Data on the number of journeys undertaken by ships between individual seaports globally are commercially sensitive and are not readily available. Therefore, we separated this parameter into the probability of an international vessel being destined for Australia and the probability of arrival at a specific Australian seaport.

Since data on the absolute number of ships were not available, we used the proportion of export trade value to Australia of all export trade value for each origin country to estimate the probability of a vessel

being bound for Australia. Export statistics were obtained from The Observatory of Economic Complexity (Observatory of Economic Complexity, no date), which utilises data from the Centre d'Études Prospectives et d'Informations Internationales (Gaulier and Zignago, 2010). Trade statistics from Hawaii were obtained from USA Trade Online (United States Census Bureau, 2022). For the Democratic People's Republic of Korea (North Korea), no trade data are available, so we conservatively estimated that 5% of sea trade was to Australia; this is likely an overestimate. We used the value for Indonesia for each of the western, central and eastern Indonesian subregions, since data for these custom subregions were not available. We obtained export trade statistics from 2015–2020 (as data were not available after quarter 1, 2021) and used the minimum, maximum and 2020 values to define a PERT distribution.

To estimate the probability of arrival at different Australian seaports we sourced the total number of port calls at Australian ports from the most recent (2018–2019) Australian sea freight report (Bureau of Infrastructure and Transport Research Economics, 2021). The raw data underlying the relevant table (Table 4.4 in that report) were not available. Thus, for each year from 2015–2019 we calculated the proportion of port calls to the top 10 ports directly, and we assumed the remaining port calls were distributed equally amongst the other 56 ports. For each port, we defined a PERT distribution taking the minimum, maximum and 2019 values over the five years analysed.

Returning live export ships

Data on all livestock exports from Australia by sea from 2017–2022 were sourced from the Department (Australian Government Department of Agriculture, Water and the Environment, 2022). Information on the absolute number of ships travelling to each port was not available. Therefore, we calculated the quantity of animals transported along each route as a proportion of total animals transported by sea by origin country by year. For example, if 50,000 head were transported from the Philippines to both Darwin and Broome then the probability of a ship from the Philippines travelling to either of these ports was 50%. We did not limit vessels to those transporting bovines, since vectors may be attracted to any returning live export ship regardless of the species being exported. We parameterised a PERT distribution taking the minimum, maximum and 2021 values, excluding 2022 data since these are incomplete.

En_8 Vector survives transport and remains infectious

We could not find experimental data describing vector survival under typical conditions on commercial vessels. Based on a New Zealand study that reported hitchhiker insect detections in shipping containers, 44.4% of mosquitoes and 25% of heavy fliers were alive at the time of detection (Border Management Group, 2003). While no *Culicoides* midges were detected in that study, six non-biting midges were found, of which two (33.3%) were found alive. Thus, we used these proportions to parameterise survival of our three vector categories.

We modelled insect infectiousness as a function of time based on peer-reviewed laboratory experiments as described for the windborne dispersal pathway (Equation 1). That same function was used to parameterise this node.

En_9 Vector disembarks without detection

We used expert opinion from the Biosecurity Operations Division within the Department's Animal Biosecurity branch to provide a qualitative estimate of the probability that a vector would disembark a vessel given the current biosecurity mitigations in place. These mitigations are different for returning live export vessels compared to other commercial ships; therefore, this node was parameterised differently in each pathway. These qualitative assessments were converted into a semi-quantitative likelihood and input into a uniform distribution (Biosecurity Australia, 2004).

En_{10} Environmental conditions suitable for vector activity at arrival destination

To estimate whether environmental conditions around Australian seaports would be conducive to vector survival and activity we first found the nearest weather station to each Australian seaport using the R script weather_script.R and the packages sf v1.0-7 (Pebesma, 2018), tidyverse v1.3.1 (Wickham et al., 2019), lubridate v1.8.0 (Grolemund and Wickham, 2011) and mapview v2.11.0 (Appelhans et al., 2022). Weather observations for these stations from 1 January 2011 to 31 December 2021 were sourced from the Bureau of Meteorology, noting that not all stations had data available for every day. For each year we estimated the proportion of suitable days based on the total number of days where data were available. The minimum, maximum and 2021 proportion of suitable days for each seaport were input into a PERT distribution.

Ex_A Susceptible bovine present at arrival destination

We modelled the probability of a bovine being present within 1 km^2 of an arriving insect vector using a negative binomial distribution as described for the windborne dispersal pathway. However, μ was parameterised using the ABS 2020–2021 SA4 cattle densities. We determined the nearest SA4 to each seaport using the R script ports2SA.R and the packages sf v1.0-7 (Pebesma, 2018) and tidyverse v1.3.1 (Wickham et al., 2019). We manually updated Botany Bay to SA4 117, as this was incorrectly assigned from the script. We did not include buffalo in our density calculations since buffalo are absent from regions where seaports are located (Australian Government Department of Sustainability, Environment, Water, Population and Communities, 2011; Saalfeld, 2014).

Ex_B Vector(s) bite bovine

This was parameterised as detailed for the windborne dispersal pathway.

Ex_C Bovine is infected

This was parameterised as detailed for the windborne dispersal pathway.

2.6.3 Torres Strait Treaty movements

En_1 Number of bovines at origin

Livestock numbers in PNG were derived as described for the windborne dispersal pathway.

En_2 Bovines infected with LSDV

This was parameterised as detailed for the windborne dispersal pathway.

En_3 Number of vectors biting each bovine per infectious day

This was parameterised as detailed for the windborne dispersal pathway.

En_4 Bovine-to-vector transmission

This was parameterised as detailed for the windborne dispersal pathway.

En_5 Vector reaches vessel

This was parameterised as detailed for the commercial shipping pathway.

En_6 Vector survives transport

We sought expert opinion from the NAQS Torres Strait and Field Operations team to qualitatively assess the probability of insect survival in the vessels involved in Torres Strait Treaty movements. We converted this to a semi-quantitative likelihood range using the previously described risk assessment methodology (Biosecurity Australia, 2004). These values were used to define a uniform distribution; there was no justification for choosing a most likely value, so a PERT distribution was not considered appropriate.

En_7 Vector disembarks without detection

As for En_6, we used expert opinion from the NAQS Torres Strait and Field Operations team to provide a qualitative assessment of a vector disembarking from a vessel without detection. This was converted to a semi-quantitative likelihood range and used to parameterise a uniform distribution.

Ex_A Susceptible bovine present at arrival destination

This was parameterised as detailed for the windborne dispersal pathway, except we did not include buffalo numbers since buffalo are reportedly absent in northern QLD (Australian Government Department of Sustainability, Environment, Water, Population and Communities, 2011).

Ex_B Vector(s) bite bovine

This was parameterised as detailed for the windborne dispersal pathway.

Ex_C Bovine is infected

This was parameterised as detailed for the windborne dispersal pathway.

2.7 Quantitative risk estimate for each pathway

Data were processed in R version 4.1.3 (R Core Team, 2021) using the tidyverse v1.3.1 (Wickham et al., 2019), mc2d v0.1-21 (Pouillot and Delignette-Muller, 2010), readxl v1.4.0 (Wickham and Bryan, 2022), sf v1.0-7 (Pebesma, 2018), scales v1.2.0 (Wickham and Seidel, 2022), parallel v4.1.3 (R Core Team, 2021) and utilsGibbs v0.0.0.9000 (Gibbs, 2022) packages. The scripts and accompanying data files are available at the Bitbucket repository https://robynhall@bitbucket.org/robynhall/awe-lsd-21_quantitative.git upon request.

Data were cleaned (as described in Section 2.6) and independent data sources were joined into a single data frame for downstream processing. A function was assigned to conduct two-dimensional Monte Carlo simulation (Figure 11). Briefly, for each variable within the final risk pathway (i.e. node in the scenario tree) a mcnode object is constructed using the package mc2d v0.1-21 (Pouillot and Delignette-Muller, 2010), which randomly generates values based on a specified number of iterations while incorporating variability and/or uncertainty dimensions as designated for each variable. We used 1,000 iterations in the variability dimension and 1,000 iterations in the uncertainty dimension (Vose Software, 2017). The mcnode objects are combined to generate a Monte Carlo object (the final risk calculation), also using the mc2d package, and summary statistics are computed for this object. This function was applied iteratively to every combination within a pathway (i.e. combination of origin country, arrival destination and vector category), and the summary statistics for each scenario were appended into a single table. This was parallelised to speed up processing. To generate the overall risk of LSDV introduction into Australia via each pathway, the summary statistics were summed across each scenario.

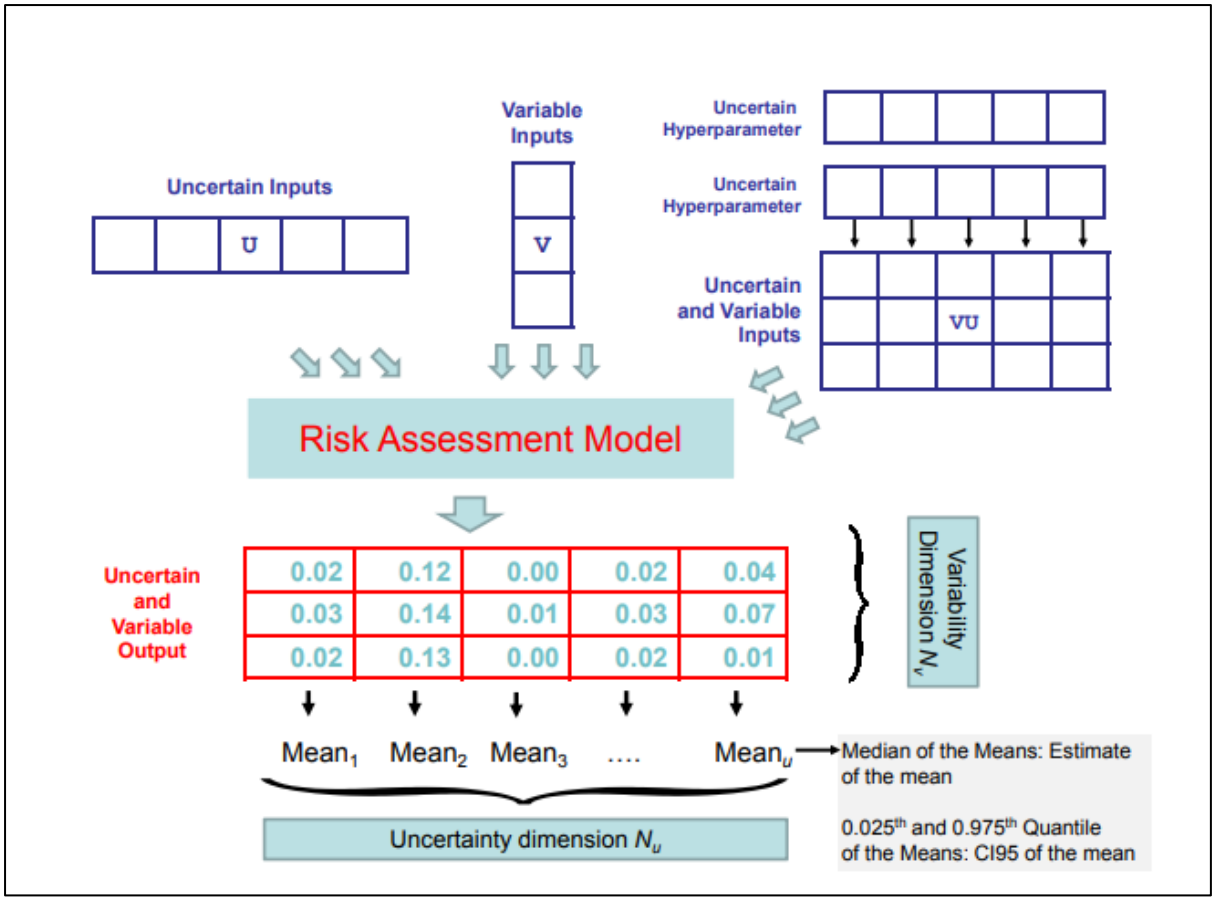

Figure 11 Schematic representation of a two-dimensional Monte Carlo simulation[6]

2.8 Sensitivity analysis

For our intermediate scenario, we conducted a global sensitivity analysis by generating tornado plots of input variables using the tornado function within the mc2d package (Pouillot and Delignette-Muller, 2010). This computes the Spearman's rho statistic for each input variable, estimating a rank-based measure of association between the output risk and each input for a scenario. We randomly selected 10 combinations (i.e. origin country, arrival destination and vector category) from each pathway for this analysis, excluding combinations with a non-zero risk. Simulations were run with 1,000 variability and 1,000 uncertainty iterations. Since the probability of LSDV entry was zero for all scenarios within the Torres Strait Treaty movements pathway, global sensitivity analyses could not be performed for this pathway.

Additionally, we conducted a comparison of the higher resolution GDAS meteorological data set with the NCEP-NCAR Reanalysis 1 data. GDAS and NCEP-NCAR models were compared for January to December 2021 using a two-sided Wilcoxon signed-rank test.

[6] From R Pouillot and ML Delignette-Muller, 'Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages', *International Journal of Food Microbiology*, 2010, 142(3):330–340.

2.9 Potential impacts of climate change

We conducted local sensitivity analyses for those parameters that we anticipated may change in a future climate scenario based on the current scientific literature. These were node En_{10} in the shipping pathways (environmental conditions suitable for vector activity) and node En_7 (vessel travels to Australian seaport) in the commercial shipping pathway excluding returning live export vessels.

We also repeated our HYSPLIT trajectory modelling under different climate scenarios to determine if the proportion of yearly trajectories suitable for windborne dispersal was changing. Initially, we intended to obtain global daily gridded meteorological data files derived from a suitable future climate model. We sought advice from meteorological experts within Commonwealth Scientific and Industrial Research Organisation – Oceans and Atmosphere as to what these suitable climate models may be. Gridded meteorological data files are the outputs of individual 'runs' from different climate models, requiring considerable expertise and computational time to generate. Thus, the selection of runs is based on the coordination efforts of the Coupled Model Intercomparison Project (CMIP) (Hausfather, 2019; IPCC, 2021). 'Runs' are parameterised from shared socioeconomic pathways (SSPs), which are future scenarios that have been modelled based on anticipated global socioeconomic changes under different climate policies up to 2100 (IPCC, 2021). For example, SSP3-7.0 is the baseline scenario that assumes 'middle of the road' outcomes produced by energy system models (Hausfather, 2019). SSP1-2.6 is a low greenhouse gas emissions scenario where warming is limited to below 2°C. SSP5-8.5 is the worst-case scenario where carbon dioxide emissions triple by 2075. When investigating the impacts of future climate change, multiple runs from several different models should be compared, to account for inter-model variation and to span the range of potential future climate trajectories. Runs from future climate models selected through CMIP6 are publicly available through project oi10 on the National Computational Infrastructure (Snow, 2020). However, to convert these data to a format usable by HYSPLIT requires additional computational resources and informatic expertise that were beyond the scope of the current project.

The meteorological experts also noted that these long-term climate change models may not be relevant to future LSD epidemiology. For example, disease prevalence could be greatly reduced through the development of new vaccines, or cattle production systems and densities may have changed due to factors such as heat stress and feed supply by the time these future scenarios are unfolding. Acknowledging that analyses over a shorter period may be more relevant, we instead assessed trends in windborne dispersal patterns by comparing HYSPLIT modelling under a 'pre-current climate' scenario to that conducted using current climate data.

Like the year 2021, 1989 was also associated with a strong La Niña (Australian Bureau of Meteorology, no date a), with a moderate Southern Oscillation Index (National Centers for Environmental Information, 2022), a negative Indian Ocean Dipole (Australian Bureau of Meteorology, no date b) and a similar number and distribution of cyclone events (Australian Bureau of Meteorology, no date c). Therefore, we took the five years up to and including each of 2021 and 1989 for our comparison. Forty-eight-hour backwards trajectories from the centroid of each NAQS risk zone were run at 6-hourly intervals (0000, 0600, 1200, 1800) for each day between 1 January 1985 to 31 December 1989 and 1 January 2017 to 31 December 2021 using NCEP-NCAR Reanalysis 1 data, as described for En_5 in the windborne dispersal pathway (Figure 10). The number of trajectories suitable for transporting particles to Australia across the two time periods were analysed as described above (Section 2.6.1) and compared between the two time periods.

3 Results and discussion

3.1 Pathways for inclusion

Potential pathways for the entry of LSDV into Australia are numerous and include both those that are heavily regulated, such as the importation of bovines and bovine products, and those that are non-regulated or less regulated, such the movement of arthropods or the movement of people and vessels between PNG and northern Australia under the Torres Strait Treaty. Risk mitigation measures (including disease surveillance) can be more difficult, complex and expensive to implement on non-regulated pathways. Risk assessment of non-regulated pathways is therefore critical in appropriately allocating resources for risk mitigation activities. For these reasons, the Department determined that this risk assessment should focus on non-regulated pathways or so-called leakage pathways; that is, the risk associated with gaps in regulated pathways. The risk pathways chosen thus focused on the entry of infected arthropod vectors.

The final four non-regulated pathways for inclusion in the analysis of LSDV entry into Australia were:

- windborne dispersal of arthropod vectors
- commercial vessels (excluding returning live export vessels) carrying hitchhiker arthropod vectors
- returning live export vessels carrying hitchhiker arthropod vectors
- Torres Strait Treaty movements carrying hitchhiker arthropod vectors

Because of both evidence of other vector-borne diseases entering via wind and speculation that this has occurred with LSDV in other regions, windborne incursion of arthropod vectors was included in this risk assessment as a non-regulated pathway.

Although commercial vessel entry is a regulated pathway, because of the potential for hitchhiker carriage of LSDV-infected arthropod vectors in freight, this pathway was considered an important potential source of leakage. Most vessels that arrive in Australia are commercial vessels. These includes bulk carriers, cruise vessels, tankers, container vessels and roll-on, roll-off (Ro-Ro) cargo ships. In this pathway we included all commercial vessels except cruise vessels and returning live export vessels (the latter was considered separately). Cruise vessels make up a relatively low level of traffic and have their own biosecurity mitigation systems.

Foreign fishing vessels and yachts (non-commercial) are assumed to arrive in insufficient volumes to pose a significant threat. Additionally, they spend extended periods of time at sea, which is not conducive to arthropod survival or retention of virus infectivity. Hence, these were not included in our assessment. While leakage can also occur from aircraft, this was not considered in our assessment because of the low volume of air freight compared to sea freight; for example, from 2017 to 2021 less than 0.5% of the imported trade volume was as air freight (National Freight Data Hub, 2022).

We determined that returning live export vessels should be assessed in a separate pathway to commercial shipping, since the risks are likely to be different due to the different mitigations employed. Between 2017 and 2021, 1,289 voyages carried live animals internationally by sea (Australian Government Department of Agriculture, Water and the Environment, 2022). Of these, 1,070 voyages carried cattle and buffalo and 219 vessels carried sheep, with Indonesia receiving the most vessels of any country (309 vessels). These vessels were required to make return journeys to Australia and may have been exposed to arthropods carrying LSDV while docked in foreign ports. Returning live export vessels are subject to strict biosecurity risk mitigation measures on re-entry into Australia and hence this could be considered a regulated pathway. Every livestock ship undergoes a pre-arrival inspection and is thoroughly cleaned, is

disinfected with soda ash, and receives two insecticide treatments on arrival into Australia. However, because of the theoretical potential for hitchhiker arthropods to enter undetected, this pathway could be an additional important source of leakage. Notably, returning live export vessels may be more attractive to biting insects than other commercial vessels due to their association with livestock, a source of blood meals and oviposition sites.

The Torres Strait Treaty was implemented in 1985 and defines a border between Australia and PNG that allows for special provisions. One of these provisions is the free movement (without visas or passports) between Australia and PNG for traditional activities. This provision is only for Torres Strait Islanders and coastal people of PNG who live in and maintain traditions of the region. There are 13 PNG villages that have free movement privileges under the Treaty (Figure 12). In general, when these movements take place, a biosecurity officer in the Torres Strait will inspect vessels and goods upon arrival. The Torres Strait Information System (TSIS) was first introduced in 2017 to facilitate issuing of permits for the movement of various goods, mainly dogs, soil and some plant material from the Torres Strait Protected Zone and the Torres Strait Permanent Biosecurity Monitoring Zone to mainland Australia (DAFF officer, pers. comm.). In February 2018, TSIS was expanded to include recording of inspection data. When pathways were being discussed for this assessment, there was some speculation around LSDV entering in animal hides brought ashore during these movements and further information was sought. However, since TSIS was introduced in 2018, no bovine hides have been seized from PNG Treaty village arrivals (DAFF officer, pers. comm.). Hence, LSDV entering in animal hides related to Treaty movements was not included as a potential pathway. Instead, hitchhiking of insects on vessels moving under the Torres Strait Treaty was considered as a potential source of leakage from an otherwise regulated pathway.

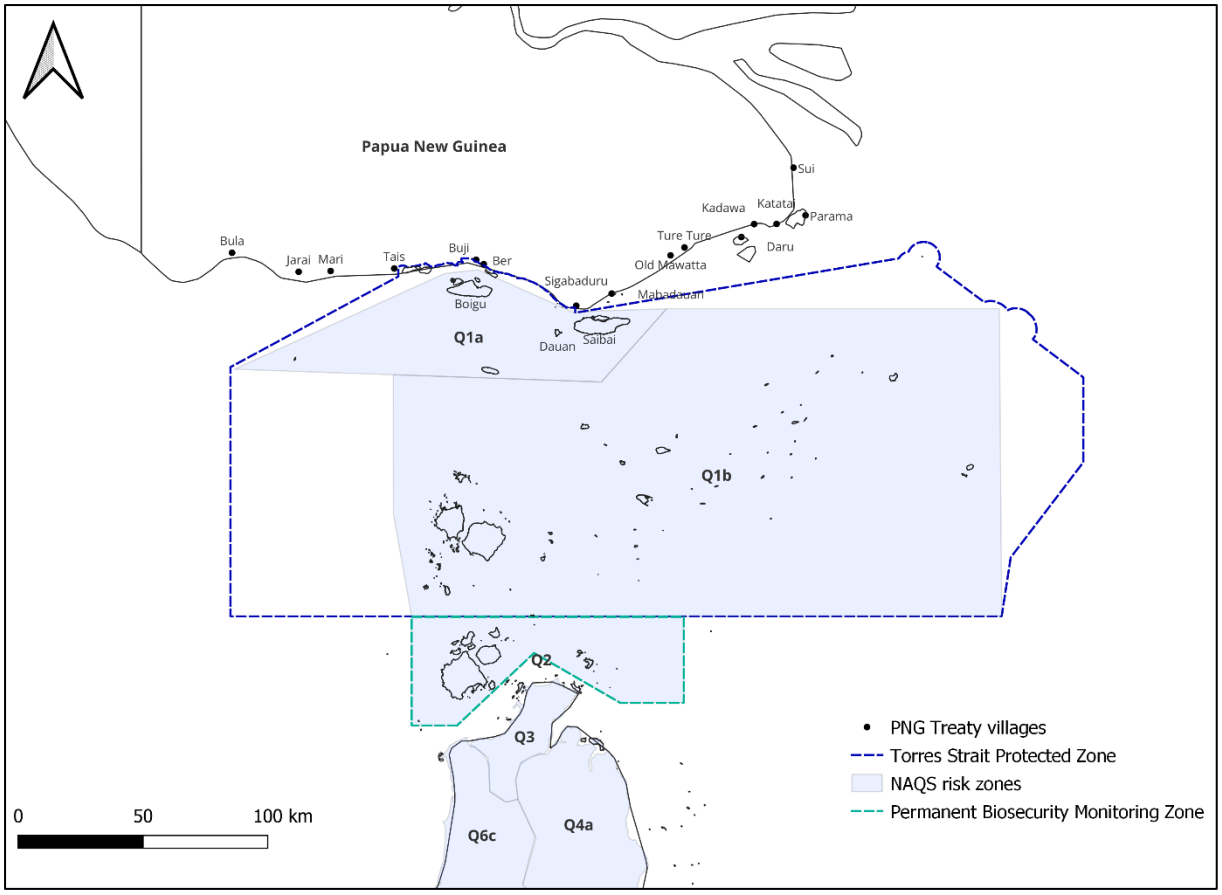

Figure 12 Relevant locations for the Torres Strait Treaty movements pathway[7]

Our assessment was limited to four non-regulated pathways for potential LSDV incursion, selected in consultation with the Department. Arguably, LSDV incursion could also occur via additional non-regulated pathways that were not considered in this analysis. For example, LSDV is known to be transmitted in hard ticks, such as those of the *Amblyomma* and *Rhipicephalus* genera (Tuppurainen et al., 2011, 2013a, 2013b; Lubinga et al., 2013, 2014a, 2014b, 2015), and migratory birds have been implicated as disseminators of ticks over long distances (Wilhelmsson et al., 2020). Therefore, it may be possible for migratory birds to introduce LSDV-infected ticks into Australia. Additionally, other species may also be able to introduce LSDV-infected ticks, such as rats on ships.

3.2 Vector categories for inclusion

We limited our analysis to three broad vector categories: heavy fliers (including stable flies, tabanids and other brachyceran flies), mosquitoes (*Culicidae*) and midges (*Ceratopogonidae*).

As previously discussed, any haematophagus arthropod species is probably capable of acquiring LSDV (Berg et al., 2015; Tuppurainen et al., 2017). Interrupted feeders that regularly parasitise cattle and are found in high abundance are of particular concern for transmission; the relative importance of different vector species is likely to vary in different regions, depending on climate, season, humidity and vegetation

[7] Shapefiles provided by T. Kerlin, NAQS.

(Berg et al., 2015). In the literature, LSDV vectors are generally categorised into brachyceran flies, mosquitoes (*Culicidae*), midges (*Ceratopogonidae*) and hard ticks. Importantly, LSDV transmission has never been demonstrated from *Culicoides* midges, despite being attempted in several studies (P. Beard, pers. comms.)[8]. Hard ticks are unlikely to be able to travel the distances required to carry LSDV into Australia in the absence of a host animal, although, arguably, migratory birds or vermin on ships could transport LSDV-infected ticks into Australia. However, we did not include hard ticks in our assessment.

Importantly, within the three broader categories that we selected there is considerable species variation. For example, anthropophilic mosquito species are of much less relevance to our assessment than zoophilic species. Yet many of the experimental studies on LSDV have utilised *Aedes aegypti*, which is known to be anthropophilic but is readily available in laboratory settings (Chihota et al., 2001; Sanz-Bernardo et al., 2021). In contrast, there is no peer-reviewed literature on the role of calliphorid or *Musca* flies beyond the *Stomoxys* genus in LSDV transmission, although these are known to be very effective mechanical vectors for diseases such as rabbit calicivirus and many human pathogens (Asgari et al., 1998; Khamesipour et al., 2018). There is also considerable variation in short-range flight distances of different species within a vector category, which would impact the probability of a vector dispersing to ports, entering a wind stream or locating a host after arrival. For example, *Aedes vigilax* can disperse up to 40 km or more while *Aedes tremulus* do not travel more than 500 metres from breeding sites (Whelan, 2010). Our entomology experts expressed concern about grouping so many species together; however, given the lack of species-specific vector information in the literature, we felt that further breakdown by vector category is unlikely to be useful.

We further assumed that LSDV was not biologically transmitted by arthropod vectors. This view is widely accepted in the literature (Chihota et al., 2001, 2003; Tuppurainen et al., 2013b; Lubinga et al., 2015; Sohier et al., 2019; Sprygin et al., 2019; Issimov et al., 2020; Paslaru et al., 2021; Sanz-Bernardo et al., 2021). A recent study reported the possibility of biological transmission of LSDV, at least in *Culicoides nubeculosus*, based on a purported increase in viral nucleic acid concentration over time and the presence of infectious virus in the wings and abdomen – that is, beyond the mouthparts (Paslaru et al., 2022). Critically, the reported increase in viral nucleic acid was determined by assaying 10 heads per time point, of which only 1–3 per time point were above the limit of detection of the assay, leading to very small sample sizes per time point with very large variance between samples. It is also unclear if these data were from a single qPCR run or multiple runs; if the latter, reporting of cycle thresholds is not appropriate and absolute quantification should be performed. Furthermore, the detection of virus beyond the mouthparts does not confirm biological transmission. For example, there is no biological transmission of rabbit caliciviruses, yet viral nucleic acid and proteins were detected on the legs as well as in the abdomen and in flyspots, even using relatively insensitive detection methods (Asgari et al., 1998). Additionally, for heavy fliers at least, once one individual in a trap is positive for rabbit caliciviruses there is very rapid contamination of other individuals within the trap, probably through contact with excreted flyspots (Hall et al., 2019). A more recent study investigating the role of houseflies and blowflies as mechanical vectors found that there was considerable overlap in the microbiome of the head, thorax, abdomen and legs plus wings, indicating that most microorganisms associated with flies are not restricted to their gastrointestinal tract (Junqueira et al., 2017). While these studies are limited to heavy fliers, they demonstrate that even mechanically transmitted microorganisms may be detected in multiple body compartments.

[8] Interim results that have generously been made available ahead of publication.

3.3 Origin countries for inclusion

We limited our analyses to origin countries within 48 hours' dispersal by wind and 10 days' travel by ship. These were considered the maximum realistic times within which a vector would survive and remain infectious with LSDV based on currently available information in the literature (Sellers, Pedgley and Tucker, 1977; Alba, Casal and Domingo, 2004; Agren et al., 2010; Burgin et al., 2013; Eagles et al., 2013; Paslaru et al., 2022). We excluded Pacific Island countries (except PNG) and territories and island nations with a land area of <3,000 km^2 from the analysis. We assumed the probability of LSDV arriving in island countries of <3,000 km^2 to be negligible (and therefore chose to exclude them from the analysis) partly because we know that ocean borders and relatively low livestock populations and trade volumes have historically kept these nations free of many infectious diseases of livestock (Brioudes, 2016). However, we cannot equivocally exclude the possibility of vectors surviving longer duration transport, or of LSDV entering via these Pacific Island countries or territories or small island nations. For example, a recent risk assessment of LSDV spread in South, East and Southeast Asia returned a negligible risk of incursion with low uncertainty for Singapore, which then experienced an outbreak within two years (Roche et al., 2020).

3.3.1 Windborne dispersal

Atmospheric dispersion modelling using HYSPLIT based on a maximum flight duration of 48 hours revealed PNG, Indonesia and Timor-Leste as potential source locations for long-distance windborne dispersal of arthropod vectors (Figure 13). Within Indonesia, windborne dispersal to Australia between 2017 to 2021 was only possible from eastern and central regions. Eagles et al. (2014) determined that windborne dispersal of *Culicoides* into Australia was possible from southern PNG westwards to Lombok, Indonesia (Lombok was classified as central Indonesia in our analysis (Figure 1)). In our analysis, dispersal was also possible from the eastern end of Java Island, noting that we used a 48-hour dispersal window instead of the 20-hour dispersal window used by Eagles et al. (2014). December to March were the highest risk months for dispersal, as reported previously (Eagles et al., 2012, 2013); additionally, we found that dispersal was also possible, although less likely, during April, May, October and November.

Countries included: Eastern and central Indonesia, PNG, Timor-Leste

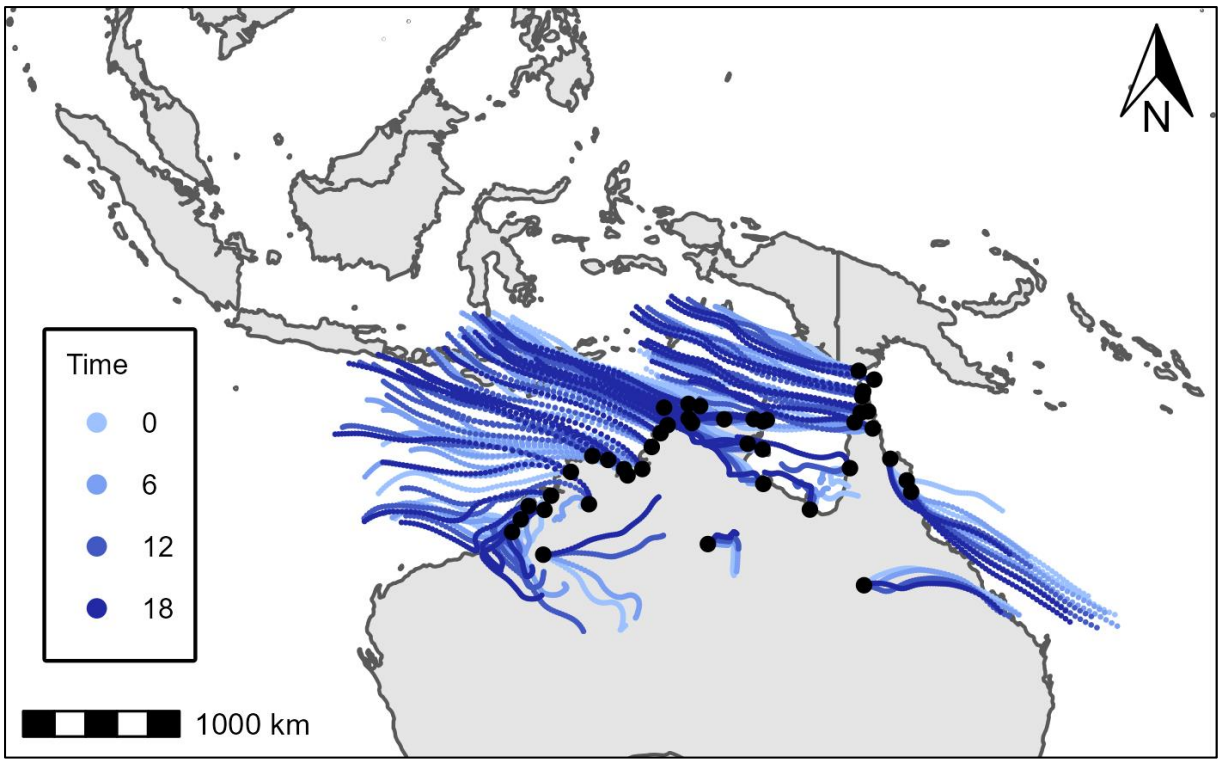

Figure 13 **Example HYSPLIT trajectory modelling from 30 January 2021**

Shown are the potential source locations (end of blue lines) for windborne dispersal based on 48-hour backwards trajectories from the centroids of arrival destinations (NAQS risk zones plus three inland regions); centroids are represented as black dots. Models were run at 6-hourly intervals (0000, 0600, 1200, 1800) using NCEP-NCAR Reanalysis 1 gridded meteorological data.

3.3.2 Commercial vessels (including returning live export vessels)

Based on a recent paper by Paslaru et al. (2022) that demonstrated retention of LSDV infectivity (by virus isolation) in insects for at least 10 days, we considered all countries within 10 days' travel time as potential origin sources for LSDV incursion (Figure 14, Figure 15). Notably, while *Aedes* mosquitoes have been shown to transmit LSDV for 2 to 6 days after feeding, other publications have suggested that other vector species such as *Stomoxys* must re-feed within 24 hours for transmission to be successful (Chihota et al., 2003; Sohier et al., 2019), making 10 days a conservative estimate. While there are sporadic reports of living Australian *Culicoides* being detected in Chinese seaports, suggesting that survival over longer durations is possible, it is unclear whether such aged insects would be in a condition to be able to seek and bite a susceptible host (Nie et al., 2005). It is also possible that these detected insects had hatched from breeding sites on board and were not the original adults that had boarded the vessel in Australia. However, we cannot equivocally exclude the possibility of travel from distances greater than the 10 days used for this analysis.

The United States of America (USA) was identified as within 10 days' travel duration for commercial ships; however, further inspection revealed that this was restricted to a single seaport in Hawaii. Thus, we considered Hawaii as a separate 'origin country' for our analysis because it was not appropriate to parameterise this single seaport using USA-wide data (i.e. bovine numbers and trade statistics).

Countries within 10 days' sea-travel time, based on vessel type, were:

Commercial shipping excluding returning live export vessels

Countries included: Bangladesh, Brunei Darussalam, Cambodia, Central Indonesia, China and associated autonomous regions, Democratic People's Republic of Korea, Eastern Indonesia, Hawaii, India, Iran, Japan, Madagascar, Malaysia, Myanmar, Oman, Pakistan, PNG, Philippines, Republic of Korea, Russian Federation, Singapore, Sri Lanka, Thailand, Timor-Leste, Vietnam, Western Indonesia, Yemen

Returning live export vessels

Countries included: Bangladesh, Brunei Darussalam, Cambodia, Central Indonesia, China and associated autonomous regions, Democratic People's Republic of Korea, Eastern Indonesia, India, Japan, Malaysia, Myanmar, PNG, Philippines, Republic of Korea, Singapore, Sri Lanka, Thailand, Timor-Leste, Vietnam, Western Indonesia

Note that whether live export vessels actually travelled to these countries was considered in En_6.

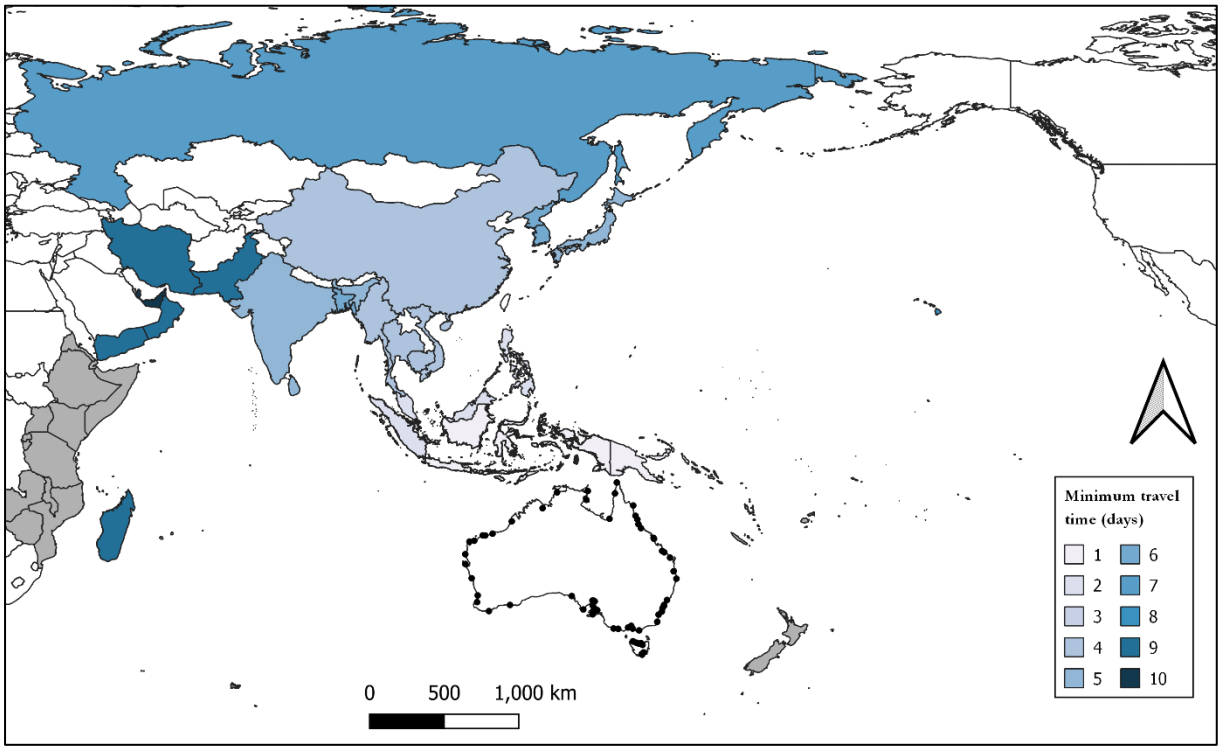

Figure 14 Map of origin countries coloured by the minimum travel time for commercial ships (excluding returning live export vessels)

Australian seaports are shown as black dots. Excluded regions are shown in grey. Scalebar accuracy may be limited due to use of a geographic coordinate system.

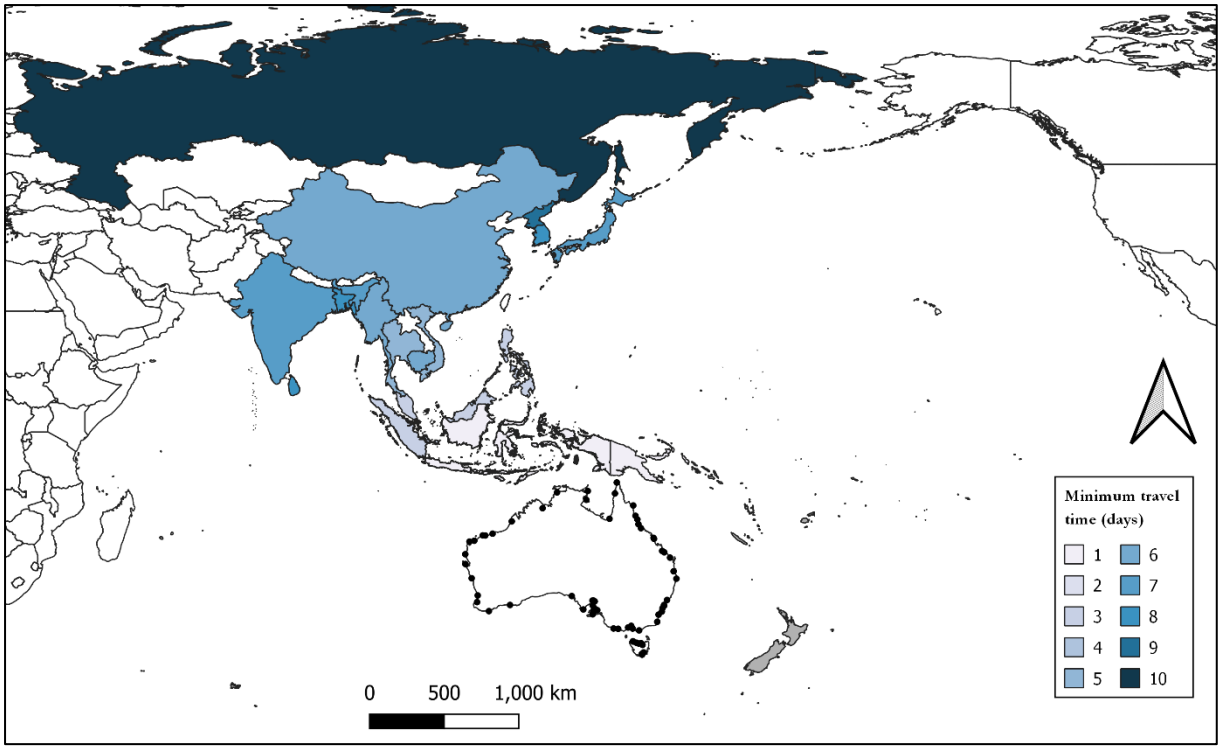

Figure 15 **Map of origin countries coloured by the minimum travel time for returning live export vessels**

Australian seaports are shown as black dots. Excluded regions are shown in grey. Scalebar accuracy may be limited due to use of a geographic coordinate system.

3.3.3 Torres Strait Treaty movements

Papua New Guinea was the only relevant origin country for the Torres Strait Treaty pathway as Treaty villages are only located in PNG (Figure 12).

Countries included: PNG

3.4 Arrival destinations for inclusion

3.4.1 Windborne dispersal

We used NAQS risk zones as potential arrival destinations for wind-dispersed arthropod vectors, as these are more useful for planning surveillance efforts, compared to other geographical divisions such as Statistical Area 2 (SA2) or natural resource management regions. Arrival destinations for the windborne dispersal pathway are shown in Figure 16, along with the corresponding livestock densities by SA2 (NAQS risk zones) or SA4 (the three additional arrival regions). There are no reports of exotic insects being recovered beyond the extent of these regions that we are aware of.

Regions included: Q1a, Q1b, Q2, Q3, Q4a, Q4b, Q5a, Q5b, Q5c, Q6a, Q6b, Q6c, Q7, rest of western QLD, N1a, N1b, N1c, N2a, N2b, N3, N4, N5, N6, N7, N8a, N8b, N9, N10, N11, N12, rest of NT, W1, W2, W3, W4, W5, W6a, W6b, W7a, W7b, W8, W9, rest of northern WA

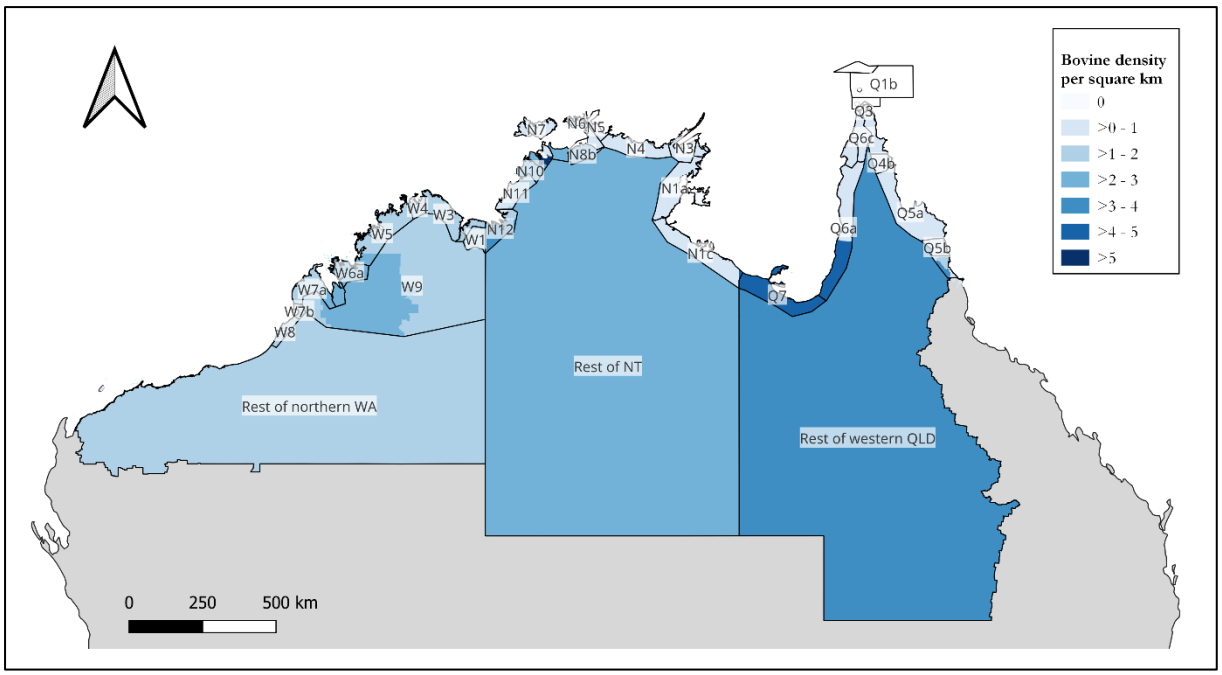

Figure 16 Map of arrival destinations for windborne dispersal pathway

Arrival destinations were based on NAQS risk zones with three additional zones, 'rest of northern WA', 'rest of NT' and 'rest of western QLD'. Bovine densities in NAQS risk zones were estimated from a discrete distribution based on the underlying SA2-level cattle densities after feral bovine densities were added. SA4-level densities were used for the three additional zones.

3.4.2 Commercial vessels (including returning live export vessels)

All Australian seaports were considered as potential arrival destinations for the shipping pathways. There are 66 seaports in Australia (Figure 17): Albany, Ardrossan, Ballina, Batemans Bay, Beauty Point, Botany Bay, Brisbane, Broome, Bunbury, Bundaberg, Burnie, Cairns, Cape Cuvier, Cape Flattery Harbor, Dampier, Darwin, Devonport, Dover, Eden, Esperance, Fremantle, Geelong, Geraldton, Gladstone, Gove, Hay Point, Hobart, Jervis Bay, Karumba, Kingscote, Klein Point, Launceston, Lucinda, Mackay, Melbourne, Milner Bay, Mourilyan Harbour, Newcastle, Onslow, Point Murat, Port Adelaide, Port Alma, Port Bonython, Port Dalrymple, Port Giles, Port Hedland, Port Huon, Port Kembla, Port Latta, Port Lincoln, Port Pirie, Port Walcott, Portland, Stanley, Sydney, Thevenard, Thursday Island, Townsville, Useless Loop, Wallaroo, Warrnambool, Weipa, Welshpool, Western Port, Whyalla and Wyndham.

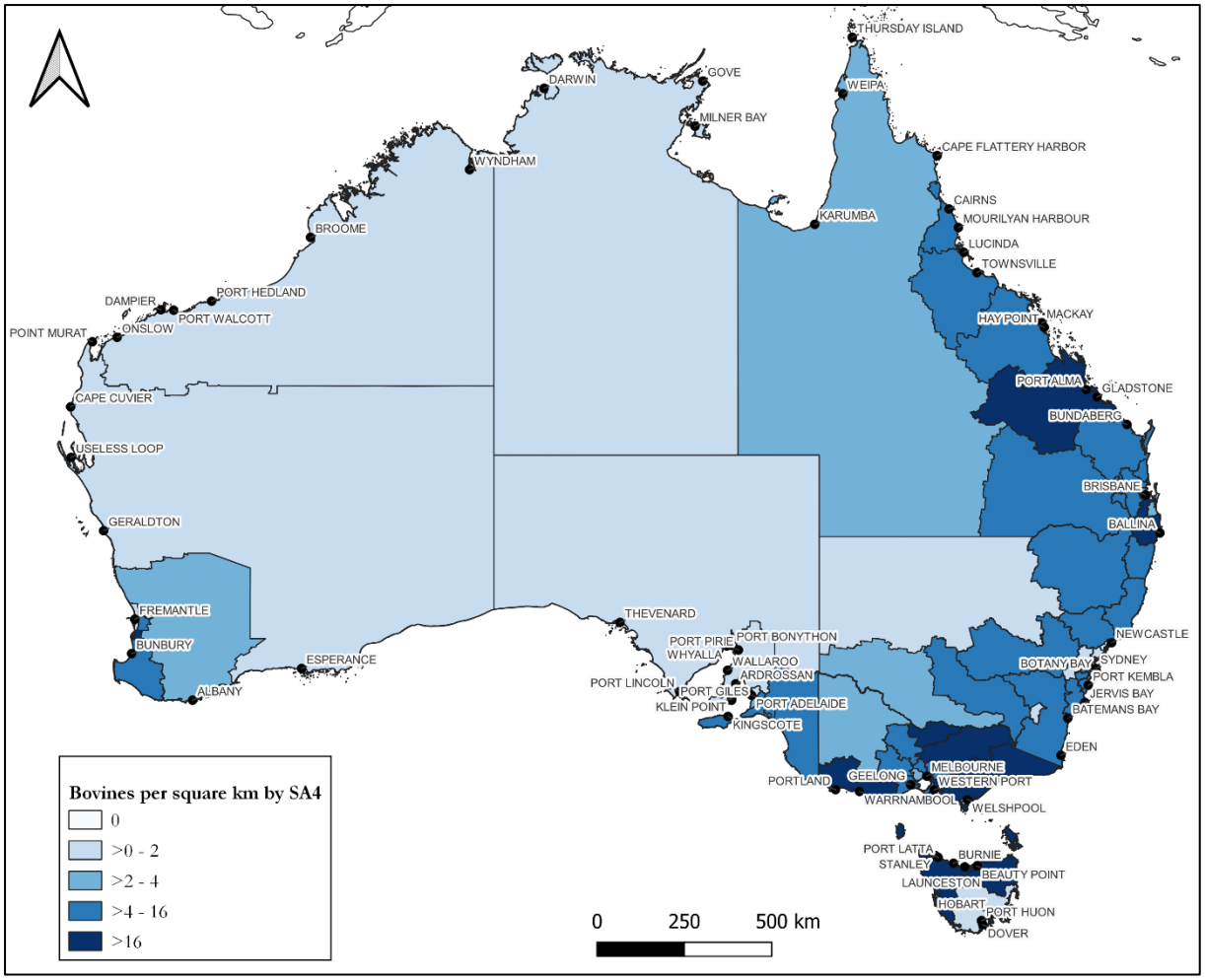

Figure 17 Map of Australian seaports and bovine densities by SA4
Scalebar accuracy may be limited due to use of a geographic coordinate system.

3.4.3 Torres Strait Treaty movements

During normal periods of traditional movements, the majority of traditional visitors (98% in 2016–2017) visit the top western islands of Boigu, Dauan and Saibai (NAQS officer, pers. comm.) (Figure 12). Other traditional visitors will travel to other islands in the Torres Strait Protected Zone but are not permitted under the Treaty to travel further south into the Torres Strait Permanent Biosecurity Monitoring Zone or to the mainland. There is some potential that illegal travel could extend beyond these destinations. Thus, we considered only the northernmost NAQS risk zones, those within the Torres Strait, as potential arrival destinations for this pathway (Figure 12).

Regions included: Q1a, Q1b, Q2

3.5 Scenario trees and development of the models

The scenario trees and incursion risk models for each pathway are presented in Section 2.5.

3.6 Parameterisation of the models

3.6.1 Windborne dispersal

En_1 Number of bovines at origin

First, we wanted to understand the potential number of infectious bovines per year in each origin country available for vectors to feed on. The methodology is described in Section 2.6.1. Bovine numbers for the windborne dispersal pathway ranged from 92,334 in PNG (2020) to 11,778,018 in central Indonesia (2020) (Figure 18).

Uncertainty: We considered this parameter to be variable (since livestock numbers may fluctuate) but not uncertain (since a curated data set from a reputable source is available).

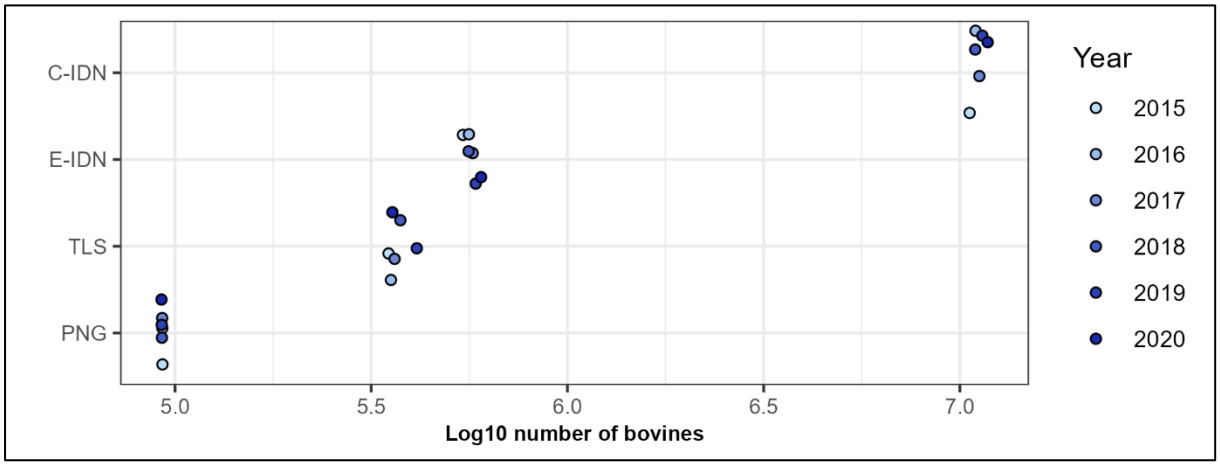

Figure 18 **Log_{10} bovine numbers by origin country for the windborne dispersal pathway per year, 2015–2020**[9]

En_2 Bovines infected with LSDV

Not all animals in an LSDV-endemic country will become infected, either because they are never exposed to sufficient infectious virus particles or because of individual variation in resistance to infection (due to intrinsic genetic or physiological factors). Likewise, an animal will only be infected at one point in time, as immunity to LSDV is widely considered to be lifelong (Kitching, 2003; European Food Safety, 2018; Namazi and Tafti, 2021). We estimated the proportion of bovines infected per year in each origin country by dividing the proportion infected at any time in their life by the average lifespan of a bovine (which represents the duration of immunity for LSDV). We then multiplied this by the duration of infectiousness to estimate the number of infectious cattle days per year by origin country, in a similar approach to that developed by the EFSA (Berg et al., 2015). We used seroprevalence to determine the proportion of animals infected during their lifetime, using 5–30% seroprevalence based on a previous risk assessment by the EFSA (Berg et al., 2015) (Figure 19). We assumed that every animal seroconverts after infection and that antibodies don't wane (e.g. that seroprevalence is an accurate reflection of the proportion of animals previously infected). This may underestimate the true proportion of animals infected. However, current

[9] The list of ISO 3166-1 alpha-3 country codes is given in Appendix 4.

LSDV seroprevalence in Australia's near neighbours is considerably lower than 5–30% given the emerging status of LSDV in these countries.

Current evidence suggests that asymptomatic animals contribute substantially less than clinically infected animals to vector-borne transmission of LSDV due to low titres of virus in the blood and skin (Sanz-Bernardo et al., 2021). Typically, only 20–60% of infected cattle develop clinical disease, even based on studies using highly susceptible *Bos taurus* breeds (Magori-Cohen et al., 2012; Tuppurainen and Oura, 2012; Sanz-Bernardo et al., 2021). This symptomatic proportion may be further reduced in more resistant breeds, although experimental data are currently lacking. To be conservative we did not distinguish between clinically and subclinically infected animals in this analysis, which will likely overestimate the final risk.

We used 5 years as the average lifespan of a bovine, also taken from the EFSA assessment (Figure 19). With a seroprevalence of 5–30% and a lifespan of 5 years, this would lead to an estimate of 1–6% of bovines infected with LSDV each year in endemic areas; if lifespans are longer, as may be the case with smallholder farming systems where farmers tend to retain animals as an asset rather than seasonally sell them to generate profit, the estimated annual incidence would decrease. Whether the kinetics of immunity (e.g. duration) vary under different farming systems or with different breeds of cattle (e.g. banteng) is not known and is an additional source of uncertainty in our analysis.

From the peer-reviewed literature, we determined that the duration of infectiousness for LSDV can range from 5–39 days, with peak infectiousness around day 14 of infection (Figure 19) (Weiss, 1968; Carn and Kitching, 1995; Tuppurainen, Venter and Coetzer, 2005; Osuagwuh *et al.*, 2007; Babiuk *et al.*, 2008; Annandale *et al.*, 2010; Berg *et al.*, 2015; Sohier *et al.*, 2019; Sanz-Bernardo *et al.*, 2021). We assumed equal infectivity over the entire infectious period, which will overestimate our final risk estimate. Studies investigating the duration of infectiousness for LSDV are typically conducted in highly susceptible *Bos taurus* breeds; more resistant breeds, such as *Bos indicus*, may have a reduced period of infectiousness, although specific laboratory studies in this breed have not yet been conducted.

We did not incorporate country-specific factors for this node, such as the density of livestock, availability of veterinary services, farm biosecurity/management type, use of LSDV vaccination or vector control practices (Ochwo et al., 2019). These data are challenging to find. In our qualitative assessment we used human development index as a proxy for the availability of veterinary services, noting that this proxy was far from ideal. Rather than trying to account for each of these factors individually, efforts to improve our assessment should rather be focused on understanding the overall yearly incidence of LSDV in relevant countries directly, which would then account for these various factors.

Uncertainty: We considered this node to be both uncertain and variable, given the cumulative uncertainty and variability in the subcomponents.

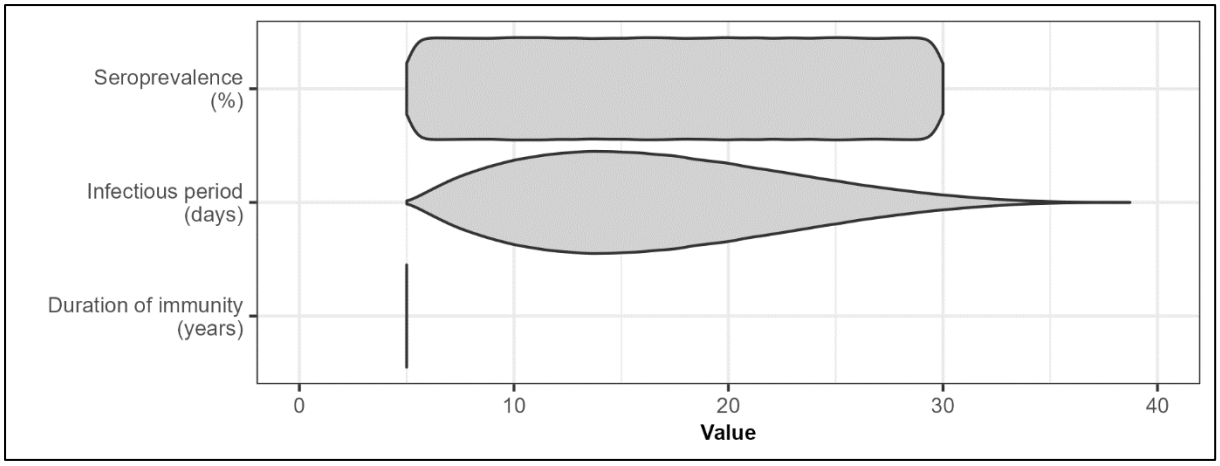

Figure 19 **Parameter values used for LSDV seroprevalence at endemicity, infectious period and duration of immunity**

En_3 Number of vectors biting each bovine per infectious day

Next, we wanted to determine the possible number of infectious vectors per year 'available' to disperse to Australia. We estimated the number of vectors feeding on a bovine per day based on the vector–host ratios derived by Gubbins et al. (2019). This ranged from 0–5,000 midges per bovine, 0–80 mosquitoes per bovine, and 30–145 heavy fliers per bovine (Figure 20). We did not consider the number of repeated bites from each insect or the feeding behaviour of the different vector species. For example, only female mosquitoes, midges and tabanids take protein meals. Many arthropods also require several days for egg maturation and oviposition after taking a blood meal, during which time they do not re-feed. In contrast, both male and female *Stomoxys* take protein meals and they feed constantly, not having the classical gonotrophic cycle typical of mosquitoes and midges. Additionally, *Stomoxys* are aggressive feeders, biting repeatedly and irritating the host, which leads to frequent host switching (Scoles et al., 2005; Baldacchino et al., 2013). This may elevate the risk associated with heavy fliers but this distinction was not captured in our model.

The distribution for each vector category is applied independently in our model, meaning that each bovine is parasitised by all three vector categories simultaneously and every bovine in a herd is equally likely to be parasitised. Furthermore, our model does not consider seasonal or diurnal changes in vector abundance, different abundances between or within countries, or species variation in feeding behaviour within a vector category, adding considerable uncertainty to these estimates. While there are many studies investigating insect biting rates directly (Todd, 1964; Schieffelin et al., 2000; Gerry et al., 2001; Bellis et al., 2004; Baldacchino et al., 2013; Ayllón et al., 2014; Elbers and Meiswinkel, 2014, 2015; Liu-Helmersson et al., 2014; Showler and Osbrink, 2015; Brugman et al., 2017; Ryan et al., 2017; Elbers, Gonzales and Meiswinkel, 2018; Rochon et al., 2021; Syahrani et al., 2022), these are typically conducted under optimal environmental conditions and sampling times for insect activity with a focus on a limited number of insect species. It is therefore challenging to extrapolate the results of these studies into a reliable estimate of the average total number of insects (and bites) per cow per day. Interestingly, there are reports of 40,000–70,000 mosquitoes feeding on cattle in a single night (Standfast and Dyce, 1968; Tatchell, 1969), 70 bites per minute from *Culicoides* (Bellis et al., 2004) and up to 1,000 *Stomoxys* per animal (Rochon et al., 2021). It is clearly not realistic to extrapolate this out to a daily rate, as cattle would not survive more than a few weeks with this level of feeding.

Uncertainty: We considered this node to be both uncertain and variable because of the requirement to extrapolate this to a daily parameter.

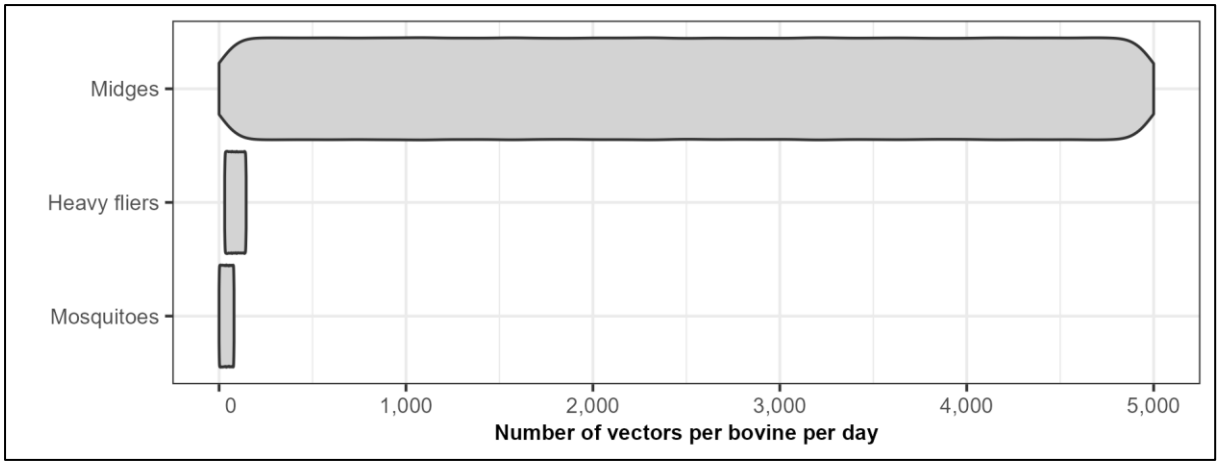

Figure 20 Range of parameter values by vector category for the number of vectors feeding on a bovine per day

En_4 Bovine-to-vector transmission

Not all vectors will acquire LSDV after feeding on an infected host. For example, feeding can be interrupted and viral loads vary depending on where an animal is bitten, with viral loads being highest in lesions and lower in the blood (Carn and Kitching, 1995b; Chihota et al., 2003; Elbers and Meiswinkel, 2014; Sanz-Bernardo et al., 2021). Viral loads may also change over the course of an infection. Several studies have investigated the proportion of insects that are LSDV-positive after feeding on an infected bovine, with considerable variation in results both within and between studies (Chihota et al., 2001, 2003; Gubbins, 2019; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). The proportion of midges acquiring LSDV after feeding ranged from 0.043–0.31, mosquitoes ranged from 0.064–1 and heavy fliers ranged from 0.024–0.9 (Figure 21).

Importantly, only one study used virus isolation to determine LSDV positivity; most studies used qPCR, which only detects viral nucleic acid, not infectious virions. Typically, experimental studies use small numbers of insects and artificial conditions, which may limit the external validity of these findings. Since time-stratified data on insect LSDV positivity are not available we assumed that the proportion of insects acquiring LSDV after feeding remained constant over the entire infectious period. Therefore, we expect that our selected values overestimate the true proportion of infectious vectors in the field.

Uncertainty: We considered this node to be variable. Since data from the literature were available in the context of LSDV, we did not incorporate an uncertainty dimension.

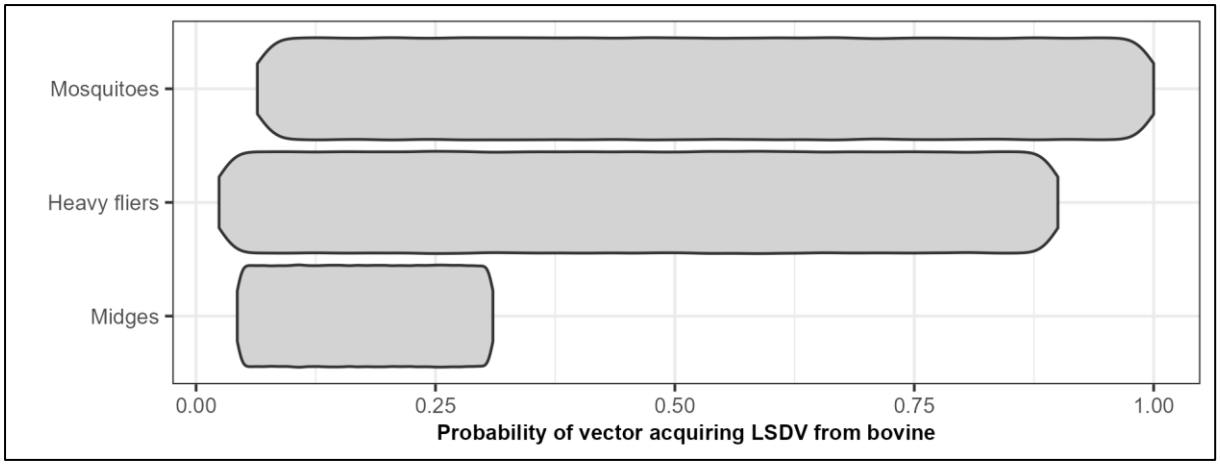

Figure 21 **Range of parameter values for a vector acquiring LSDV from an infected bovine after feeding**

En_5 Vector transported to Australia by wind

Mosquitoes, midges and heavy fliers undertake self-directed flight when seeking hosts, nectar, mates, shelter and oviposition sites (Verdonschot and Besse-Lototskaya, 2014; Elbers, Koenraadt and Meiswinkel, 2015). These intentional, active daily flights (as opposed to passive dispersal via prevailing winds) are typically over short distances, although this varies considerably by species (Service, 1997; Whelan, 2010; Verdonschot and Besse-Lototskaya, 2014). A recent meta-analysis on mosquitoes reported average flight ranges between 25 metres and 6 kilometres (Verdonschot and Besse-Lototskaya, 2014). Similarly, midges can reportedly travel up to 5 km over several days (Elbers, Koenraadt and Meiswinkel, 2015). For heavy fliers such as stable flies, studies have shown that most disperse over distances less than 13 km (Showler and Osbrink, 2015). While long-distance dispersal of mosquitoes, midges and heavy fliers by wind is known to occur, these events are widely considered to be rare accidents (Service, 1997; Elbers, Koenraadt and Meiswinkel, 2015). This view has recently been challenged, with reports of windborne migration of African malaria vectors over distances up to 300 km (Huestis et al., 2019). However, there is no evidence suggesting self-directed migration of arthropods from Southeast Asia into Australia.

Small insects generally undertake directed flight within the flight boundary layer, close to the Earth's surface, or within the vegetation layer where winds are low (Srygley and Dudley, 2007). For example, short distance flights of stable flies are typically restricted to heights of around 1 metre above ground level (Showler and Osbrink, 2015). At higher altitudes, wind speeds increase exponentially so that insects are unable to undertake self-directed flight (Elbers, Koenraadt and Meiswinkel, 2015). Generally, *Culicoides* become inactive at wind speeds above 4 km/h (Elbers, Koenraadt and Meiswinkel, 2015). Likewise, wind velocity is inversely correlated with mosquito activity (Bidlingmayer, Day and Evans, 1995). When ambient winds are above these speeds, smaller insects typically seek shelter. However, mosquitoes and midges can ascend above the flight boundary layer to altitudes of ten to several hundred metres to enter fast-moving air streams for long-distance dispersal (Verdonschot and Besse-Lototskaya, 2014; Klausner, Fattal and Klement, 2017). In contrast, stable flies are reportedly restricted to within 60 metres of the ground, even during wind-assisted dispersal (Showler and Osbrink, 2015). Importantly, to survive high-altitude transportation, air temperature must remain above 15°C (Elbers, Koenraadt and Meiswinkel, 2015). Using visual, thermal and olfactory cues, insects can then descend when resources are available, or can be dropped to the ground by downdrafts (Verdonschot and Besse-Lototskaya, 2014; Showler and Osbrink, 2015).

There are three subcomponents that must align for windborne dispersal to occur over sea. Vectors must:

- enter the wind stream
- enter at a date and time conducive to transport along an appropriate trajectory
- reach land in the destination country.

Considering the literature discussed above, we assessed the probability of an insect entering high-altitude wind streams as extremely low. Since no empirical data were available to estimate this value directly, we derived a semi-quantitative estimate (1×10^{-5}–1×10^{-3}) according to a previously reported risk assessment methodology by converting our qualitative assessment (Biosecurity Australia, 2004). We considered that all vector categories were capable of being swept into high-altitude wind streams, although as described above this may not be true of heavy fliers (which would then lead to an overall risk of zero for this vector category).

We estimated the proportion of the year where winds were suitable for vector dispersal to Australia using atmospheric modelling of wind trajectories. The proportion of trajectories per year when winds were suitable for dispersal to Australia ranged from 0 to approximately 30% (Figure 22).

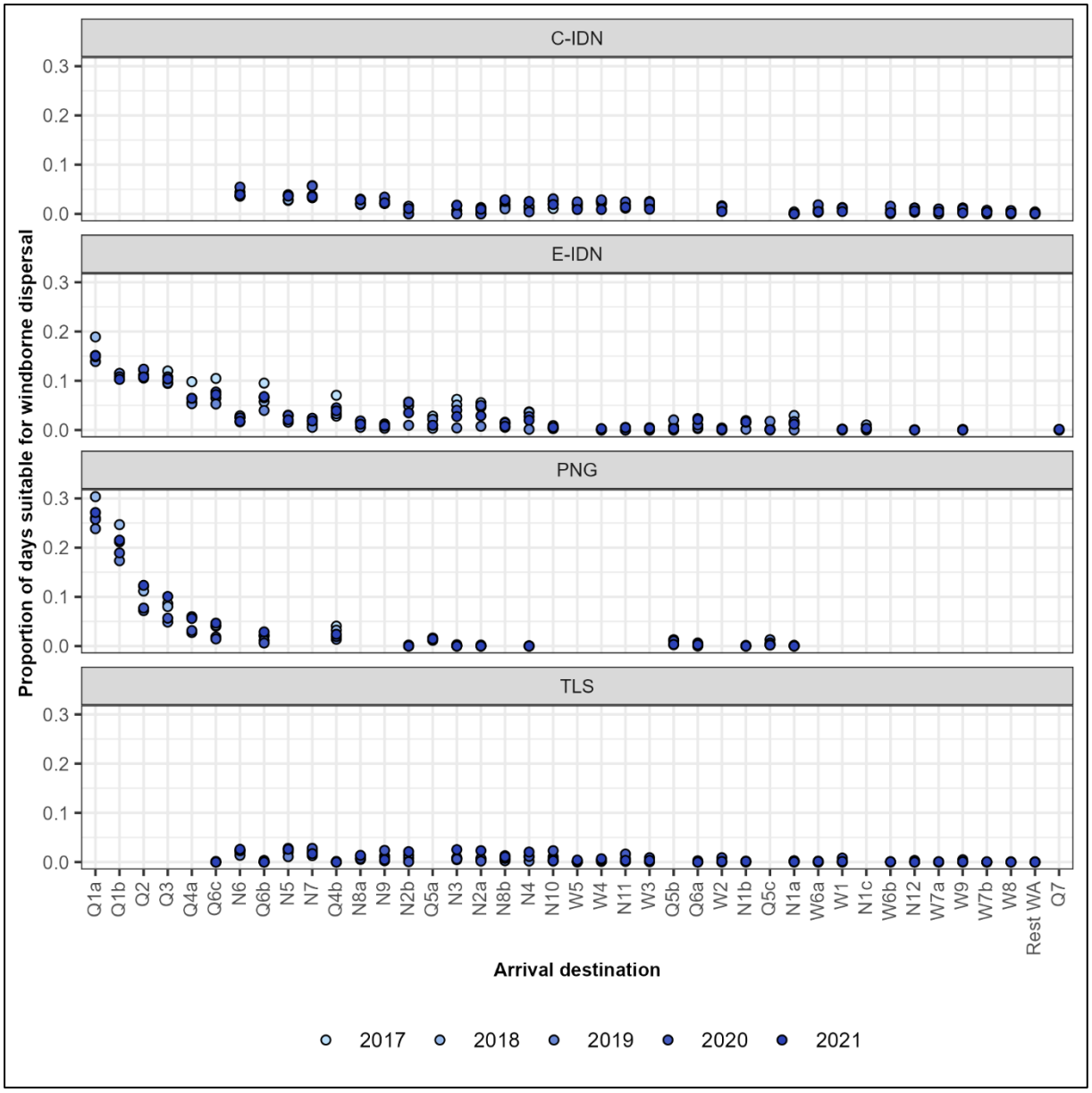

Figure 22 **Proportion of trajectories per year suitable for windborne dispersal to Australia, by origin country and arrival destination, 2017–2021[10]**

To estimate the probability of a single particle (i.e. insect) reaching land in Australia, we used atmospheric concentration/dispersion modelling to simulate the forward dispersion of 10,000 theoretical particles (i.e. sufficient numbers to simulate a distribution) from various source locations within each origin country at times when winds were suitable for dispersal to Australia (e.g. Figure 23).

[10] NAQS risk zones for which windborne dispersal was not possible from any origin country are not shown.

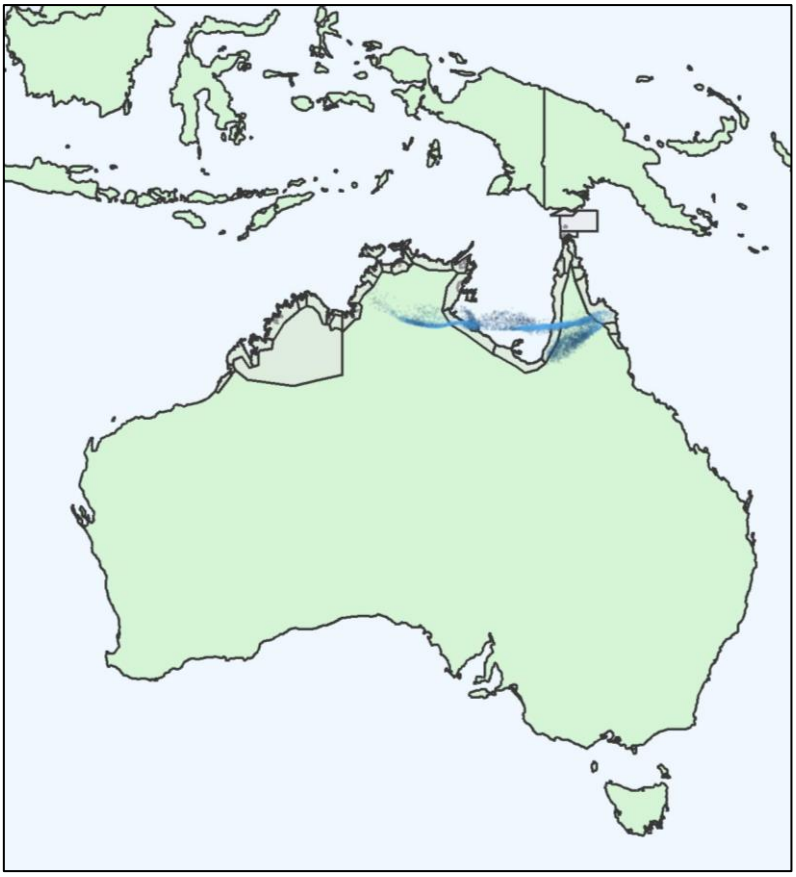

Figure 23 A representative forward concentration dispersion run of 10,000 particles (i.e. insects) viewed 48 hours after 'release' from central Indonesia (-9.658, 124.436) at 18:00 on 25 December 2021

NAQS risk zones are shown in grey. Individual particles/insects are represented as blue dots coloured by altitude.

The proportion of particles arriving on suitable days ranged from 1/10,000 to 9,696/10,000 (Figure 24). However, this is clearly an overestimate of the true proportion; because we could not be certain where a vector would choose to land, particles were counted in every arrival destination with which they intersected. For example, many of those 9,696 particles would also be counted in other arrival destinations, yet clearly an individual insect would only be able to arrive in one of these destinations. More complex modelling may be able to assign relative probabilities for each risk zone, based possibly on distance from origin country or particle height, but this was beyond the scope of the current project.

The choice of point source locations, for both the trajectory and concentration modelling, may also influence these results. For example, for our concentration modelling we selected source locations within each origin country that were close to Australia and that were clearly in line with prevailing winds based on our trajectory modelling, likely overestimating the risk from the origin country overall. If we had instead selected centroids as source locations, or locations that were more northerly or at the edges of the prevailing wind streams, it is probable that fewer particles would have successfully reached Australia.

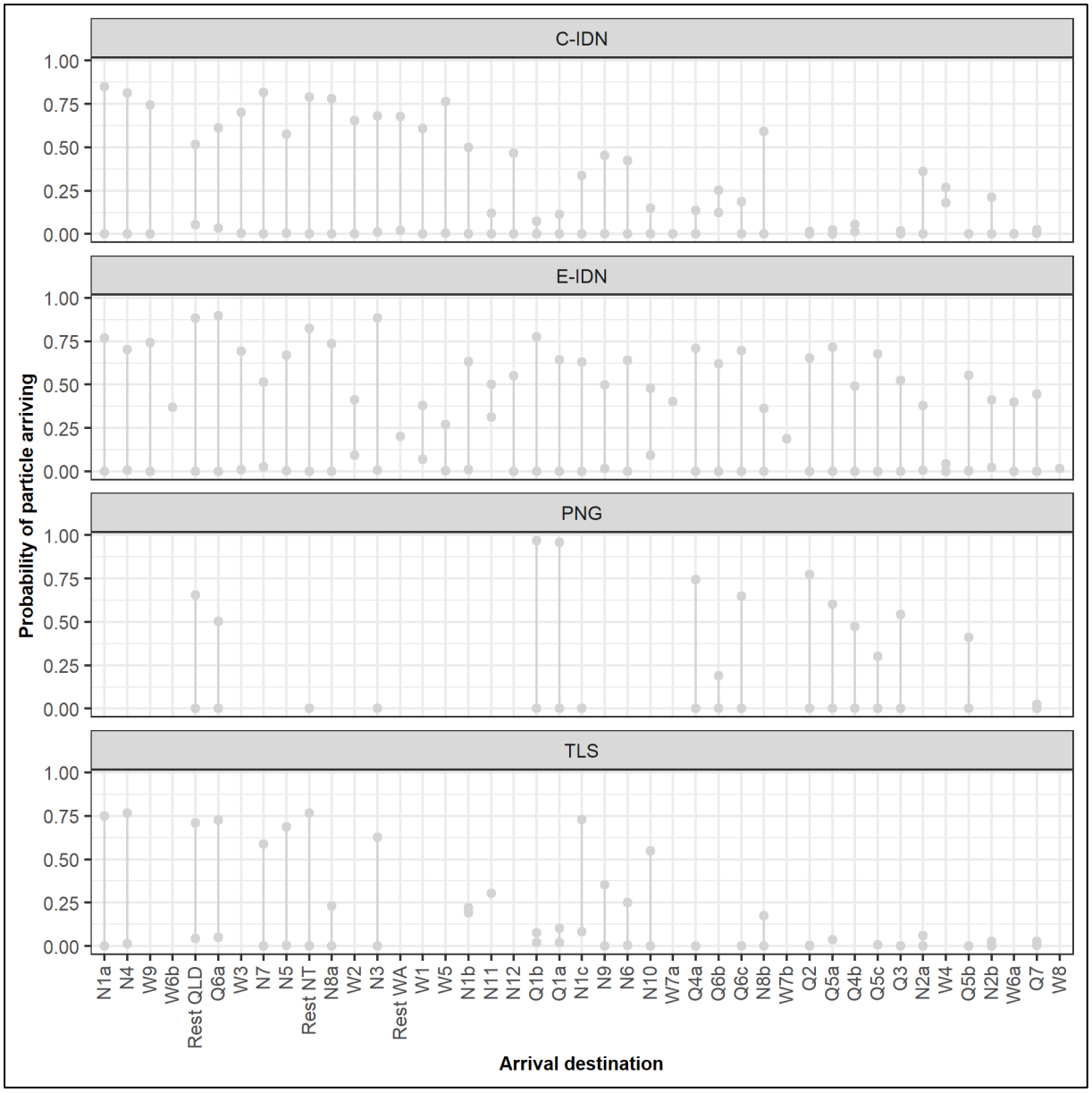

Figure 24 Minimum and maximum probabilities for wind-dispersed particles arriving in NAQS risk zones by origin country, 2021

Additionally, several limitations of our HYSPLIT modelling lead to high uncertainty at this node. The technical limitations of HYSPLIT are described in detail elsewhere (NOAA-Air Resources Laboratory, no date b). In addition to these fundamental limitations, HYSPLIT was developed to model atmospheric dispersion of gases and fine particles such as dust and volcanic ash. When applied to larger 'particles' (i.e. insects, in this instance) several key parameters are not modelled. For example, particles are reflected at the top model height defined in the HYSPLIT parameters, while in the case of insects they would likely perish at this altitude and should thus be 'deposited' once reaching this height. The model does not account for self-directed flight or direction changes. While there is an advanced parameter (VBUG) that is able to add a horizontal speed to trajectories, this does not apply to concentration runs; this is applied equally over the entire trajectory, whereas self-directed flight would only occur in certain conditions (such as when windspeeds and altitude are sufficiently low). We didn't consider air temperature, relative humidity, damaging wind velocities, or other climatic factors that may influence vector deposition. We also didn't consider environmental conditions at the source location to determine whether insects would be active under those conditions, which would influence the probability of entering high-altitude wind

streams in the first place. Since these environmental variables are present in the meteorological data files they presumably could be incorporated into more advanced models, but this was beyond the scope of the current project.

The deposition parameters used here were based on the previous peer-reviewed literature and default HYSPLIT settings, without supporting empirical data. While deposition can be computed based on particle diameter, air density and particle density, this does not account for intentional settling by insects undertaking self-directed flight.

Taken together, these limitations led to our HYSPLIT modelling not differentiating between the three vector categories; this is likely to considerably overestimate the probability of windborne dispersal particularly for *Stomoxys*, which may not even enter high-altitude wind streams.

For some combinations of origin country and arrival destination no suitable days were identified based on trajectory simulations, yet arriving particles were detected based on the concentration runs. While this shows that trajectories may underestimate the number of suitable days for dispersal, computationally it was not considered feasible to run dispersal runs from a comprehensive grid of source locations within each country for every time point and day. The discrepancy between trajectory and concentration runs is presumably due to several factors (Figure 10). Firstly, NAQS centroids were used for backwards trajectory runs but polygon boundaries were used for forward dispersion runs. Conversely, precise source locations within origin countries were used for dispersion runs while polygon boundaries were used for trajectory intersections. Secondly, particles were released from a single time point on suitable days, rather than every six hours as for the trajectory runs. Additionally, there may be discrepancies between the higher-resolution GDAS meteorological data used for concentration runs and the NCEP-NCAR data used for calculating trajectories. More detailed HYSPLIT modelling focussing on specific geographical regions of interest could resolve these issues but was beyond the scope of the current project. Conversely, there were several scenarios where dispersal was reportedly possible based on trajectory results, yet no particles were detected from concentration runs, suggesting that trajectory runs may also sometimes overestimate the number of suitable days.

Finally, HYSPLIT has extensive advanced parameterisation options, for example, to modify the horizontal and vertical mixing parameters, compute the vertical velocity, adjust the time steps and Lagrangian time scales, compute the mixing layer depth and adjust for turbulence anisotropy. In particular, how much a plume will disperse, and thus the likelihood of multiple insects being deposited together, is strongly dependent on the mixing parameters, which we kept at default settings. Sensitivity analysis of these different parameters was beyond the scope of this project. Previous studies that utilised HYSPLIT to model windborne vector dispersal in the context of infectious disease spread also did not report empirically testing these advanced configuration parameters (García-Lastra et al., 2012; Eagles et al., 2014; Durr, Graham and van Klinken, 2017; Klausner, Fattal and Klement, 2017; EFSA Panel on Animal Health and Welfare et al., 2020).

Uncertainty: We considered this to be both uncertain and variable because of the lack of empirical data and the various limitations associated with the HYSPLIT modelling.

En_6 Vector survives dispersal and remains infectious

Vectors undergoing long-distance windborne dispersal will encounter many hazards that increase insect mortality, including low temperatures at higher altitudes, damaging winds and precipitation that can impair flight performance. Empirical data on insect survival during long-distance windborne dispersal is scarce. One study from Mali reported 30% survival of *Anopheles gambiae* mosquitoes in net- or cloth-covered tubes held at 250 metres above ground level for 11 hours (Sanogo et al., 2020). Wind speeds at this altitude reached 7–9 m/s (25–32 km/hr) during the study. While monthly wind speeds during the Australian summer monsoon average 3–4 m/s, they can sometimes be as high as 10–15 m/s (Suppiah et

al., 2011), which would further reduce insect survival. The study also did not assess time points beyond 11 hours (such as the 48 hours used for our analysis), so extrapolation beyond this is limited. Furthermore, in that study mosquitoes were able to rest on the surfaces of the tube, although the authors argued that buffeting against surfaces would result in lower survival in the tubes than during free flight in a wind stream. In contrast, Elbers et al. (2015) and Verdonschot et al. (2014) both considered that only a few specimens would survive long-distance windborne dispersal events. Accordingly, for our analysis we considered insect survival during windborne dispersal to be extremely low. Notably, this parameterisation for all vector categories may not be ideal, as mosquitoes are relatively fragile and heavy fliers more robust, for example. For windborne dispersal, we also did not consider whether environmental conditions at the arrival destination would be conducive to vector activity, since all arrival destinations fell within a reasonably narrow latitude band within one of two climate classifications (i.e. tropical savanna and semi-arid climates).

We modelled insect infectiousness as a function of time, deriving input data on the proportion of infectious insects at different days post-feeding from the peer-reviewed literature (Figure 25, Appendix 3) (Chihota et al., 2001; Issimov et al., 2021; Paslaru et al., 2022). Here, we restricted studies to those that demonstrated infectiousness by virus isolation (i.e. we excluded those studies only reporting qPCR results) because true infectivity is of key importance for this node. Because of the paucity of data, we were not able to generate individual models for each vector category. This model showed a general decline in infectiousness over time, although there was considerable discrepancy between individual studies (Appendix 3). Notably, very small numbers of vectors were tested, particularly at later time points, and outputs from this model should be interpreted cautiously. For example, Paslaru et. al. (2022) reported 2/2 midges positive for LSDV at 10 days post-feeding but 0/3 midges positive at 7 days post-feeding, demonstrating the challenges around interpreting results from small sample sizes. Further details are given in Appendix 3.

Of note, recent unpublished studies from the Pirbright Institute found that *Aedes* mosquitoes were capable of transmitting LSDV to recipient cattle four days after feeding on LSDV lesions on donor animals (P. Beard, pers. comm.)[11]. However, transmission from *Stomoxys* was only successful on the day of feeding, and not at 2 or 4 days post-feeding. There was no evidence for transmission from *Culicoides nubeculosus*, noting that *Culicoides* will not re-feed within four days. These results are in agreement with those previously reported by Chihota et al. (2001, 2003).

[11] Interim results that have generously been made available ahead of publication.

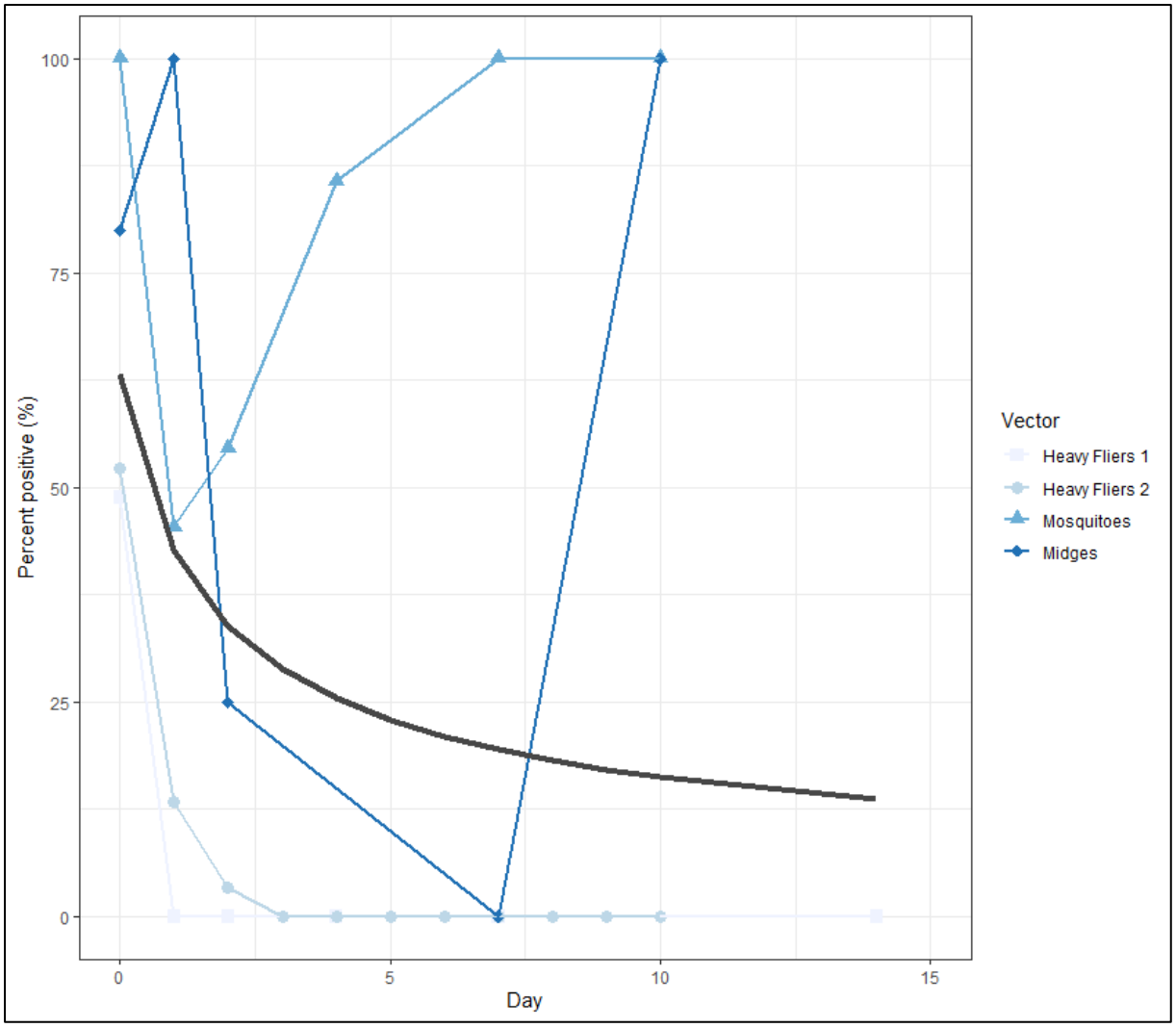

Figure 25 Non-linear least squares model of vector infectiousness over time for LSDV
The model (black line) was fitted using data from four studies (blue lines). Details in Appendix 3.

Uncertainty: We considered this to be uncertain and variable, due to the small sample sizes and artificial conditions in the experimental studies.

Ex_A Susceptible bovine present at arrival destination

After reaching Australia, any arriving vector must be capable of seeking out a host. The probability of reaching a susceptible host depends on the bovine density near the arrival site and the physiological condition of the arriving vector. As discussed above under En_5, mosquitoes, midges and heavy fliers can undertake short-distance self-directed flights of up to 5–13 km. However, we assumed that this range may be reduced after experiencing the harsh environmental conditions associated with windborne transport. Thus, we modelled the probability of a bovine being present within 1 km² of an arriving insect vector. Feral bovine densities (feral cattle, buffalo and Banteng) obtained from NAQS field surveys were added to domestic cattle densities, resulting in final bovine densities from 0–5.55 per km² (Figure 16).

Feral bovine density estimates from NAQS field surveys ranged from 0 per km² (in several NAQS risk zones) to 1.62 per km² in N6. This is broadly comparable to estimates determined from a 2014 survey in Arnhem Land (Saalfeld, 2014). In that study, the mean buffalo density was estimated to be 1.07 per km²; however, densities of 10–50 per km² were observed in some areas. Presumably, higher densities are seen around water points or other locations attractive to bovines. There are several limitations to the feral

bovine estimates used in this assessment. For example, multiple surveys were combined over several years, thus the same animal may have been counted multiple times (either within the same or across multiple NAQS risk zones) or may no longer be present in the population. Additionally, these numbers were not corrected for sampling/perception bias.

Uncertainty: We considered this to be variable since bovine density will not be homogeneous within arrival destinations. We also considered this to be uncertain because reliable fine-scale estimates of feral buffalo numbers are not available and because of the limitations of extrapolating densities between SA2s and NAQS risk zones.

Ex_B Vector(s) bite bovine

The minimum number of vectors necessary to transmit a minimum infectious dose of LSDV is not definitely known. Therefore, we assumed three distinct scenarios for our analyses:

1. At least 30–50 insects are necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors are necessary for transmission
3. A single insect is sufficient for transmission

We assumed that the probability of at least 30 vectors arriving simultaneously is extremely low (1×10^{-5}–1×10^{-3}) and that the probability of 3–5 vectors arriving together is very low (1×10^{-3}–0.05), relative to the probability of a single vector arriving.

We used expert opinion from entomologists to estimate the probability of a vector biting an available bovine (Table 2). Notably, despite only female mosquitoes and midges taking blood meals, one expert rated the maximum probability as 100% for all vector categories. Expert opinion varied and both noted that this was species-dependent within our vector categories. For example, *Aedes aegypti* are anthropophilic, so may be less likely to bite a bovine than other mosquito species. One expert also noted that the lifecycle of several arthropod species involves a gonotrophic cycle, where females are not always seeking a protein meal. We further assumed that a single insect would bite a single bovine and did not account for a single insect potentially transmitting to multiple animals, although not all insect species will take multiple successive blood meals.

Table 2 Probability of an arriving vector biting an available bovine

	Midges			Mosquitoes			Heavy fliers		
	Min.[1]	**ML[2]**	**Max.[3]**	**Min.[1]**	**ML[2]**	**Max.[3]**	**Min.[1]**	**ML[2]**	**Max.[3]**
Expert 1	0.25	0.33	0.33	0.25	0.33	0.33	0.25	0.62	1.0
Expert 2	0.1	0.5	1.0	0.1	0.5	1.0	0.1	0.5	1.0

[1] minimum; [2] most likely; [3] maximum

Uncertainty: We considered this to be variable and uncertain given the lack of empirical data and the discrepancies between the two expert opinions.

Ex_C Bovine is infected

We estimated the vector-to-bovine transmission rate for LSDV based on the peer-reviewed literature. We considered transmission as the development of clinical disease, since current evidence suggests that asymptomatic animals contribute minimally to LSDV transmission due to low viral loads in the blood and skin (Sanz-Bernardo et al., 2021). Notably, LSDV transmission experiments have all used large batches of insects (36–300 insects per batch), so these estimates are 'batch' transmission rates and not individual insect transmission rates (Weiss, 1968; Chihota et al., 2001, 2003; Magori-Cohen et al., 2012; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). Based on experimental transmission studies, the

minimum number of infectious insects thus far demonstrated to successfully transmit LSDV to recipient cattle is 36 horseflies (*Haematopota* spp.) (Sohier et al., 2019), 50 *Aedes* mosquitoes (Chihota et al., 2001) or >200 *Stomoxys* stable flies (Sohier et al., 2019; Issimov et al., 2020). However, recent unpublished studies from the Pirbright Institute found that as few as 14 *Aedes* mosquitoes could transmit LSDV to recipient cattle and *Aedes* mosquitoes remained capable of transmitting LSDV for up to 4 days post-feeding (P. Beard, pers. comm.)[12]. Transmission of LSDV from *Culicoides* midges has never been demonstrated experimentally; thus, we used a modelled transmission parameter for *Culicoides* (Sanz-Bernardo et al., 2021). Since stratified data were not available, we used these 'batch' transmission rates to inform all three of our scenarios (i.e. at least 30 vectors necessary, 3–5 vectors necessary and a single vector sufficient for LSDV transmission).

We modelled the vector-to-bovine transmission rate using a uniform distribution for each vector category, taking the minimum and maximum observed values (not those derived in the 95% confidence interval) from the literature (Figure 26).

Uncertainty: We considered this node to be variable and uncertain. Notably, as discussed above, data on the probability of a single insect transmitting LSDV are critically lacking.

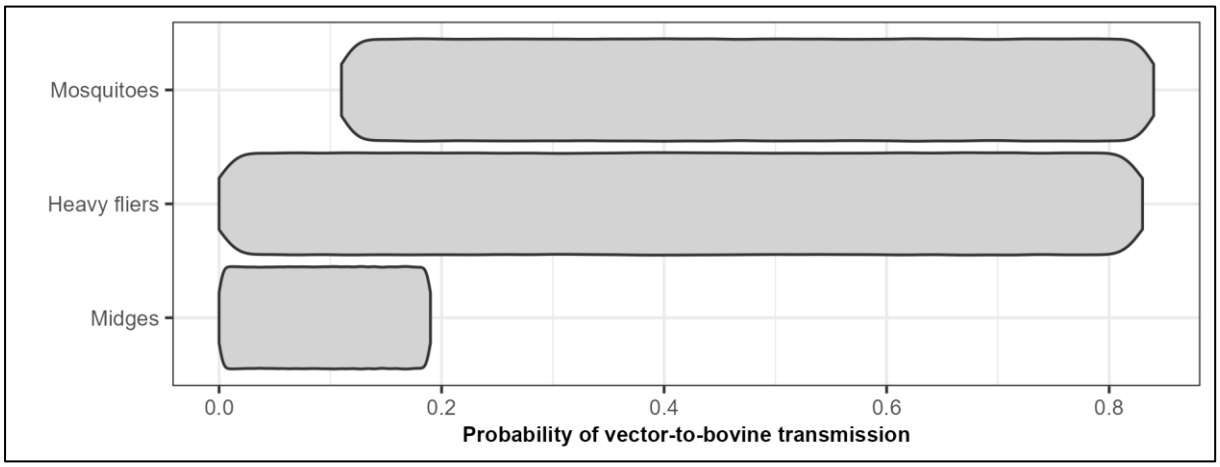

Figure 26 Range of parameter values for a bovine acquiring LSDV after being bitten by a 'batch' of infectious vectors

3.6.2 Commercial vessels (including returning live export vessels)

En_1 Number of bovines at origin

Across the relevant origin countries for the shipping pathways, bovine numbers ranged from 174 in Singapore (2020) to 304,201,366 in India (2016) (Figure 27). The methodology is described in Section 2.6.1.

Uncertainty: We considered this parameter to be variable (since livestock numbers may fluctuate) but not uncertain (since a curated data set from a reputable source is available).

[12] Interim results that have generously been made available ahead of publication.

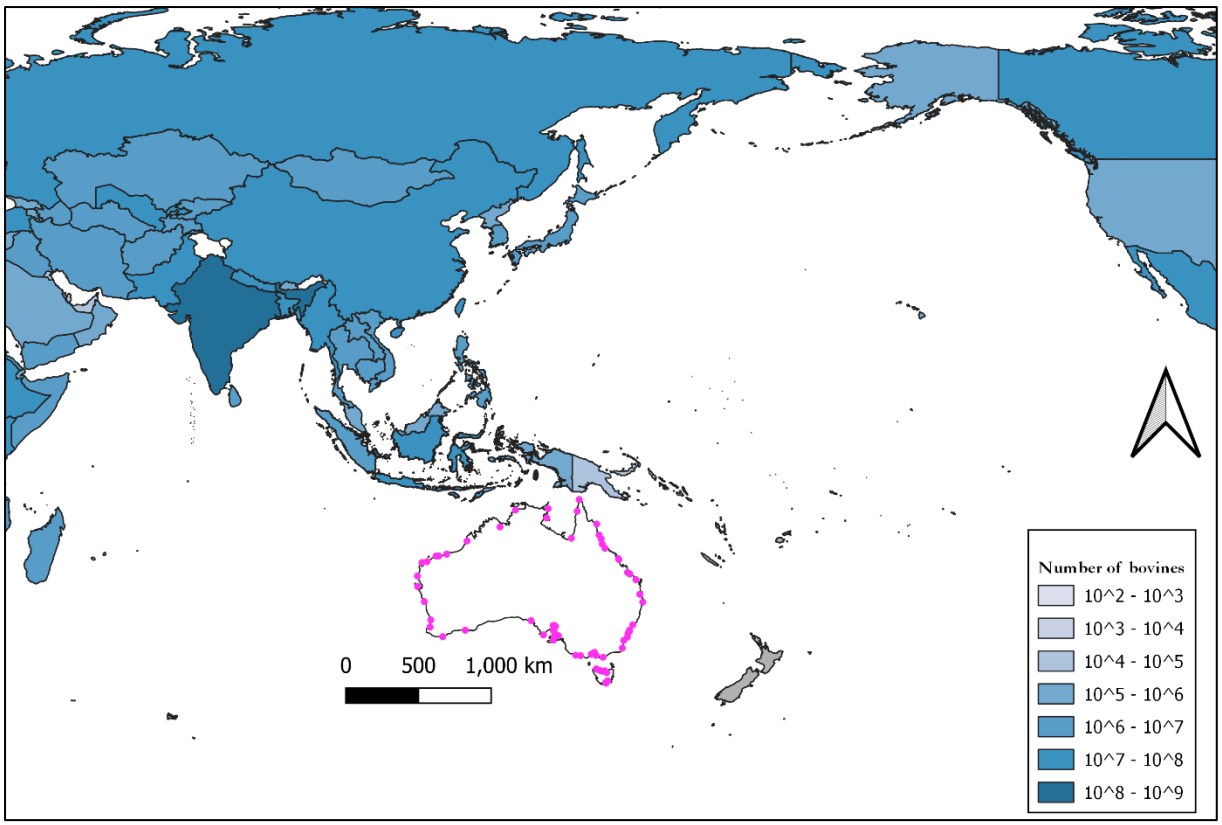

Figure 27 Map of origin countries coloured by number of bovines, 2020

This includes countries beyond 10 days' travel time from an Australian seaport, which were later excluded. Australian seaports are shown as pink dots. Excluded regions are shown in grey. Scalebar accuracy may be limited due to use of a geographic coordinate system.

En_2 Bovines infected with LSDV

This was parameterised as detailed for the windborne dispersal pathway.

En_3 Number of vectors biting each bovine per infectious day

This was parameterised as detailed for the windborne dispersal pathway.

En_4 Bovine-to-vector transmission

This was parameterised as detailed for the windborne dispersal pathway.

En_5 Vector flies to seaport

We assessed the probability of a vector flying to a seaport after feeding on a bovine as very low, given that most self-directed flights are over relatively short distances and are guided by visual, olfactory and thermal cues as described for En_5 in the windborne dispersal pathway (Service, 1997; Verdonschot and Besse-Lototskaya, 2014; Elbers, Koenraadt and Meiswinkel, 2015). Presumably, if a zoophilic vector is already in close proximity to bovines there would be little stimulus to travel to a seaport. Critically, this was not informed by empirical data. This qualitative assessment was converted to a semi-quantitative value (1×10^{-3}–0.05) using a previously reported risk assessment methodology (Biosecurity Australia, 2004) and the most likely value was scaled based on urbanisation index (Figure 28).

Uncertainty: Because of the lack of empirical data used to inform this parameter we considered it to be both variable and uncertain.

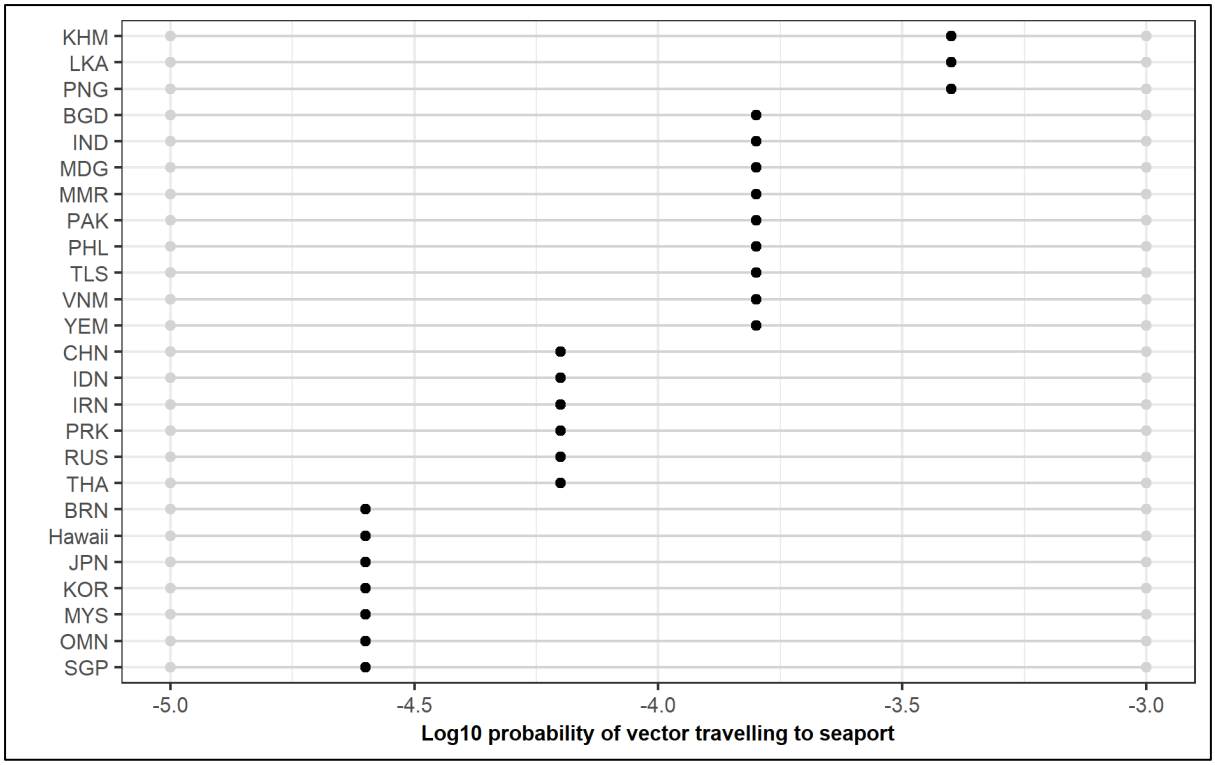

Figure 28 Minimum (left grey dot), most likely (black dot) and maximum (right grey dot) log_{10} probability of a vector travelling to a seaport by origin country, scaled by urbanisation index[13]

En_6 Vector lands on vessel

When seeking shelter or oviposition sites, insects may be attracted to ships by carbon dioxide and other volatiles emitted by both people and livestock, and by the lights of the vessel at night, depending on species-specific behaviours (NAQS & DAFF officers, pers. comm.). While arthropods are regularly found in insectocutors on ships arriving in Australian ports, it is thought that these are frequently of Australian origin and are attracted to the ships on approach to the port, rather than originating in the exporting country (NAQS & DAFF officers, pers. comm.). These traps are only turned on once in Australian waters and remain on while in port, thus preferentially sampling local coastal species (NAQS officer, pers. comm.). One study from New Zealand inspected 11,265 arriving container ships and detected a total of nine mosquitoes, 28 heavy fliers and zero *Culicoides* midges (Border Management Group, 2003). This supports the assertion that, overall, insects rarely enter ships, given the presumably very large source vector population (i.e. denominator). For this analysis, we were interested in the probability of a vector landing on a ship, not the probability of a ship having a vector on board. Critically, we do not know the denominator of number of insects in the vicinity of the port. In the absence of empirical data, we considered the likelihood of a vector landing on a vessel to be extremely low and assigned a semi-quantitative value (1×10^{-5}–1×10^{-3}) using the previously described risk assessment methodology (Biosecurity Australia, 2004).

Uncertainty: Because of the lack of empirical data used to inform this parameter we considered it to be both variable and uncertain.

[13] The list of ISO 3166-1 alpha-3 country codes is given in Appendix 4.

En_7 Vessel travels to Australian seaport

Commercial vessels excluding returning live export ships

Data on the number of journeys undertaken by ships between individual seaports globally are commercially sensitive and are not readily available. Therefore, we separated this parameter into the probability of an international vessel being destined for Australia and the probability of arrival at a specific Australian seaport.

Since the absolute number of ships was not available, for each origin country we used the proportion of export trade value to Australia of all export trade value to estimate the probability of a vessel being bound for Australia. We appreciate that the use of trade values rather than number of ships will bias our results. For example, where Australia receives high-value goods from certain countries, such as gold or oil, then the proportion of trade value to Australia will be high even if we receive few ships. Conversely, where Australia receives predominantly low value or bulky goods from countries the risk would be underestimated; we may receive many ships of these goods, but this would not be reflected in the export trade value data. The proportion of export trade value to Australia ranged from 7.3×10^{-4}% from Yemen in 2017 to 67.4% from Hawaii in 2015 (Figure 29).

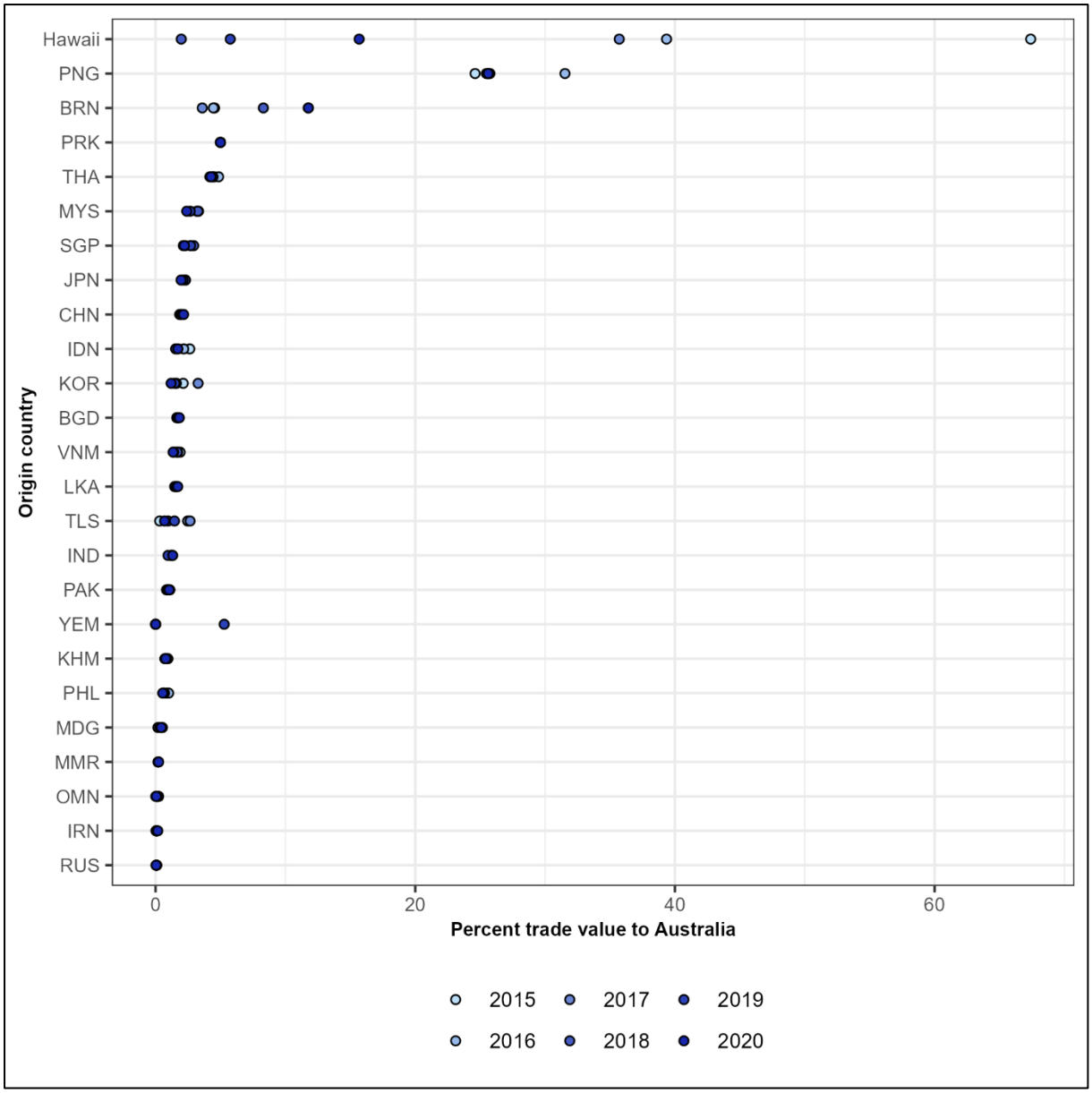

Figure 29 **Percentage of export trade value to Australia of all export trade value by origin country per year, 2015–2020[14]**

To estimate the probability of arrival at a specific Australian seaport, we calculated the proportion of port calls to each Australian seaport. This ranged from 0.0043 (i.e. 0.43%) for all ports except the top ten in 2017 to 0.1689 (i.e. 16.89%) for Port Hedland in 2018 (Figure 30).

[14] The list of ISO 3166-1 alpha-3 country codes is given in Appendix 4.

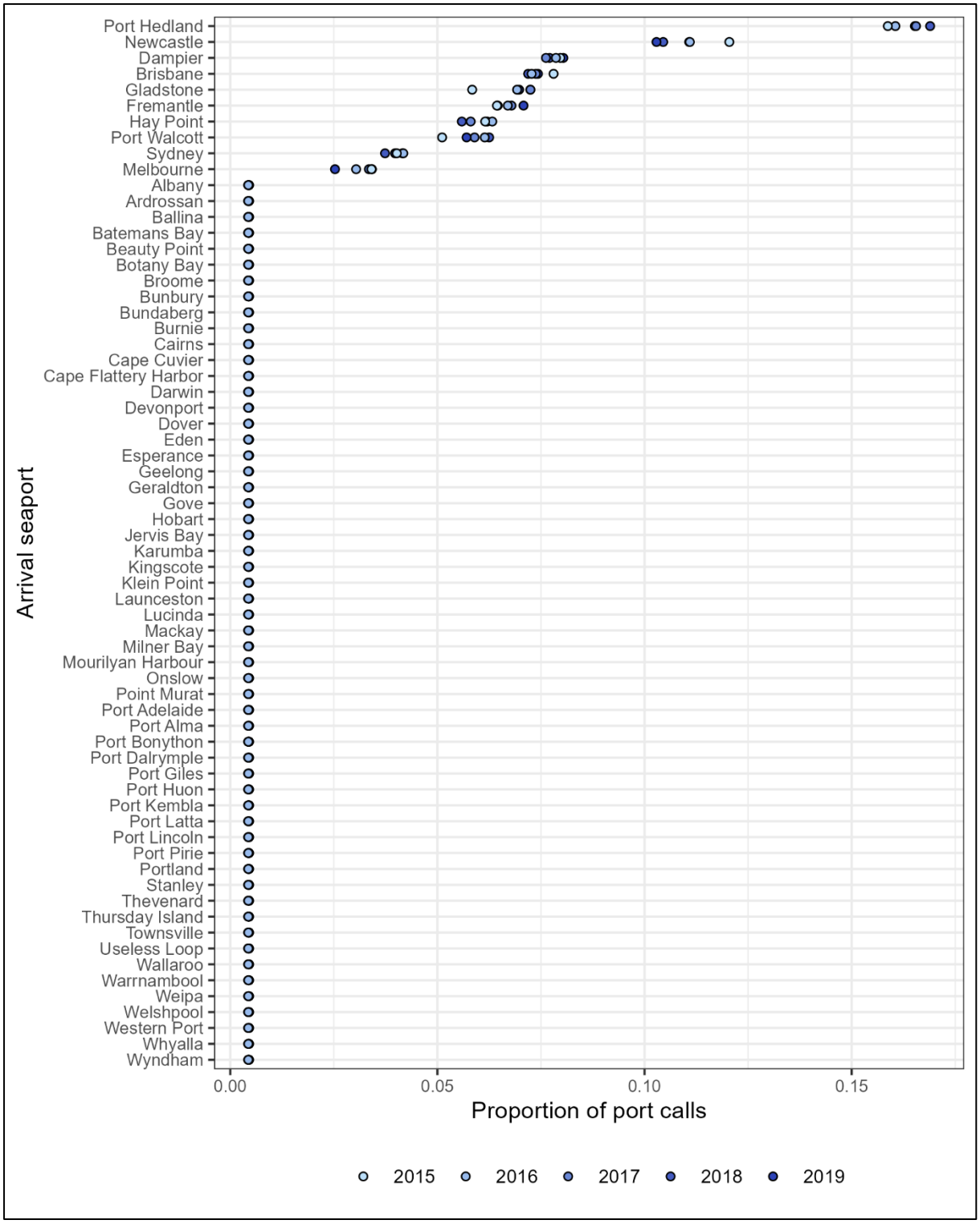

Figure 30 Proportion of port calls by Australian seaport per year, 2015–2019

Returning live export ships

We considered that live export data would reflect all entering livestock vessels, since Australia does not import livestock. Many ports don't receive returning live export ships; therefore, the probability of LSDV entry (via returning live export vessels) at many Australian seaports is zero. The Australian ports receiving returning live export vessels based on routes of less than 10 days' travel duration were Broome, Darwin, Fremantle, Geelong, Geraldton, Karumba, Port Alma, Port Hedland, Portland, Townsville and Wyndham. Many origin countries only received live export vessels from a single port each year (which varied by year). Probabilities for this node ranged from 0–100%, depending on route (Figure 31).

Since the number of vessels are not reported, we weren't able to calculate the proportion of vessels travelling along each route; instead, we used the proportion of animals transported along each route as a proxy.

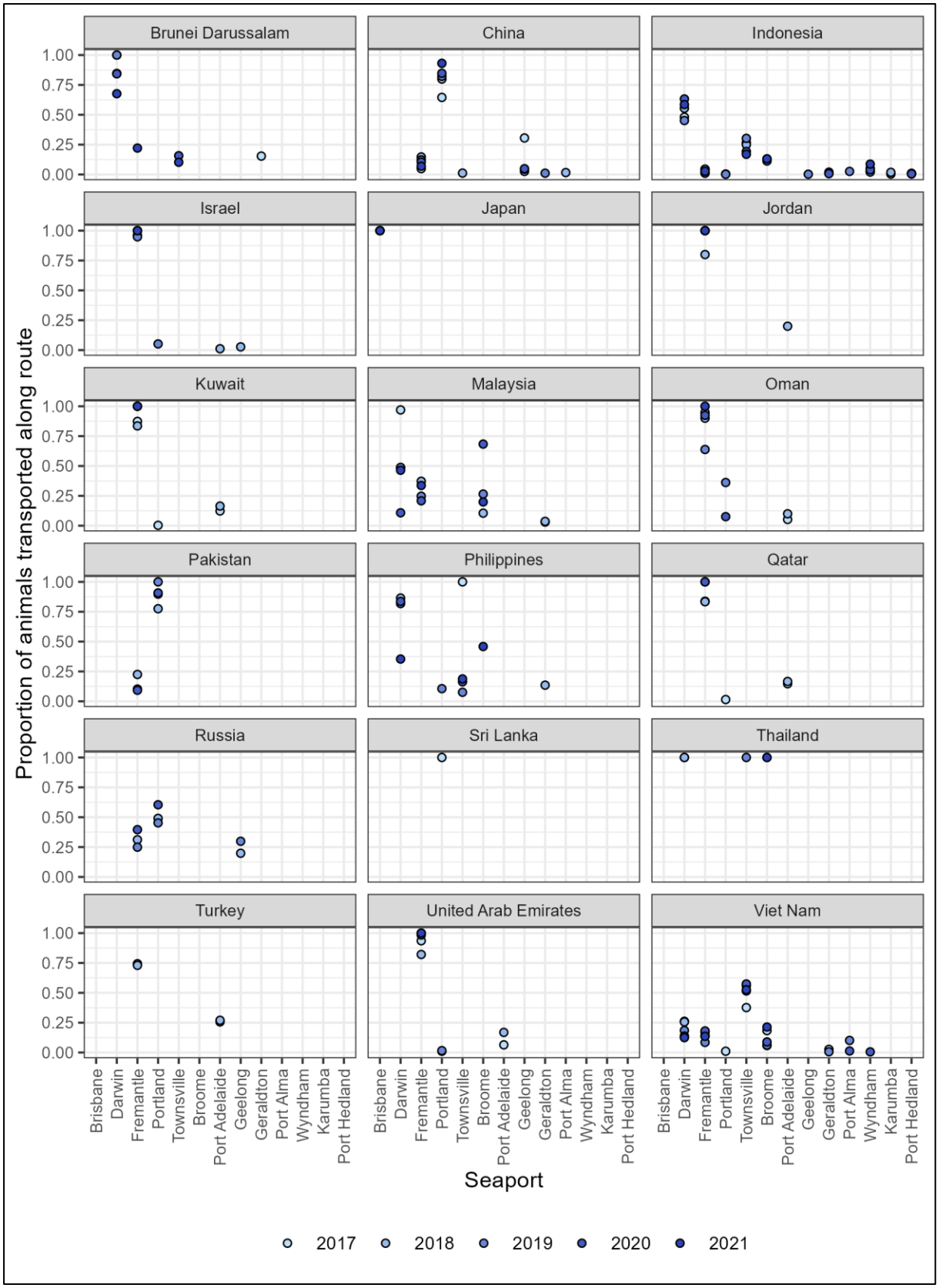

Figure 31 **Proportion of animals transported on live export vessels from Australian seaports by origin country per year, 2017–2021**

Uncertainty: This parameter was considered variable but not uncertain since curated data are available.

En_8 Vector survives transport and remains infectious

We could not find experimental data describing vector survival under typical conditions on commercial vessels. Based on the New Zealand study described above for En_5, of the relevant insects detected on commercial vessels, 44.4% of mosquitoes and 25% of heavy fliers were alive at the time of detection (Border Management Group, 2003). While no *Culicoides* midges were detected, six non-biting midges were found, of which two (33.3%) were found alive. Thus, we used these proportions to parameterise survival of our three vector categories. Importantly, this does not account for travel time, so may underestimate the survival from close destinations and may overestimate the survival from more distant destinations.

We modelled insect infectiousness as a function of time based on peer-reviewed laboratory experiments as described for the windborne dispersal pathway (Figure 25, Equation 1). That same function was used to parameterise this node.

Uncertainty: We considered this node to be both variable and uncertain, as discussed for En_6 of the windborne dispersal pathway.

En_9 Vector disembarks without detection

We used expert opinion from the Biosecurity Operations Division within the Department's Animal Biosecurity branch to estimate the probability that a vector would disembark a vessel given the current biosecurity mitigations in place. The experts from the Biosecurity Operations Division estimated the likelihood of not detecting a vector on board a vessel (excluding returning live export vessels) as very low, which was converted into a semi-quantitative likelihood (minimum 0.001, maximum 0.05) (Biosecurity Australia, 2004). They considered the risk to be lower for returning live export vessels compared to other commercial ships, given the additional mitigations employed. Therefore, we qualitatively assessed this node as extremely low for the returning live export vessels pathway, using minimum (1×10^{-5}) and maximum (1×10^{-3}) values.

Uncertainty: We considered this node to be variable and uncertain, given the lack of empirical data.

En_{10} Environmental conditions suitable for vector activity at arrival destination

In our qualitative assessment we noted that it was critical to consider the environmental conditions at the arrival destination to avoid artificially elevating the probability of LSDV incursion at some ports (such as Hobart and Launceston). Different vector species require different environmental conditions to undertake flight, feeding, oviposition, development and other physiological processes: mosquitoes are only active above 15°C (Reinhold, Lazzari and Lahondère, 2018), midges require temperatures above 18°C (Murray, 1987a) and heavy fliers are active above 14.4°C (Bailey and Meifert, 1973). While relative humidity, precipitation and other environmental factors also impact vector activity, we only considered temperature in our analysis. We estimated the proportion of days per year where the minimum daily temperature remained above that reported to be necessary for activity for the different vector categories (Figure 32).

Uncertainty: We considered this to be variable but not uncertain since reliable data were available.

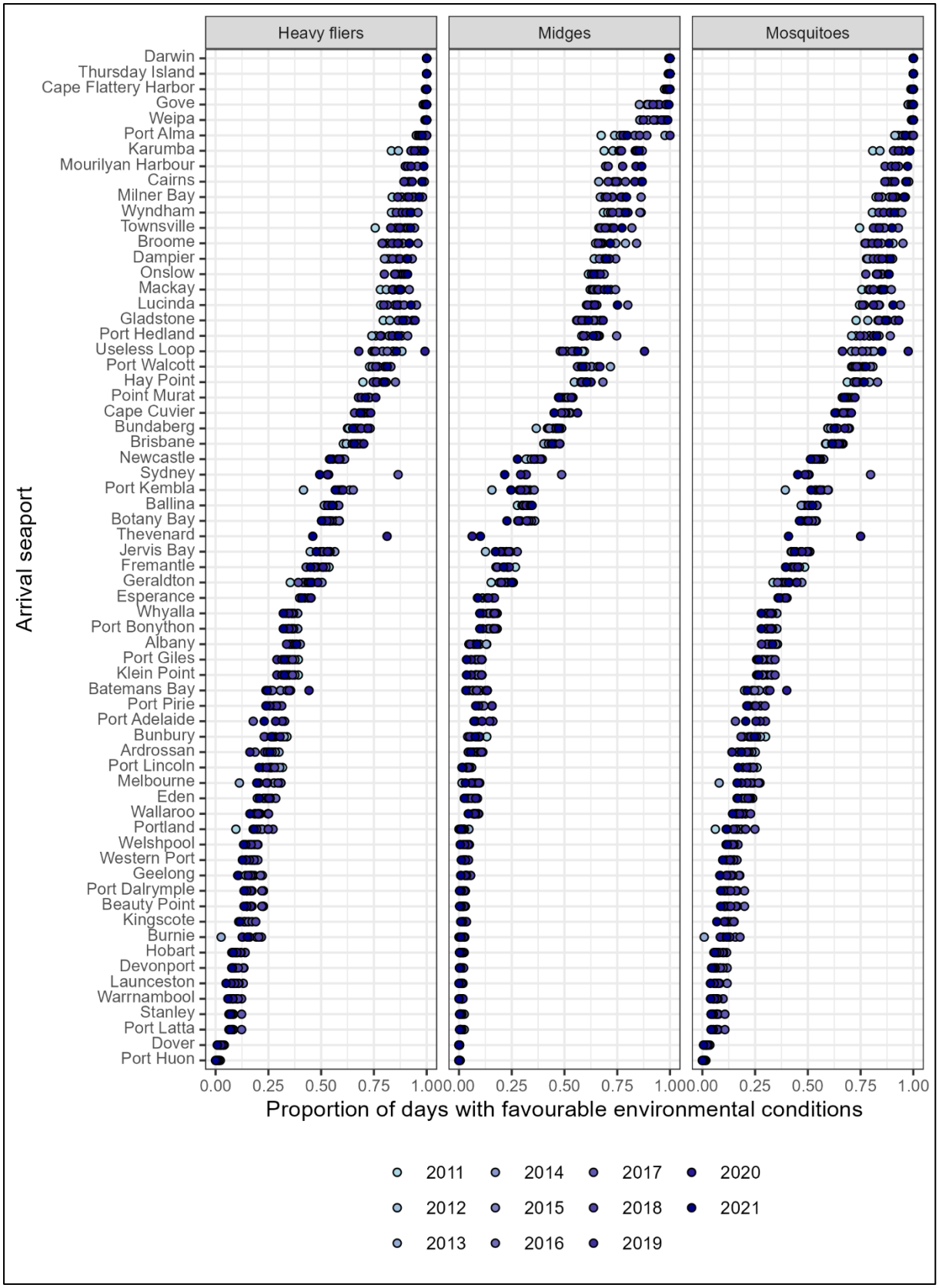

Figure 32 Proportion of days per year where environmental conditions were favourable for vector activity, by arrival seaport, 2011–2019

Ex_A Susceptible bovine present at arrival destination

We used SA4-level cattle densities to inform the availability of cattle around arrival seaports. We initially intended to use SA2 level densities for improved geographical resolution. However, when we interrogated the data, we found that many (24/66) seaports were located in SA2s that reportedly contained 0 cattle, including ports such as Gladstone, Mackay, Broome and Darwin. Since SA2s are based on human population density they can cover relatively small geographical areas in populated regions, yet susceptible cattle may still be present within flying distance of a vector. Thus, we considered SA4 level densities to be more appropriate. We did not include feral buffalo in our density calculations since buffalo are absent from regions where seaports are located (Australian Government Department of Sustainability, Environment, Water, Population and Communities, 2011; Saalfeld, 2014). Final cattle densities varied from 0 per km^2 in the major capital cities to 44.8 per km^2 around Portland and Warrnambool (in Southwest Victoria) (Figure 17).

Uncertainty: We considered this to be variable and uncertain due to the low geographic resolution of SA4 data.

Ex_B Vector(s) bite bovine

This was parameterised as detailed for the windborne dispersal pathway.

Ex_C Bovine is infected

This was parameterised as detailed for the windborne dispersal pathway.

3.6.3 Torres Strait Treaty movements

En_1 Number of bovines at origin

Livestock numbers in PNG were derived as described for the windborne dispersal pathway, ranging from 92,334 in 2020 to 92,905 in 2015 (Figure 18). The methodology is described in Section 2.6.1.

En_2 Bovines infected with LSDV

This was parameterised as detailed for the windborne dispersal pathway.

En_3 Number of vectors biting each bovine per infectious day

This was parameterised as detailed for the windborne dispersal pathway.

En_4 Bovine-to-vector transmission

This was parameterised as detailed for the windborne dispersal pathway.

En_5 Vector reaches vessel

This was parameterised as detailed for the commercial shipping pathway.

En_6 Vector survives transport

While typical travel times within the Torres Strait are short, most vessels making this journey are open (NAQS officer, pers. comm.). Hence, we assumed that insects would likely be blown out while vessels are motoring. However, because of the short travel times it is likely that any surviving vectors would still be infectious on arrival; thus, we didn't include infectivity in this pathway. Expert opinion from the NAQS Torres Strait and Field Operations team suggested that the probability of survival in these small open vessels would be low. We converted this to a semi-quantitative likelihood range (i.e. 5–30%) using the previously described risk assessment methodology (Biosecurity Australia, 2004). We did not consider environmental conditions at the arrival destination since all destinations are located within the Torres Strait with very similar climatic conditions.

Uncertainty: Because of the lack of empirical data used to inform this parameter we considered it to be both variable and uncertain.

En_7 Vector disembarks without detection

There is very limited intervention for goods on vessels travelling in the Torres Strait (NAQS officer, pers. comm.). Some, but not all, high-risk material for mosquitoes from the Torres Strait is flagged for intervention, but inspection is generally of limited value for detecting mosquitoes (NAQS officer, pers. comm.). The primary risk is transport of immature stages, which are not considered to be of relevance for LSDV transmission. Currently, a cordon sanitaire is in place around Thursday Island and Horn Island to prevent dispersal of *Aedes albopictus* to mainland Australia (Muzari et al., 2017). This approach involves harbourage spraying, source reduction, insecticide treatment of containers, placement of lethal tire piles, mosquito population monitoring and public awareness campaigns, although these measures will have only a limited effect on non-container-breeding mosquitoes (NAQS officer, pers. comm.). Other insect species are monitored in Thursday Island and Horn Island port environs, although this does not imply control or intervention, especially for non-container-breeding species (NAQS officer, pers. comm.). Expert opinion from the NAQS Torres Strait and Field Operations team assessed the probability of adult vectors disembarking from these vessels as moderate, as minimal specific interventions are in place. As for En_6, we converted this to a semi-quantitative likelihood range (30–70%) and used this to parameterise a uniform distribution.

Uncertainty: Because of the lack of empirical data used to inform this parameter we considered it to be both variable and uncertain.

Ex_A Susceptible bovine present at arrival destination

This was parameterised as detailed for the windborne dispersal pathway, except we did not include feral buffalo numbers since buffalo are reportedly absent in northern QLD (Australian Government Department of Sustainability, Environment, Water, Population and Communities, 2011). Critically, there are reportedly no bovines in any of the relevant NAQS risk zones for this pathway (i.e. Q1a, Q1b, Q2).

Uncertainty: This node was neither variable nor uncertain.

Ex_B Vector(s) bite bovine

This was parameterised as detailed for the windborne dispersal pathway.

Ex_C Bovine is infected

This was parameterised as detailed for the windborne dispersal pathway.

3.7 Quantitative risk estimate for each pathway

For this QRA, we estimated the number of LSDV incursions into Australia per year through four non-regulated pathways, assuming a situation where LSDV is endemic throughout Southeast Asia and PNG.

Each pathway comprised multiple combinations of origin country, arrival destination and vector category.

We defined an LSDV incursion as the clinical infection of a single animal, regardless of whether this animal transmitted onwards to other susceptible bovines. To estimate the overall probability of LSDV entry into Australia for each of the four pathways we summed the probability of entry across all combinations within each pathway. There were 516 independent combinations for the windborne dispersal pathway, 3,285 combinations for the commercial shipping excluding returning live export vessels pathway, 1,722 combinations for the returning live export vessels pathway and 9 combinations for the Torres Strait Treaty movements pathway.

Since we did not know the minimum number of vectors necessary to initiate an LSDV infection in a bovine, each pathway was assessed under three separate scenarios:

1. At least 30–50 insects necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors necessary for transmission
3. A single insect is sufficient for transmission

Based on our intermediate scenario where 3–5 vectors are necessary for successful transmission, we estimated the median number of LSDV incursions per year to be 0.002 per year (or one incursion every 403 years) via windborne dispersal, 8×10^{-4} per year (or one incursion every 1,229 years) via arrival of hitchhiker vectors on commercial vessels excluding returning live export ships and 2×10^{-4} per year (or one incursion every 4,899 years) via arrival of hitchhiker vectors on returning live export ships. Based on our analysis, no incursions are expected via Torres Strait Treaty movements. In other words, on average the incursion probability via all pathways was very low. Critically, while parameterising our assessment we encountered considerable uncertainty at multiple nodes, leading to exceptionally wide CIs around the estimated number of LSDV incursions each year. Our 95% CIs ranged from 6×10^{-6} to 0.15 per year via windborne dispersal, 3×10^{-6} to 0.057 per year via commercial vessels excluding returning live export ships and 6×10^{-7} to 0.0141 per year via returning live export ships. While we report the estimated median risks in this study, it is essential to bear in mind these very wide CIs when interpreting our results. Further research is required to constrain the uncertainty around these parameters to better understand the true risk of LSDV incursion into Australia via these pathways.

When we repeated our assessment assuming that an individual insect was sufficient to initiate an infection, the estimated LSDV incursion probability increased substantially to one incursion every 7–8 years via windborne dispersal (median 0.13 per year, 95% CI 4×10^{-4} to 5 per year), every 25 years via commercial vessels (median 0.04 per year, 95% CI 2×10^{-4} to 2 per year) and every 95 years via returning live export ships (median 0.01 per year, 95% CI 4×10^{-5} to 1 per year). Under the scenario that at least 30–50 insects are necessary for successful vector-to-bovine transmission of LSDV, the estimated incursion probability became negligible, reducing to one incursion every 20,706 years (median: 5×10^{-5} per year, 95% CI 1×10^{-7} to 0.003 per year) via windborne dispersal, every 62,807 years (median: 2×10^{-5} per year, 95% CI 5×10^{-8} to 0.001 per year) via commercial vessels excluding live export ships and one incursion every 247,871 years (median: 4×10^{-6} per year, 95% CI 1×10^{-8} to 3×10^{-4} per year) via returning live export vessels.

Under all scenarios, the highest risk combinations were windborne dispersal of midges from central Indonesia to NAQS risk zones N6, N8a, N8b, N7, N5 and N4. These risk zones cover the Tiwi Islands and the region east of Darwin up to and including the Cobourg Peninsula.

To further understand the comparative risks between origin countries, arrival destinations and vector categories, we stratified each pathway separately by these variables. While the absolute estimated number of LSDV incursions changed under the three different scenarios, the comparative risks remained unchanged. The list of ISO 3166-1 alpha-3 country codes for origin countries is given in Appendix 4.

Windborne dispersal

The highest probability of LSDV incursion for the windborne dispersal pathway was via midges, followed by heavy fliers and then mosquitoes (Figure 33). To identify which parameter/s drove this result, we looked at the nodes that were parameterised by vector category, namely: the number of vectors biting a bovine per infectious day (En_3), the probability of bovine-to-vector transmission (En_4), the probability of a vector biting a bovine (Ex_B) and the probability of vector-to-bovine transmission (Ex_C). Importantly, our long-distance aerial dispersal modelling did not distinguish between vector categories. For En_3, the number of midges feeding on a bovine per infectious day (0–5,000) greatly exceeded that of either mosquitoes (0–80) or heavy fliers (30–145), driving the observed ranking of vector categories (Figure 21).

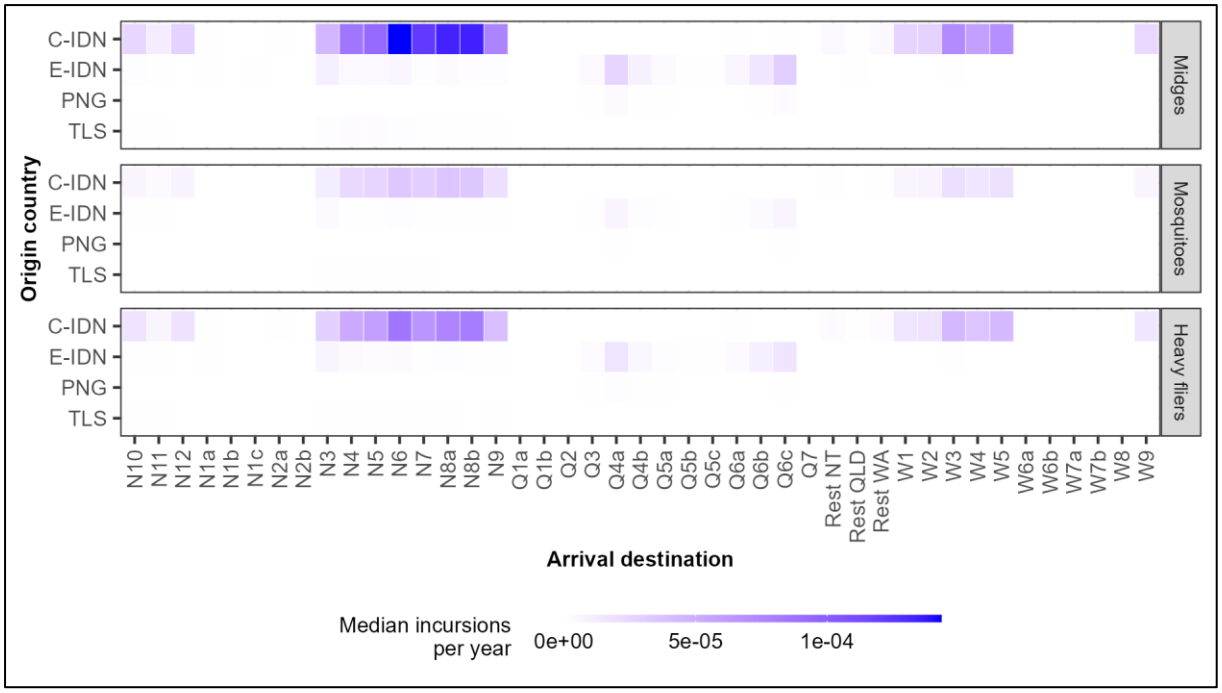

Figure 33 Median LSDV incursions per year into Australia by windborne dispersal of vectors, assuming several (i.e. 3–5) insects are necessary for LSDV transmission

The origin county of highest risk was central Indonesia, followed by eastern Indonesia, Timor-Leste and then PNG (Figure 33). The nodes that were parameterised by origin country were: the number of bovines at origin (En_1), the proportion of days suitable to disperse vectors to Australia and the proportion of vectors arriving (En_5) and the probability of a vector being infectious on arrival (En_6), which was based on distance. Central Indonesia has considerably more bovines than the other origin countries, which primarily drove the observed rankings (Figure 18). PNG had the highest proportion of wind trajectories suitable for windborne dispersal, especially to NAQS zones within the Torres Strait (Figure 22); however, the low number of bovines in PNG and in the NAQS zones within the Torres Strait (comprising the relevant arrival destinations) contributed to the low overall incursion risk. Like PNG, eastern Indonesia also had a substantial number of suitable days for windborne dispersal. Dispersal was possible from eastern Indonesia to most NAQS risk zones apart from those along the western coastline, although the highest risk arrival zones were in Queensland (Figure 33). Timor-Leste generally had fewer days suitable for windborne dispersal to Australia; additionally, typically only a limited proportion of released particles from Timor-Leste made landfall in Australia (Figure 22, Figure 24). These factors both contributed to the overall low potential for windborne incursions from Timor-Leste.

When aggregated by vector species and origin country, the highest risk arrival destinations were the Tiwi Islands and regions around the Van Diemen Gulf and Cobourg Peninsula east of Darwin (i.e. NAQS risk zones N6, N8b, N8a, N7, N5 and N4) (Figure 33). These risk zones had the highest cumulative probability of the proportion of days suitable for windborne dispersal and the probability of particles arriving from central Indonesia; since the probability of incursion from central Indonesia was relatively high due to the high bovine numbers there, these arrival destinations were also high.

Commercial shipping excluding returning live export vessels

As for the windborne dispersal pathway, our analysis identified midges as the highest risk vector category for LSDV incursion, followed by heavy fliers and then mosquitoes (Figure 34). Again, the disproportionate number of midges feeding on bovines drove this comparative risk.

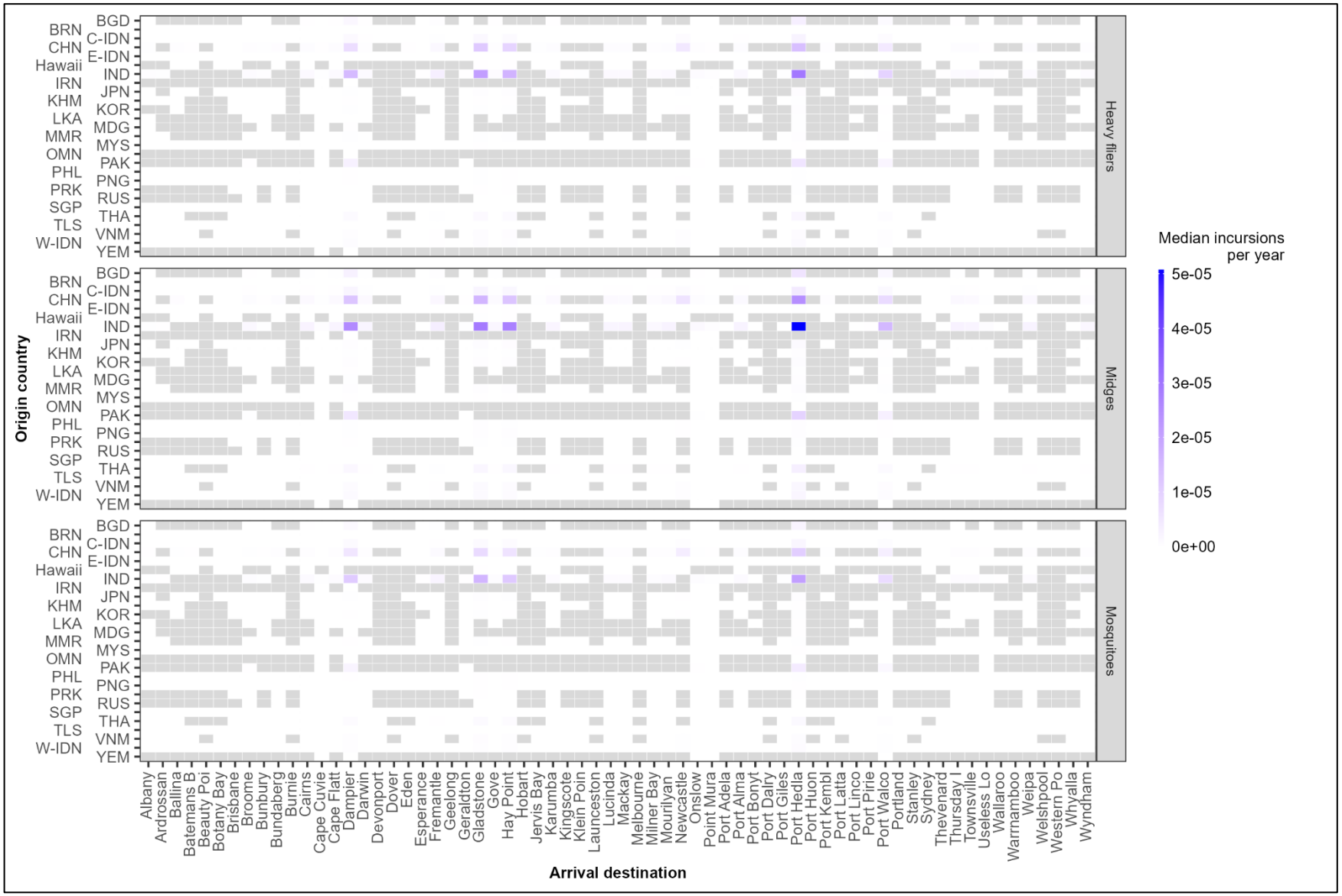

Figure 34 Median LSDV incursions per year into Australia by hitchhiker vectors on commercial ships (excluding returning live export vessels), assuming several (i.e. 3–5) insects are necessary for LSDV transmission

Grey boxes represent excluded scenarios (i.e. >10 days' travel time between the origin country and arrival destination). Arrival destination names have been truncated for visualisation purposes. The list of ISO 3166-1 alpha-3 country codes is given in Appendix 4.

The origin countries with the highest probability for LSDV incursion into Australia via commercial shipping (excluding returning live export vessels) were India and China (Figure 34). Notably, travel from these origin countries was only possible to certain Australian seaports within the 10-day maximum duration assumed in this assessment (shown as non-grey squares in Figure 34). Ships from Singapore, Oman, Brunei Darussalam, Iran, Yemen, Timor-Leste, the Russian Federation, Sri Lanka, Hawaii, Madagascar and eastern Indonesia had an exceptionally low median cumulative risk of LSDV incursion. The nodes that depended on origin-country-specific parameterisation were: the number of bovines in the origin country (En_1), the probability of a vector flying to a seaport, which depended on urbanisation index (En_5), the probability of a vessel travelling to an Australian seaport (En_7) and the probability of a vector being infectious on arrival (En_8). India, Pakistan and China had the highest bovine numbers (Figure 27), although vector survival and infectiousness from Pakistan was lower due to the longer travel duration (Figure 14).

By arrival seaport, Brisbane, Sydney, Botany Bay, Melbourne and Port Adelaide had zero risk of LSDV incursion due to the absence of cattle from the SA4 regions in which these ports were located. The highest risk seaport was Port Hedland, followed by Gladstone, Dampier, Hay Point and Port Walcott (Figure 34). These ports had a high proportion of port calls in addition to having a high proportion of days with suitable environmental conditions for vector activity (Figure 32).

Returning live export vessels

Again, midges were the highest risk vector category because of the large number of midges that could potentially feed on a bovine per day relative to other vectors (Figure 35).

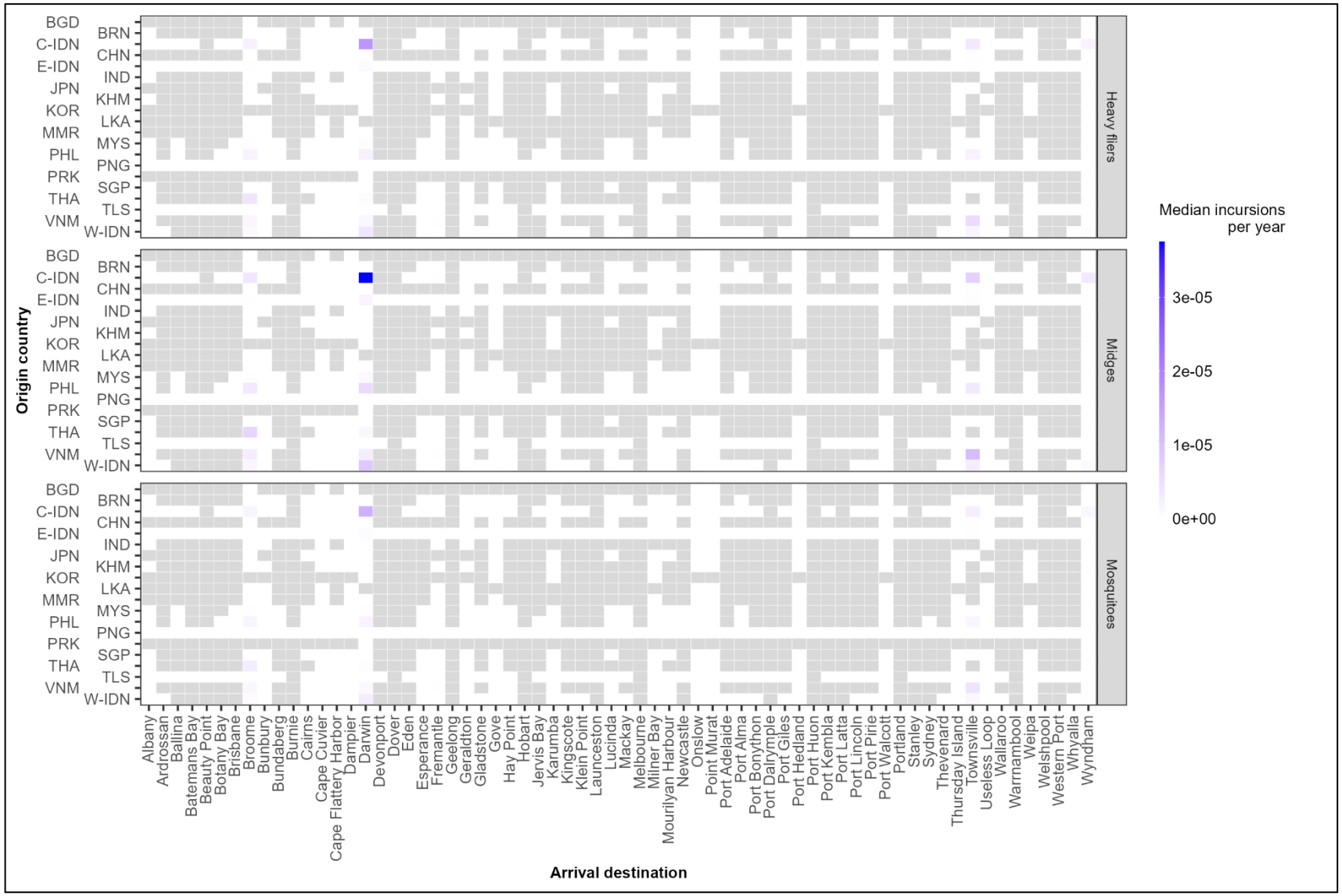

Figure 35 Median LSDV incursions per year into Australia by hitchhiker vectors on returning live export vessels, assuming several (i.e. 3–5) insects are necessary for LSDV transmission

Grey boxes represent excluded scenarios (i.e. >10 days' travel time between origin country and arrival destination). The list of ISO 3166-1 alpha-3 country codes is given in Appendix 4.

For returning live export vessels, the origin countries of highest risk were central Indonesia, Vietnam, western Indonesia, the Philippines and Thailand. These countries had relatively high livestock numbers (Figure 27), traded with those Australian seaports that were within 10 days' travel time (Figure 15) and had environmental conditions generally favourable for vector activity (Figure 32). Notably, Australia only exports livestock along a limited number of routes (shown as non-grey squares in Figure 35), so the probability of LSDV entry via returning live export vessels for many of the origin countries considered in this assessment was zero.

The highest risk arrival seaports were Darwin, Townsville and Broome. These are the three most heavily trafficked Australia seaports by live export vessels (Australian Government Department of Agriculture, Water and the Environment, 2022).

Torres Strait Treaty movements

Due to there being no bovines in the potential arrival destinations considered in this pathway (i.e. NAQS risk zones Q1a, Q1b, Q2) the probability of LSDV entry for each scenario, and therefore the cumulative entry probability, was zero.

3.8 Sensitivity analysis

Based on our global sensitivity analysis, the most influential nodes in our windborne dispersal model were: the number of vectors feeding on a bovine per infectious day (En_3), the probability of a vector being transported to Australia by wind ($En_{5.1, 5.2, 5.3}$), the probability of a vector surviving windborne dispersal ($En_{6.2}$), the probability of bovine-to-vector transmission (En_4), the number of insects necessary to initiate an infection ($Ex_{B.1}$) and the probability of vector-to-bovine transmission (Ex_C) (Appendix 5). For both shipping pathways, the most influential nodes based on the scenarios examined were: the probability of a vector flying to a seaport (En_5), the probability of a vector landing on a vessel (En_6), the probability of a vector disembarking without detection (En_9), the number of vectors feeding on a bovine per infectious day (En_3), the probability of bovine-to-vector transmission (En_4), the number of insects necessary to initiate an infection ($Ex_{B.1}$) and the probability of vector-to-bovine transmission (Ex_C). Sensitivity analyses could not be conducted for the Torres Strait Treaty movements pathway since the probability of incursion was zero for all scenarios. Importantly, En_3, En_4, Ex_B and Ex_C were highly influential nodes across all the pathways analysed.

Additionally, we conducted a comparison of the higher resolution GDAS meteorological data set with the NCEP-NCAR Reanalysis 1 data. The proportion of suitable days for windborne dispersal was significantly higher when using NCEP-NCAR meteorological data compared to GDAS data (p = <0.00001, two-sided Wilcoxon signed-rank test). Thus, our use of the NCEP-NCAR model may be conservative and overestimate the probability of windborne incursion of LSDV vectors into Australia. This contrasts with Eagles et al. (2013) who found no significant difference between the two meteorological models for the period December 2007 to March 2008.

3.9 Potential impacts of climate change

To assess how climate-change-driven variation in wind conditions may impact dispersal of LSDV vectors to Australia, we compared the proportion of trajectories suitable for windborne dispersal to Australia under pre-2000s and current climate conditions. The median proportion of suitable trajectories across all arrival destinations generally increased in the 2010s (2017–2021) compared to the 1980s (1985–1989). This trend was seen for central and eastern Indonesia and PNG (Figure 36). However, this increase was only statistically significant for PNG (p = 0.032, Wilcoxon rank sum test). Overall, this may suggest a general trend towards increased risk of windborne incursion over time.

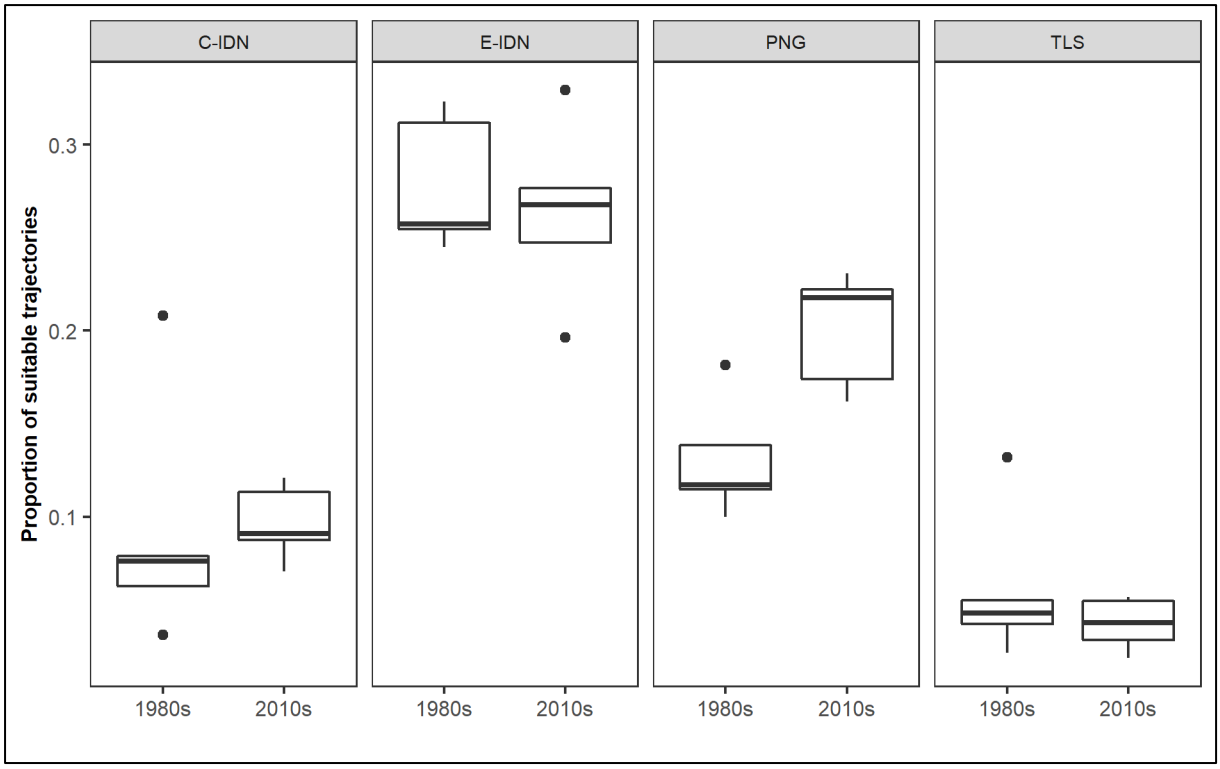

Figure 36 Proportion of trajectories suitable for windborne dispersal to Australia by origin country, 1985–1989 and 2017–2021

We additionally conducted local sensitivity analysis for those parameters that we anticipated may change in a future climate scenario based on the current scientific literature. Substantial future warming is projected with very high confidence for northern Australia. Mean warming is predicted to increase by 0.5–1.3°C above 1986–2005 levels, and maximum and minimum temperatures are also projected to increase (Suppiah et al., 2011; Moise et al., 2015). Environmental temperatures were considered in our shipping scenarios in node En_{10}. Through our global sensitivity analyses, we found that neither shipping pathway was particularly sensitive to this node. When we increased the minimum daily temperature by 1.3°C the median number of LSDV incursions by the commercial shipping pathway increased by around 2.5 times, from 8×10^{-4} per year to 2×10^{-3} per year. For the returning live export pathway the median number of incursions increased from 2×10^{-4} per year to 5×10^{-4} per year. Notably, we did not consider maximum temperatures in this analysis, where an increase may in fact limit vector survival. We also did not consider other environmental variables such as rainfall or relative humidity, as these are not parameterised in our model and the projected changes through the impacts of climate change are less certain than the temperature changes.

The potential future changes to international shipping were also investigated. As discussed in the introduction, more frequent and extreme weather events are likely to delay ships, reducing the risk of LSDV incursion. We did not specifically model this scenario. Overall, the demand for international shipping is expected to continue to grow into the future, independent of the impacts of climate change (Kuhn and Beaufoy, 2009). This will likely increase the volume of shipping travel to Australia. Our global sensitivity analysis showed that our commercial shipping model was not sensitive to node En_7 (the probability of a vessel travelling to an Australian seaport) (Appendix 5). When we conducted a local sensitivity analysis whereby we increased the probability of a ship travelling to Australia by 50%, the median risk of an LSDV incursion increased from an estimated median 8×10^{-4} incursions per year to 1×10^{-3} incursions per year. Critically, this node is defined as the probability of an individual ship travelling to Australia, not the absolute volume of sea traffic to Australia. Thus, if sea traffic increases equally across

all importing countries this probability, and therefore the risk estimate, will not change. Furthermore, we do not know how realistic our choice of a 50% increase in traffic specifically to Australia is. Importantly, shipping is also likely to become more expensive until a workable global solution to reducing carbon emissions is established (Kuhn and Beaufoy, 2009). This could lead to reduced demand for international freight if local alternatives are available.

3.10 General limitations and assumptions

Many of the assumptions and limitations around individual nodes have been discussed in detail above. Here, we consider some of the broader limitations and assumptions of our assessment.

For this risk assessment, we assumed the consequences of entry and establishment of LSD in Australia to be extreme, due to its marked impacts on production and trade; therefore, we did not conduct a specific consequence assessment.

We conducted this assessment based on a future hypothetical scenario of LSDV being endemic throughout Southeast Asia and PNG. As of October 2022, reports of LSDV outbreaks in Australia's northern neighbours were restricted to Sumatra and Java Islands, Indonesia, while other areas of Indonesia, Timor-Leste and PNG currently remain free of LSDV to our knowledge. The probability of LSDV arriving from a country or region that is free from the disease is clearly negligible; however, we conducted this analysis to inform surveillance and mitigation strategies in the event of LSDV being widespread throughout our northern neighbours. Furthermore, we assumed that LSDV may spread in this area before being officially declared. The main risk pathways for long-distance spread of LSDV are widely considered to be informal movements of infected live animals with subsequent short-distance dispersal by arthropod vectors (Tuppurainen and Oura, 2012); notably, most livestock movements through Indonesia occur when livestock are transported from outer regions to Jakarta (M. Patching, Boralis group, pers. comm.). This would presumably slow the spread of LSDV to our closer neighbouring regions, such as Timor-Leste and PNG; however, we assume that these areas will be affected in time (Roche et al., 2020). For example, classical swine fever virus spread from Malaysia down to Sumatra, Java, Kalimantan and other central Indonesia islands, and then into Timor-Leste and PNG, between 1994 and 2007 (Sawford, 2015).

We classified points of origin at the country level, which arguably may not provide appropriate geographic resolution. Parameters such as livestock density in the country of origin (En_1), LSDV incidence (En_2), vector abundance (En_3), likelihood of vectors flying to a seaport (En_5) and likelihood of a vessel travelling to an Australian seaport (En_7) will vary considerably between regions within a country. For all pathways, LSDV incidence may vary depending on local environmental conditions (lowland vs highland, rainfall, humidity, proximity to vector breeding sites), farming systems and biosecurity, availability of veterinary services and other factors (Ochwo et al., 2019). For the shipping pathways, some ports are likely to be in closer proximity to cattle and are therefore likely to have a higher vector abundance; ports with fewer ships may have a higher likelihood of vectors landing on a specific ship; transport to Australia will be more likely from certain ports. Similarly, for windborne dispersal, the risk from more distant regions within an origin country may be lower than from the closest land border and airflow is also known to be more turbulent over land, likely increasing deposition (Gloster et al., 2008). We also assumed homogeneous distribution of bovines throughout origin countries; if bovines are overdispersed away from seaports and coastlines then the incursion risks reported here will be overestimated. More detailed analyses at higher spatial resolution should be conducted for specific origin regions and subregions of interest.

Likewise, we didn't consider temporal factors in our analysis. Vector abundance, especially, will vary both on diurnal and seasonal scales, which will likely impact the number of vectors that feed on a bovine daily

(En_3). Critically, our model was highly sensitive to this node. LSDV incidence, and therefore the number of infectious bovines available to be fed on, will also vary seasonally (Tuppurainen and Oura, 2012). Bovine distribution may vary seasonally, for example, due to congregation around watering points in the drier months or due to localised flooding during the wet season. We estimated the overall incursion risk over an entire year, rather than identifying high-risk times for each pathway. Additional detailed analyses at finer temporal resolution may help to identify specific high-risk periods to better inform targeted surveillance strategies.

We considered an LSDV incursion to be the clinical infection of a single Australian bovine. This didn't consider whether onward transmission occurred from this animal or whether infection was detected. A study based on the LSDV outbreak in Albania in 2016 estimated a median basic reproduction number (R_0) across all affected villages of greater than 1 in only 35% of villages, suggesting that disease would fade out in most introductions (European Food Safety et al., 2019). Simulation modelling using the Australian Animal Disease Spread Modelling framework modified for LSDV may shed additional light on the likelihood of onward transmission and outbreak sizes resulting from an incursion.

We made several key assumptions about vector categories at various nodes that, if incorrect, would lead to a negligible risk for these categories. For example, transmission of LSDV has never been demonstrated from *Culicoides* to bovines. If the modelled parameter that we used for Ex_C is incorrect, then *Culicoides* may not present a risk in the context of LSDV incursion. Additionally, *Stomoxys* may not enter high-altitude wind streams at all; thus, our estimated risk for windborne dispersal of *Stomoxys* may be overestimated. Finally, only *Aedes* mosquitoes have been demonstrated to transmit LSDV to bovines with a delay in re-feeding; transmission from *Stomoxys* was only successful on the day of feeding, and not at 2 or 4 days post-feeding (P. Beard, pers. comm.)[15]. We parameterised our model of infectiousness over time on virus isolation studies, which may overestimate the probability of infectiousness to bovines, particularly for midges and heavy fliers.

[15] Interim results that have generously been made available ahead of publication.

4 Conclusions

We previously estimated the probability of LSDV incursion into Australia via infectious arthropod vectors through four non-regulated pathways to be negligible using a qualitative risk assessment framework, but noted that this assessment did not account for the volume of vector movements into Australia (Zalcman, Hall and Cowled, 2022). Critically, even a negligible risk can become significant when multiplied by a large entry volume. Furthermore, we identified several limitations to our qualitative analysis that made it prudent to additionally undertake a QRA where we could incorporate uncertainty and variability. For each pathway we defined a scenario tree comprising successive nodes, each of which must occur for the incursion of LSDV into Australia. We did not assess the consequences of entry, as these were assumed to be extreme. Since we did not know the minimum number of vectors necessary to initiate an LSDV infection in a bovine, each pathway was assessed under three separate scenarios:

1. At least 30–50 insects necessary for successful vector-to-bovine transmission of LSDV
2. Several (i.e. 3–5) vectors necessary for transmission
3. A single insect is sufficient for transmission

Each pathway comprised multiple combinations of origin country, arrival destination and vector category. Overall, there were 5,532 individual combinations assessed across the four pathways.

For every node within each scenario tree we defined a probability distribution parameterised depending on origin country, destination and/or vector category. Parameter values were obtained from the peer-reviewed literature, grey literature and expert opinion. Two-dimensional Monte Carlo simulation was used to compute the output probability distribution (representing the estimated number of LSDV incursions into Australia per year) for each combination within each pathway, where an incursion was defined as a single Australian bovine becoming clinically infected. The estimated risks for each scenario were summed to obtain an overall risk for each pathway.

4.1 Key findings

The key finding from this QRA was the marked uncertainty associated with many of the parameter values used in the analysis. This resulted in an extremely wide CIs around the estimated number of possible LSDV incursions into Australia. Noting this uncertainty, the estimated median risk of LSDV incursion into Australia via our three scenarios was:

1. One incursion every 14,652 years if at least 30–50 vectors are necessary for LSDV transmission (or 7×10^{-5} entries per year; 95% CI of 2×10^{-7} to 0.004 per year)
2. One incursion every 286 years if multiple, fewer vectors are necessary (median: 0.003 entries per year, 95% CI 9×10^{-6} to 0.22 per year)
3. One incursion every 5–6 years if a single insect is sufficient (median: 0.18 entries per year, 95% CI 6×10^{-4} to 8 per year)

In our previous qualitative risk assessment, the probability of LSDV entry into Australia via all pathways was negligible, which we defined in that assessment as a 0 to 1×10^{-5} likelihood of occurrence, without considering temporal factors. This is slightly lower than the most conservative scenario in our QRA, where many vectors (i.e. 30–50) are necessary for LSDV transmission. As mentioned in our qualitative report, even a negligible risk can become possible when insect numbers are high.

Current evidence suggests that multiple infectious vectors are required to initiate an LSDV infection in a bovine; however, the minimum number required to transmit infection is not conclusively known and we cannot definitively state that a single insect cannot transmit LSDV under the right circumstances. Given

the many highly uncertain parameters, it is critical to emphasize that undue attention should not be focused on the actual number of incursions estimated in this QRA. The utility of this assessment is principally in identifying the comparative risks between different origin countries, arrival destinations and vector categories to better prioritise surveillance and mitigation efforts. Furthermore, our assessment identifies key critical research priorities that must be addressed to more accurately estimate the true risk of LSDV incursion into Australia.

The overall highest risk pathway was windborne dispersal of infectious vectors, which was possible from central or eastern Indonesia, Timor-Leste or PNG between October and May, assuming a maximum dispersal time of 48 hours. This is generally in agreement with previous studies (Eagles et al., 2013, 2014). The NAQS risk zones incorporating the Tiwi Islands and regions east of Darwin extending to and including the Cobourg Peninsula (i.e. N4, N5, N6, N7, N8a and N8b) were associated with the highest incursion likelihoods and should be prioritised for future surveillance, particularly during the high-risk months of December to March. Furthermore, our modelling identified that the probability of LSDV incursion may be increasing over time due to the impacts of climate change.

For the windborne dispersal pathway and both shipping pathways, midges were assessed as posing a higher risk of LSDV incursion than the other vector categories. However, this was driven strongly by the highly uncertain node En_3, the number of vectors feeding on a bovine, where the number of midges feeding daily greatly outnumbered both heavy fliers and mosquitoes. Multiplied over the several days that a bovine remains infectious with LSDV, this leads to vastly more midges being potentially infectious than other vector categories. Because of the uncertainty associated with this parameter this finding should be interpreted cautiously. Additionally, transmission from *Culicoides* to bovines has never been demonstrated experimentally.

Interestingly, we estimated a comparatively higher risk of LSDV incursion via heavy fliers compared to mosquitoes. Yet, while detections of exotic midges and mosquitoes are not infrequently reported in northern Australia, there are no such reports of the incursion of exotic larger flies (G. Bellis, Charles Darwin University, pers. comm.). Like LSDV, Surra (caused by *Trypanosoma evansi*) is also transmitted by biting flies; while Surra is endemic in Southeast Asia and PNG it has not yet been detected in Australia. Additionally, several species of heavy fliers, such as *Hippobosca* sp. and *Chrysomya bezziana* (old-world screwworm fly), have remained restricted to Southeast Asia and/or PNG without dispersing to Australia (G. Bellis, Charles Darwin University, pers. comm.). This suggests that our QRA may have overestimated the risk posed by heavy fliers, likely because our atmospheric dispersion modelling simulates dispersal of particles without considering insect-specific parameters (such as deposition rates or weight). Thus, the probability of windborne dispersal was assumed to be equal between midges, mosquitoes and heavy fliers, likely overestimating the risk for heavy fliers. This is a key priority area for future research.

In our assessment, the transport of hitchhiker vectors on commercial vessels, including returning live export vessels, was strongly correlated with the number of livestock in the country of origin. Importantly, we assumed homogeneous distribution of bovines throughout origin countries; if bovines are more densely populated away from seaports and coastlines then the incursion risks reported here will be overestimated. A QRA for the introduction of JEV into the United States via hitchhiker mosquitoes on cargo ships reported a negligible annual risk of incursion for that virus (Oliveira et al., 2018). That assessment did not incorporate the number of livestock in the origin country, but rather utilised the proportion of JEV-infected mosquitoes for each origin region multiplied by the number of mosquitoes per cargo ship multiplied by the number of ships per year from each origin region to the US. Vector infection rates by origin country are not available for LSDV.

Unsurprisingly, arrival seaports with higher maritime traffic were associated with higher incursion risks in our assessment. Our findings confirm the importance of the current hitchhiker pest mitigation measures in place for both commercial vessels and returning live export ships.

For both our qualitative and quantitative models, we determined that there was no risk of LSDV incursion via Torres Strait Treaty movements. This is due to the absence of bovines in the Torres Strait NAQS risk zones. The introduction of bovines, either domestic or feral, into these regions would necessitate re-assessment.

4.2 Recommendations

Given the significant and increasing threat posed to Australia's livestock industries by LSDV, it is critical to maintain preparedness work to ensure that risks are managed appropriately. As discussed above, a key outcome of this QRA was to determine knowledge gaps around LSDV in the Australian context. We identified several key nodes that would benefit from additional research.

Our model was most sensitive to the following parameters: the probability of vector-to-bovine transmission (Ex_C), the probability of multiple vectors arriving (Ex_B), the number of vectors feeding on a bovine per infectious day (En_3), the probability of bovine-to-vector transmission (En_4), the probability of a vector being transported to Australia by wind (wind $En_{5.1, 5.2, 5.3}$), the probability of a vector surviving windborne dispersal (wind $En_{6.2}$), the probability of a vector flying to a seaport (shipping En_5) and landing on a vessel (shipping En_6) and the probability of a vector disembarking without detection (shipping En_9).

Arguably, the most prominent gap in our analysis is the number of vectors necessary to initiate an infection. Experimental transmission studies on LSDV have all used batches of insects (36–300 insects per batch) (Weiss, 1968; Chihota et al., 2001, 2003; Magori-Cohen et al., 2012; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021). Sprygin et al. (2019) state that:

> Because vector transmission is considered to be of a mechanical nature and the numbers [*sic*] of infective viruses on insects' mouthparts is likely to be low, in the absence of other supporting factors, air currents would need to transfer hundreds of contaminated vectors onto a single susceptible animal to induce full clinical disease.

However, no studies have directly investigated the ability of a single insect to transmit LSDV. Typically, when exotic *Culicoides* have been detected in Australia only single specimens have been recovered per sampling occasion (Eagles et al., 2014). Transmission of LSDV from *Culicoides* midges to bovines has not yet been demonstrated experimentally. Recent unpublished studies from the Pirbright Institute again found no evidence of LSDV transmission from *Culicoides nubeculosus* midges; however, it was found that as few as 14 *Aedes* mosquitoes could transmit LSDV to recipient cattle (P. Beard, pers. comm.)[16]. One study calculated that an individual *Stomoxys calcitrans* could only transfer $10^{-0.8}$ $TCID_{50}$ of LSDV (Sohier et al., 2019), while the minimum infectious dose was estimated at $>10^1$ $TCID_{50}$ (Carn and Kitching, 1995a). The authors therefore concluded that it seemed improbable that one stable fly could transmit sufficient virus to induce LSD (Sohier et al., 2019). Furthermore, this calculation was based on recently fed flies, prior to any significant viral decay; the time required for windborne dispersal or ship travel to Australia would likely further reduce the viral load present in vectors. Critically, unlike BTV there is no evidence for active replication of LSDV in the arthropod vector, so it is problematic to extrapolate parameters for LSDV based on experiences with BTV. However, our findings show that if a single vector is indeed sufficient to initiate an LSDV infection, the incursion risk is substantially increased. This suggests that individual PCR-positive insects will likely arrive in Australia and may be detected during surveillance activities. Likewise, nucleic acid of FMDV and African swine fever virus are frequently detected in seized pork products;

[16] Interim results that have generously been made available ahead of publication.

however, this does not necessarily equate with infectious virus or sufficient viral loads to initiate an infection. We strongly suggest that future research is directed towards examining the role of single insects in LSDV transmission.

Accurate data on the biting rates of relevant vector species and the variability in biting rates diurnally and seasonally are required to better parameterise En_3. This node constrains the number of potentially infectious vectors, and therefore the number of potential incursion events, and is thus highly influential in our model.

While several experimental studies have been published investigating the dynamics of LSDV transmission between bovines and vectors (En_4, Ex_C), critically, many of those studies relied on qPCR for virus detection (as well as using batches of vectors rather than single insects). This assay detects viral nucleic acid, not infectious virus particles, and therefore is not appropriate when assessing infectivity. Furthermore, many experimental studies have used vector species that are readily available in the laboratory, such as the anthropophilic *Aedes aegypti*. Future research should focus on zoophilic vector species relevant to the Australian context, such as buffalo fly (*Haematobia irritans exigua*), *Stomoxys calcitrans, Culicoides brevitarsis, Musca* spp. and *Culex* mosquitoes, and should utilise virus isolation (or animal infection where possible and ethically appropriate) for assessment of viral infectivity.

Further investigations into the relevant biology of vector species in the context of these non-regulated pathways would be useful for diseases beyond just LSDV. For example, the survival of these species under typical conditions on commercial ships and during windborne dispersal would help to inform $En_{6.2}$ and En_8. Understanding the short-range dispersal behaviour of key species would help to constrain En_5 and En_6 within the shipping pathways and Ex_A (the probability of a susceptible bovine being present at the arrival destination). Population genomics studies could be conducted on key insect species across northern Australia to better understand the frequency of introduction events.

LSDV transmission studies have generally involved highly susceptible *Bos taurus* breeds (Weiss, 1968; Chihota et al., 2001, 2003; Magori-Cohen et al., 2012; Sohier et al., 2019; Issimov et al., 2020; Sanz-Bernardo et al., 2021), while *Bos indicus* and buffalo are common in northern Australia (although free-ranging shorthorns and other *Bos taurus* breeds are present in smaller numbers). LSDV transmission is widely thought to be driven by symptomatic animals, since average viral titres in the blood and skin of asymptomatic animals are very low (Sanz-Bernardo et al., 2021). Typically, only 20–60% of infected animals develop clinical disease (Magori-Cohen et al., 2012), and this may be further reduced in more resistant breeds. Likewise, vector feeding behaviour and viral loads may vary between cattle breeds. Further research should be directed towards investigating breed-specific differences in the dynamics of LSDV pathogenesis, transmission and immunity.

Additionally, it is possible that emerging LSDV variants, such as the recombinant and vaccine-derived viruses reported from Kazakhstan, Russia, China and Asia, may differ phenotypically from the 'classical' variants. It is critical to ascertain which viruses are circulating in Indonesia and to characterise the pathogenesis of these variants specifically.

Our analysis highlighted the many complexities associated with modelling long-distance windborne dispersal of insect vectors across extended spatial and temporal scales. As such, we strongly encourage ongoing development of a user-friendly, web-based implementation of the Tool for Assessing Pest and Pathogen Aerial Spread (TAPPAS) (Durr, Graham and van Klinken, 2017). This would facilitate near-real-time monitoring of meteorological conditions, accelerating identification of likely source and arrival destinations and enabling enhanced risk-based surveillance for vector incursions when conditions are identified as suitable for vector dispersal to Australia. Further modifications of atmospheric dispersion models to better incorporate insect-specific parameters is also required.

More detailed information on livestock densities across at-risk areas of northern Australia would be extremely valuable. Since surveillance efforts in these regions are based on NAQS risk zones, having livestock number and density data aggregated at this spatial scale would be particularly useful. Alternatively, information on total cattle numbers and station areas could be obtained directly from individual stations in the relevant risk areas. Notably, the paucity of detailed information on feral bovine numbers across northern Australia proved challenging for this assessment.

Critically, current mitigation measures, such as insecticide treatments of incoming vessels, must be maintained and adapted as the disease situation changes in potential origin countries. Periodic re-evaluations of this assessment are strongly recommended to maintain our understanding of the probability of LSDV incursion via non-regulated pathways.

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Appendix 1

Consultation meeting participants

The following participants attended the consultation meeting on 25 March, 2022.

NAQS: Dr Skye Fruean, Dr Charlotte Burgoyne, Dr Michele Byers, Dr Tiffanie Huynh, Mr Tim Kerlin, Dr Josef Schmidt, Mr Matt Shields, Dr Guy Weerasinghe, Dr Cassandra Wittwer

The Department: Dr Jenny Baird, Dr Erin Davis, Dr Janene Kingston

Ausvet Pty Ltd: Dr Emma Zalcman, Dr Brendan Cowled

Appendix 2

Parameters used for HYSPLIT modelling

Trajectories

CONTROL FILE
21 12 31 18 #start date and hour
1 # number of starting locations
-9.434 142.209 1 # starting latitude and longitude and level in metres above ground level
-48 # run time
0 # vertical motion method
2000 # top of model in metres above ground level. Trajectories terminate at this level.
1 # number of meteorological grids
C:/Users/RobynHall/Downloads/NCEP_NCAR// # meteorological files parent directory
RP202112.gbl # meteorological file name
C:/Hysplit/HYSPLIT_win64U_v5.2.1/working/
traj-ncep-bwd-21-12-31-18-43lat_-9p434_lon_142p209-hgt_1-48h

Concentration/dispersion runs

CONTROL FILE
21 01 01 18 # start date and hour
1 # number of starting locations
-8.063 140.184 1.0 # starting latitude and longitude and level in metres above ground level
48 # run time
0 # vertical motion method
2000.0 # top of model in metres above ground level. Particles reflect back once they reach this level.
1 2 # number of meteorological grids and files
C:/Users/RobynHall/Downloads/gdas1.0/ # meteorological files parent directory
gdas1.dec20.w5
C:/Users/RobynHall/Downloads/gdas1.0/
gdas1.jan21.w1
1 # number of pollutants
TEST # name of pollutant
3333.3 # emission rate per hour
3.0 # hours of emission
00 00 00 00 00 # release start
1 # number of grids
0.0 0.0 # grid centre lat lon
1.0 1.0 # grid spacing lat lon
30.0 60.0 # grid span lat lon
./ # output grid directory
cdump # output grid file name
1 # number of vertical levels
100 # height of levels
00 00 00 00 00 # sampling start
00 00 00 00 00 # sampling stop
02 48 00 # av/now/max hrs min
1 # number of pollutants
1.0 1.0 1.0 # diameter density shape, not used because deposition specified below.
0.005 0.0 0.0 0.0 0.0 # vel m/s mol wt A-rat D-rat Henry - dry deposition
0.0 8.0E-05 8.0E-05 # Henry's in-cloud below-cloud - wet deposition
0.0 # radioactive decay days
0.0 # resuspension factor

SETUP.CFG

```
&SETUP
tratio = 0.75 # advection stability ratio
initd = 0 # initial distribution, particle, puff, or mix
kpuff = 0 # horizontal puff dispersion
khmax = 9999 # maximum particle duration
kmixd = 0 # mixing layer depth computation option
kmix0 = 150 # mixing layer depth minimum
kzmix = 0 # vertical mixing adjustments
kdef = 0 # horizontal turbulence
kbls = 1 #boundary layer stability computation option
kblt = 0 # boundary layer turbulence parameterization
idsp = 1 # particle dispersion scheme
conage = 24 #t1 for particle-puff release mode conversion after t1
gemage = 48 #t2 particle or puff transfer to the global model t2
numpar = 10000 #particles released per cycle
qcycle = 0.0 # cycling of emissions
efile = '' # temporal emission file name
tkerd = 0.18 # unstable turbulent kinetic energy ratio
tkern = 0.18 # stable turbulent kinetic energy ratio
hscale = 10800.0 # horizontal Lagrangian time scale
vscales = 5.0 # horizontal Lagrangian time scale for stable planetary boundary layer
vscaleu = 200.0 # horizontal Lagrangian time scale for unstable planetary boundary layer
ninit = 3 # particle initialisation option. 3=replace
ndump = 1 # particle file first output hrs
ncycl = 3 # particle file repeat outputs hrs
pinpf = 'PARINIT' # particle initialisation file name
poutf = 'PARDUMP' # particle output file name
mgmin = 10 # meteorological subgrid minimum horizontal size
kmsl = 0 # starting height in metres above ground level or above sea level
maxpar = 100000 # maximum number of particles
cpack = 1 # binary concentration grid packing
cmass = 1 # output units concentration or mass
dxf = 1.0 # horizontal X-grid adjustment factor for ensemble
dyf = 1.0 # horizontal Y-grid adjustment factor for ensemble
dzf = 0.01 # vertical factor for ensemble
ichem = 5 # chemistry conversion modules
kspl = 1 # puff split frequency
krnd = 6 # puff enhanced merging interval
frhs = 1.0 # puff horizontal merge distance sigma
frvs = 0.01 # puff vertical merge distance sigma
frts = 0.1 # puff temporal merge distance fraction
frhmax = 3.0 # puff max horizontal merge sigma
splitf = 1.0 # puff horizontal split factor
cmtfn = '' # centre-of-mass trajectory output file name
wvert = .TRUE. # vertical interpolation scheme for WRF fields
/
```

HYSPLIT.BAT

```
@echo off
setLocal EnableDelayedExpansion

set WRK=%CD%

set DIR=c:
set PGM=%DIR%\Hysplit\HYSPLIT_win64U_v5.2.1
cd %PGM%\working


IF EXIST hysplit2.bin DEL hysplit2.bin
IF EXIST cdump DEL cdump
IF EXIST PARDUMP DEL PARDUMP
%PGM%\exec\hycs_std

IF EXIST partplot.html DEL partplot.html
ECHO 'TITLE^&','### %0 ### ^&'      >LABELS.CFG
ECHO 'MAPID^&','Particle Number^&' >>LABELS.CFG
ECHO 'LAYER^&','Maximum^&'         >>LABELS.CFG
ECHO 'UNITS^&','Particles^&'       >>LABELS.CFG
ECHO 'VOLUM^&','^&'                >>LABELS.CFG
ECHO 'RELEASE^&','^&'              >>LABELS.CFG

ECHO 'TITLE^&','### %0: cross-section ### ^&' >LABELS.CFG
%PGM%\exec\parxplot +g1 -iPARDUMP -k1 -z80 -j%PGM%\graphics\arlmap

%PGM%\exec\par2asc.exe -iPARDUMP -oPARDUMP.txt -a1
```

Appendix 3

Modelling retention of infectiousness of potential vectors of LSDV

A literature review was conducted to obtain information on the retention of infectiousness of potential vectors of LSDV. Data from articles where infectiousness was determined by virus isolation was used to model the probability of vector infectiousness per day. Vectors from three categories were used in the analysis:

- Heavy fliers – *Stomoxys calcitrans, Stomoxys sitiens, Stomoxys indica*
- Mosquitoes – *Culicidae*
- Midges – *Culicoides* spp.

Data for heavy fliers were sourced from two papers (Chihota et al., 2001; Issimov et al., 2020). The number of infectious heavy fliers decreased to 0 two days after an infectious blood meal (Table 3).

Table 3 Number of heavy fliers positive for LSDV by virus isolation and total tested per day

Day	Paper	Positive	Total	Percent positive (%)
0	1	44	90	49
0	2	47	90	52
1	1	0	45	0
1	2	4	30	13
2	1	0	45	0
2	2	1	30	3
3	2	0	30	0
4	1	0	45	0
4	2	0	30	0
5	2	0	30	0
6	2	0	30	0
7	1	0	45	0
7	2	0	30	0
8	2	0	30	0
9	2	0	30	0
10	2	0	30	0
14	1	0	45	0

Data for both mosquitoes and midges were sourced from a single study (Paslaru et al., 2022). The number of infectious mosquitoes decreased over 10 days; however, infectious mosquitoes and midges were still present at day 10 post-feeding (Table 4, Table 5). Notably, very small numbers of vectors were tested, particularly at later time points.

Table 4 Number of mosquitoes positive for LSDV by virus isolation and total tested per day

Day	Positive	Total	Percent positive (%)
0	5	5	100
1	5	11	45
2	6	11	55
4	6	7	86
7	1	1	100
10	1	1	100

Table 5 Number of midges positive for LSDV by virus isolation and total tested per day

Day	Positive	Total	Percent positive (%)
0	4	5	80
1	5	5	100
2	1	4	25
7	0	3	0
10	2	2	100

Due to the limited data available, values from all species were combined for modelling.

The data do not follow a linear structure, therefore non-linear least squares models were used to find the most appropriate model to estimate the probability of vector infectiousness over time.

Firstly, to estimate the starting parameters (a: the y value when x = 0, b: the decay rate) for the non-linear least squares model, a linear model was fit. By taking logs of both sides of this model, the slope and intercept are estimates of parameters a and b.

$$\log(percent) \sim \log(Day)$$

The values are then fitted in a non-linear least squares model:

$$y(percent) \sim \exp(\log(a)) * Day\text{^}\log(b)$$

Where:

- a is the intercept determined above (Intercept (log(a)) = 3.72)
- b is the decay rate determined above (Decay rate (log(b)) = 0.18)

The probability of infectiousness at each day is calculated using the estimates from the non-linear least squares model (Table 6, Table 7).

Table 6 Results from non-linear least squares model.

Value for a	Value for b
63.24	–0.57

Therefore:

$$y(percent) \sim 63.24 * Day\text{^} - 0.57$$

Table 7 Probability of a vector being infectious per day, based on non-linear least squares model

Day	Probability (%)
0	63.24
1	42.60
2	33.81
3	28.70
4	25.27
5	22.77
6	20.86
7	19.33
8	18.07
9	17.02
10	16.12
11	15.34
12	14.66
13	14.05
14	13.51
15	13.02

Due to lack of data and both mosquitoes and midges not reaching 0% positivity on virus isolation, it is recommended that these results be interpreted with caution. Further data collection and analysis is recommended.

Appendix 4

Table 8 ISO 3166-1 alpha-3 country codes used in this study

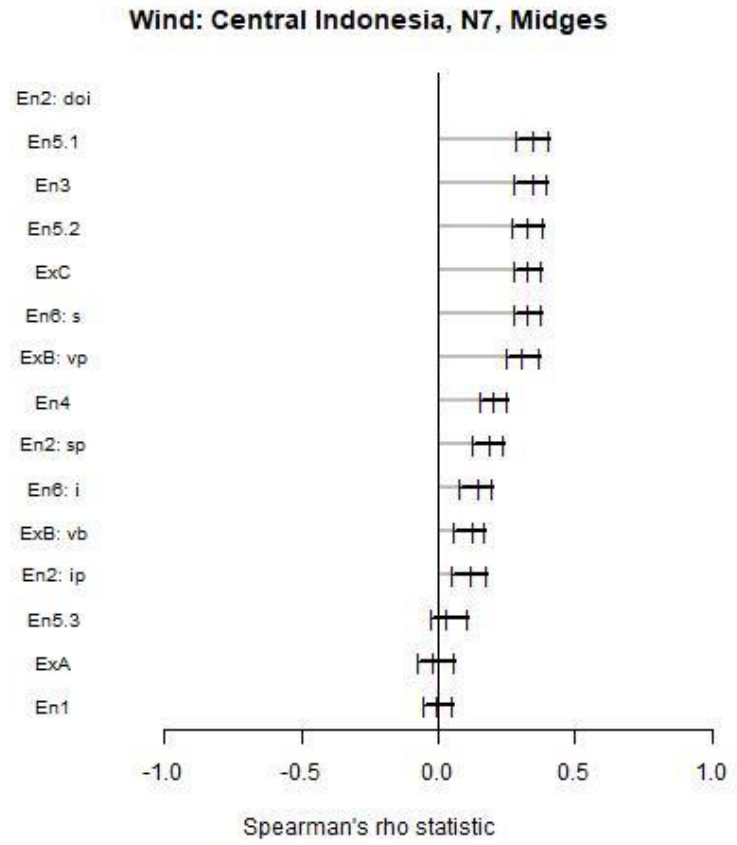
Alpha-3 code	Origin country
BGD	Bangladesh
BRN	Brunei Darussalam
C-IDN	Central Indonesia
CHN	China and autonomous regions
E-IDN	Eastern Indonesia
IND	India
IRN	Iran
JPN	Japan
KHM	Cambodia
KOR	Korea, Republic of
LKA	Sri Lanka
MDG	Madagascar
MMR	Myanmar
MYS	Malaysia
OMN	Oman
PAK	Pakistan
PHL	Philippines
PNG	Papua New Guinea
PRK	Democratic People's Republic of Korea
RUS	Russian Federation
SGP	Singapore
THA	Thailand
TLS	Timor-Leste
USA	Hawaii
VNM	Vietnam
W-IDN	Western Indonesia
YEM	Yemen

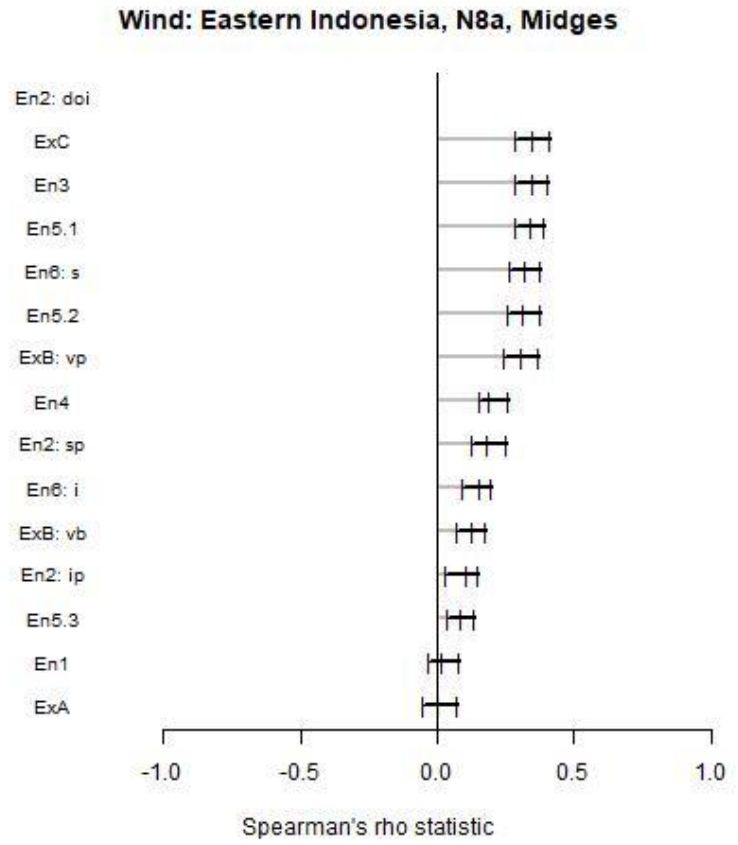
Appendix 5

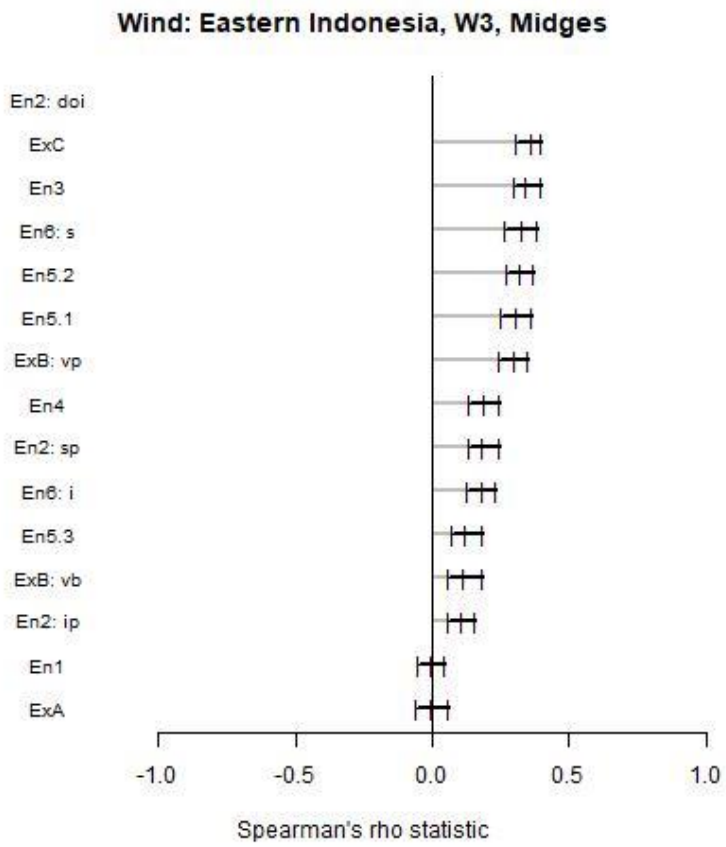
Sensitivity analysis

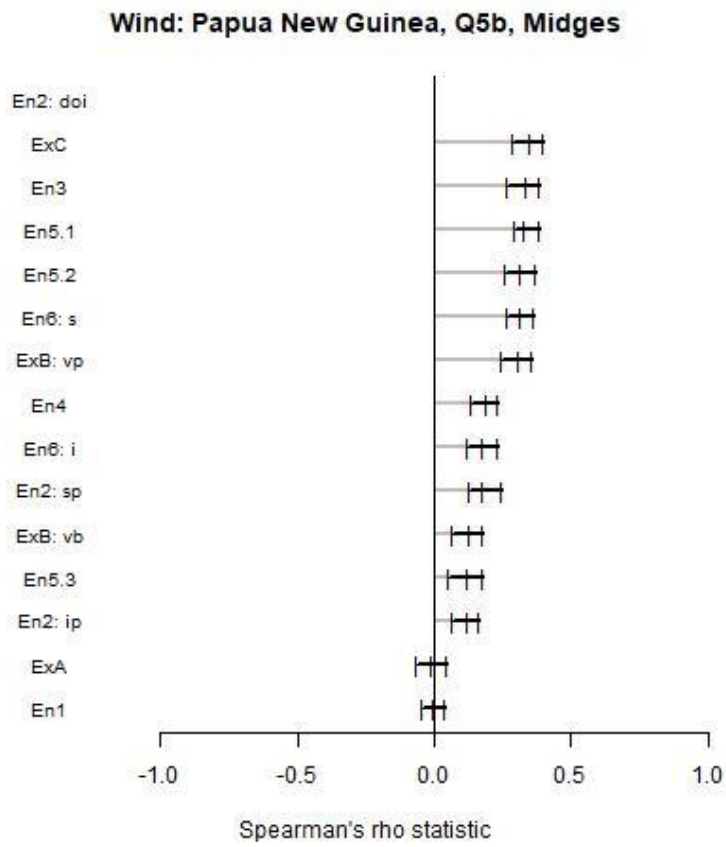
doi duration of immunity; s survives; vp vector(s) present; sp seroprevalence; i infectious; vb vector(s) bite; ip infectious period; A to Australian waters; p to seaport

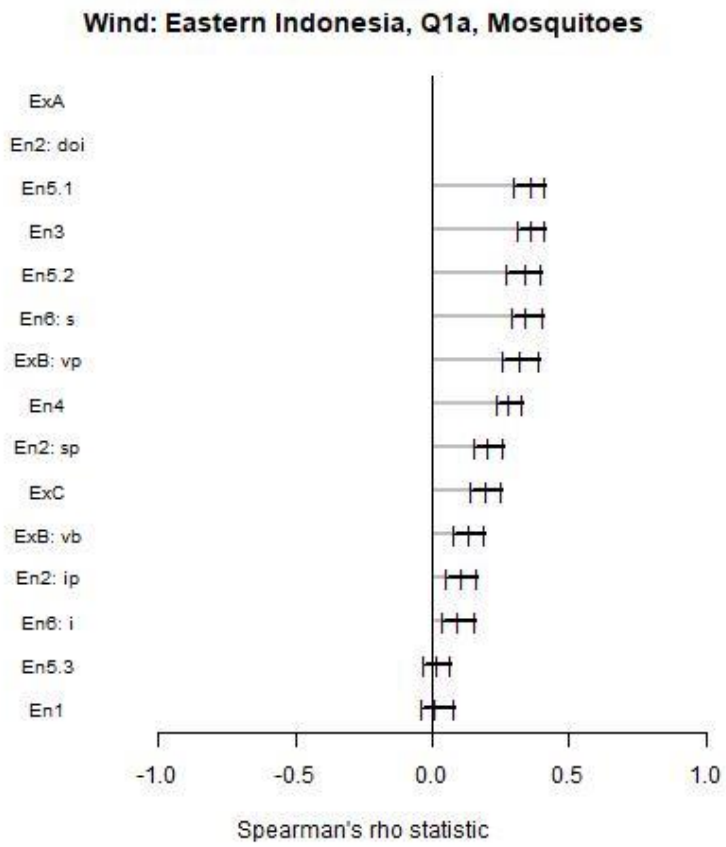
Windborne dispersal

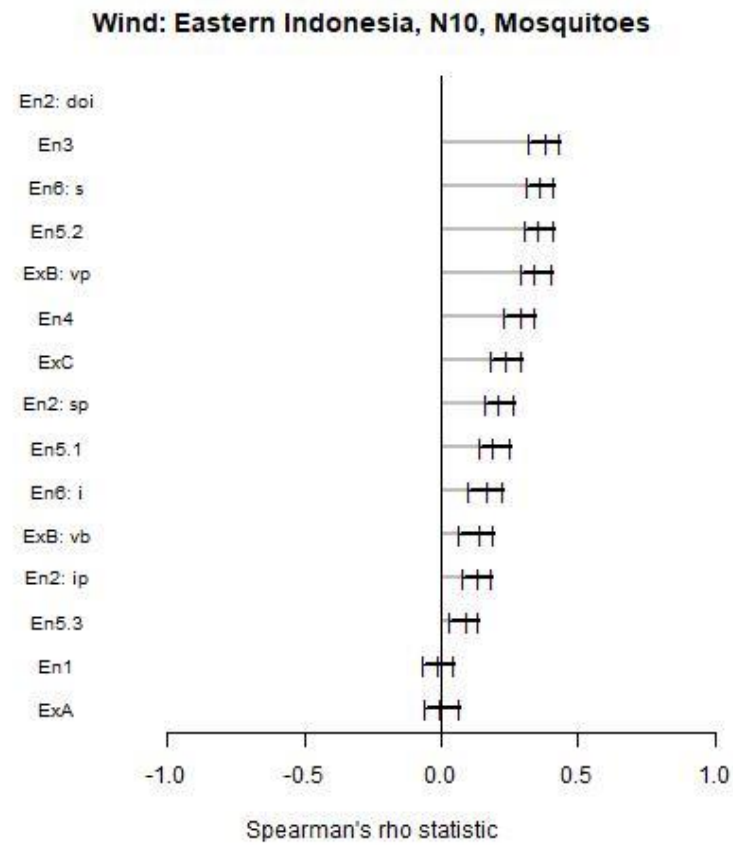


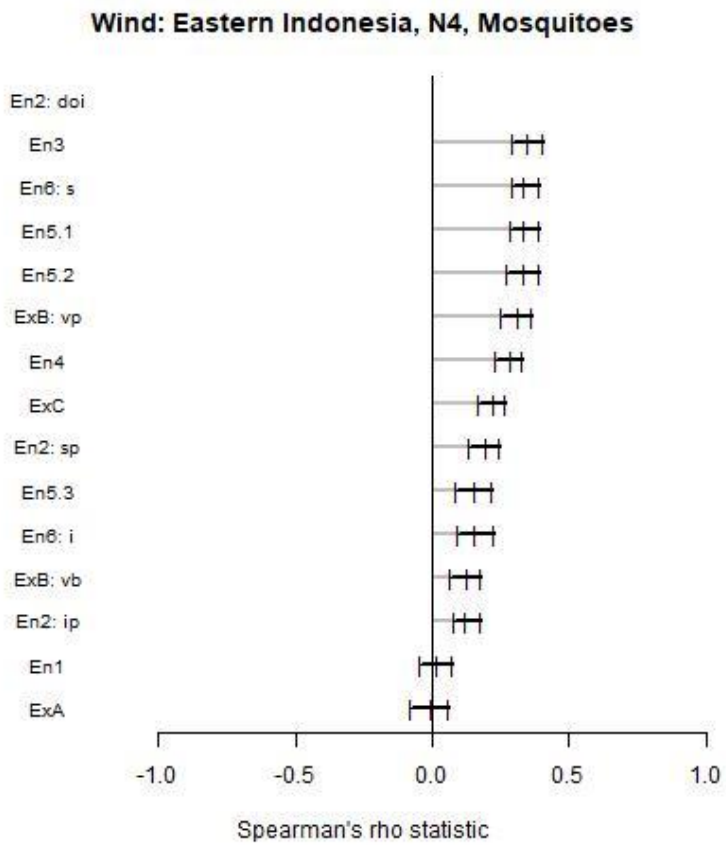


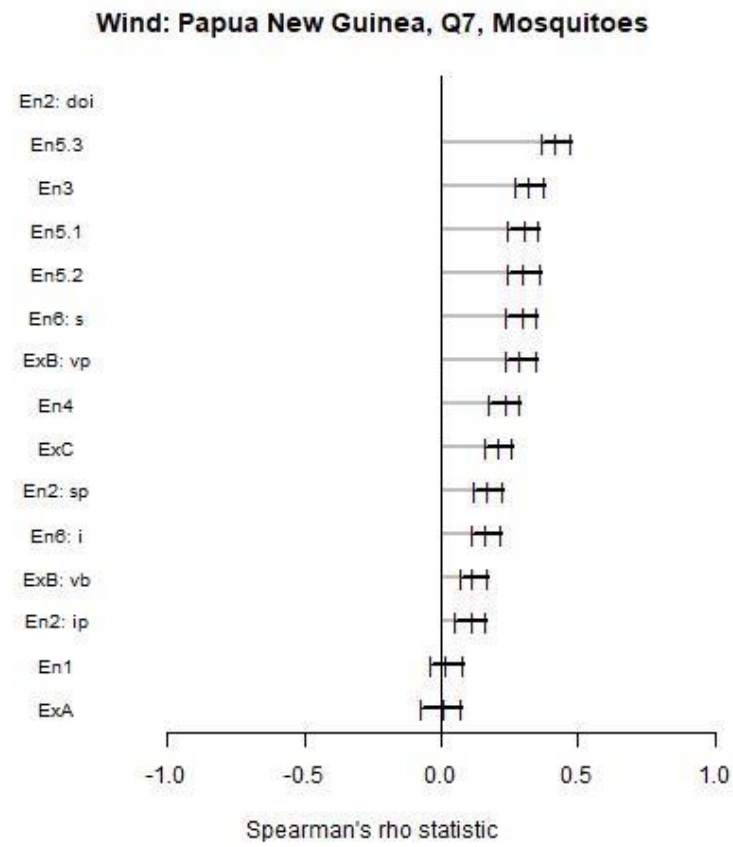

Wind: Eastern Indonesia, W3, Midges
En2: doi
ExC
En3
En6: s
En5.2
En5.1
ExB: vp
En4
En2: sp
En6: i
En5.3
ExB: vb
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

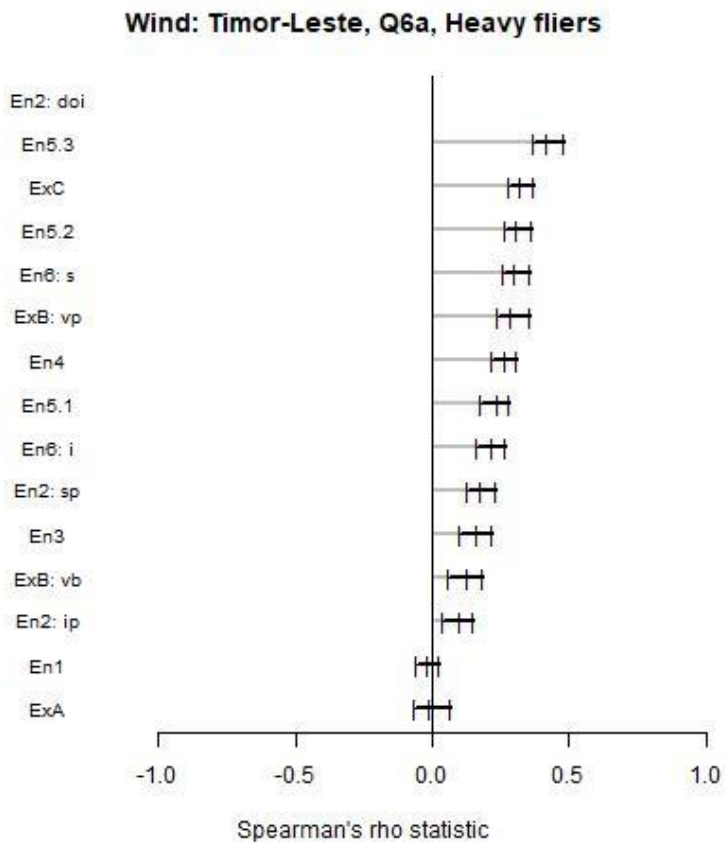

Wind: Papua New Guinea, Q5b, Midges
En2: doi
ExC
En3
En5.1
En5.2
En6: s
ExB: vp
En4
En6: i
En2: sp
ExB: vb
En5.3
En2: ip
ExA
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

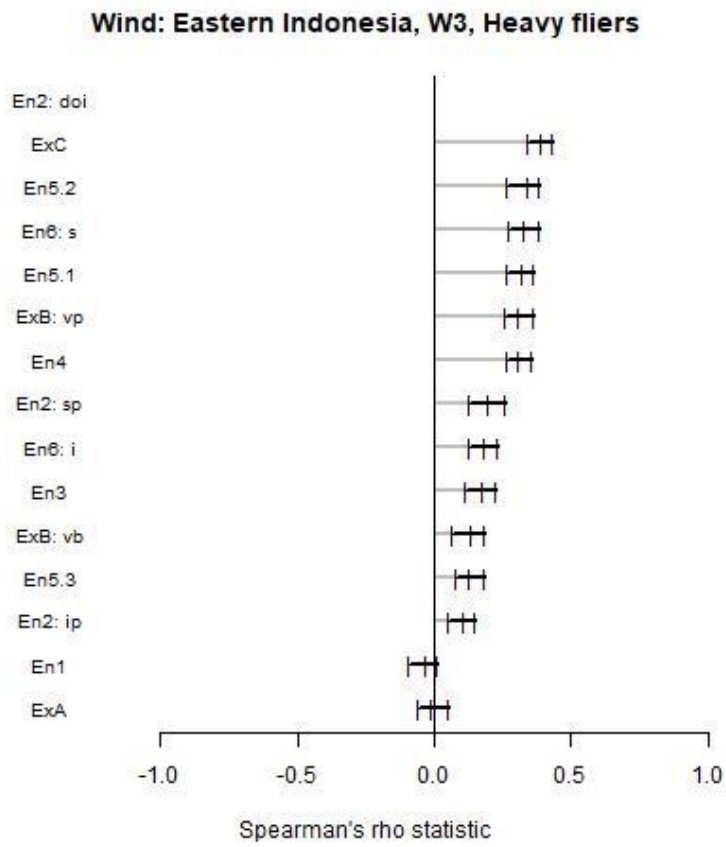

Wind: Eastern Indonesia, Q1a, Mosquitoes
ExA
En2: doi
En5.1
En3
En5.2
En6: s
ExB: vp
En4
En2: sp
ExC
ExB: vb
En2: ip
En6: i
En5.3
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


Wind: Eastern Indonesia, N10, Mosquitoes
En2: doi
En3
En6: s
En5.2
ExB: vp
En4
ExC
En2: sp
En5.1
En6: i
ExB: vb
En2: ip
En5.3
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

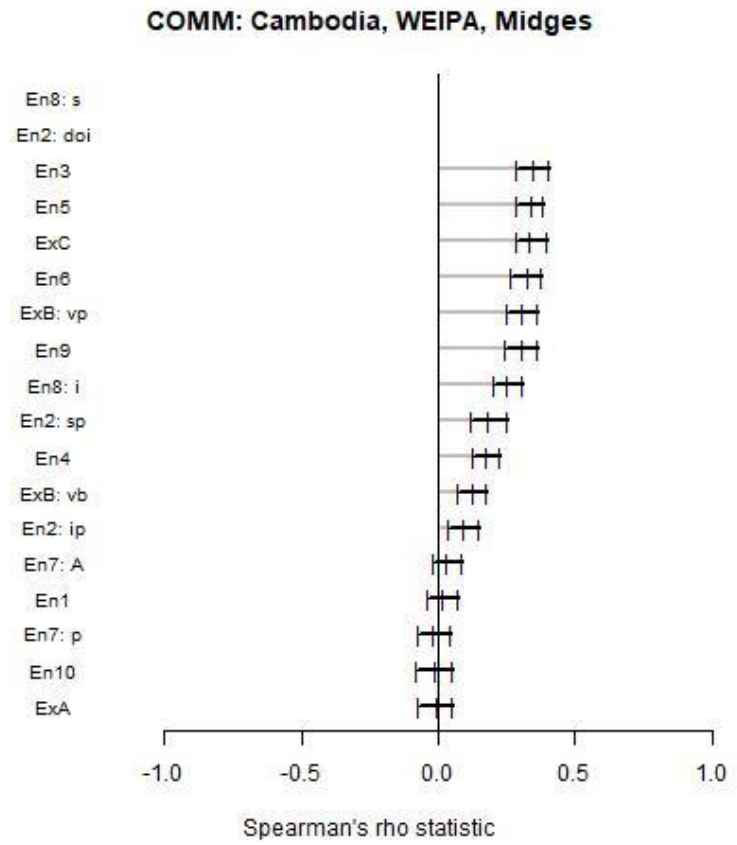

Wind: Eastern Indonesia, N4, Mosquitoes
En2: doi
En3
En6: s
En5.1
En5.2
ExB: vp
En4
ExC
En2: sp
En5.3
En6: i
ExB: vb
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

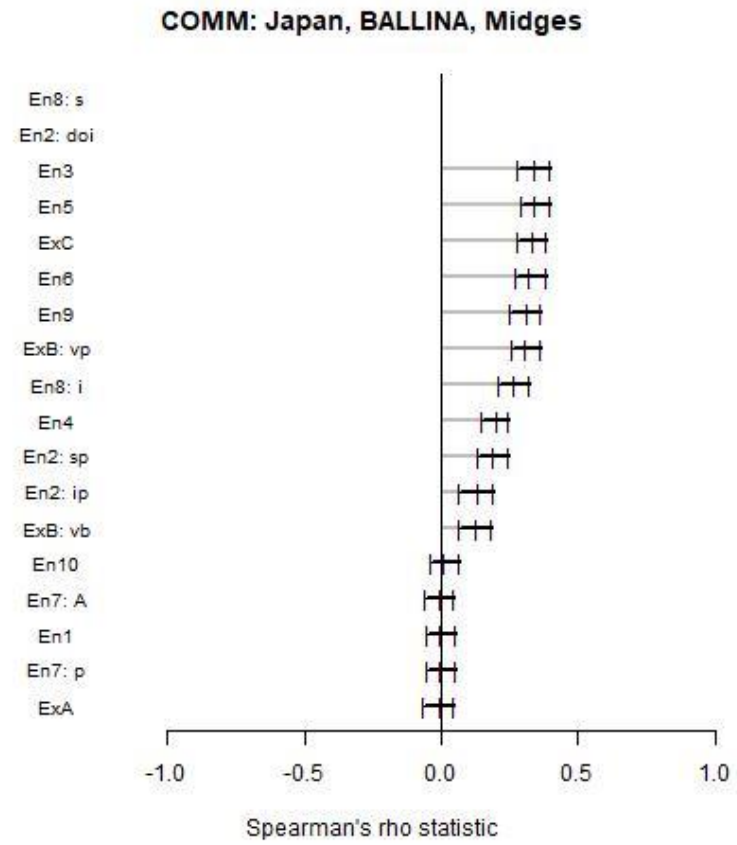

Wind: Papua New Guinea, Q7, Mosquitoes
En2: doi
En5.3
En3
En5.1
En5.2
En6: s
ExB: vp
En4
ExC
En2: sp
En6: i
ExB: vb
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


Wind: Timor-Leste, Q6a, Heavy fliers
En2: doi
En5.3
ExC
En5.2
En6: s
ExB: vp
En4
En5.1
En6: i
En2: sp
En3
ExB: vb
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

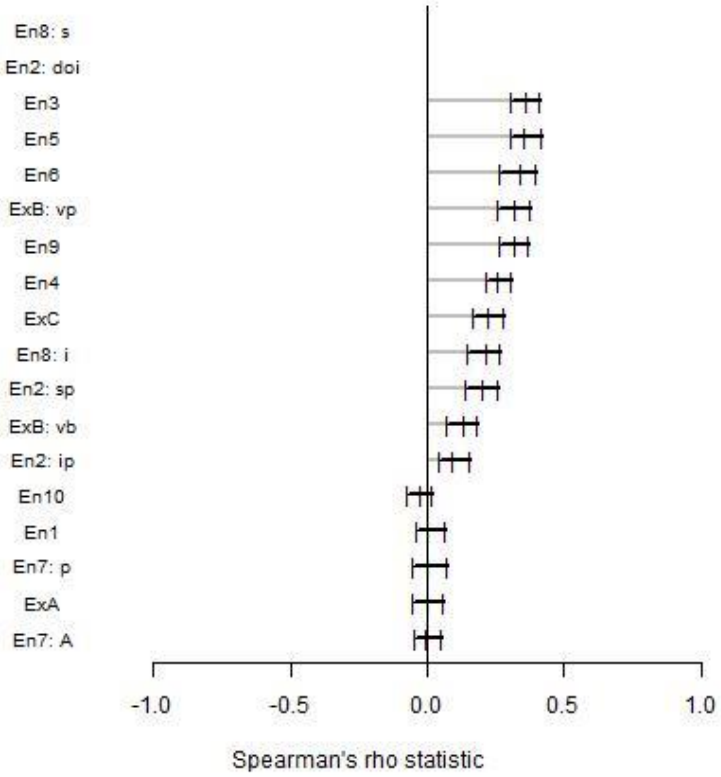

Wind: Eastern Indonesia, W3, Heavy fliers
En2: doi
ExC
En5.2
En6: s
En5.1
ExB: vp
En4
En2: sp
En6: i
En3
ExB: vb
En5.3
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

Commercial shipping (excluding returning live export vessels)

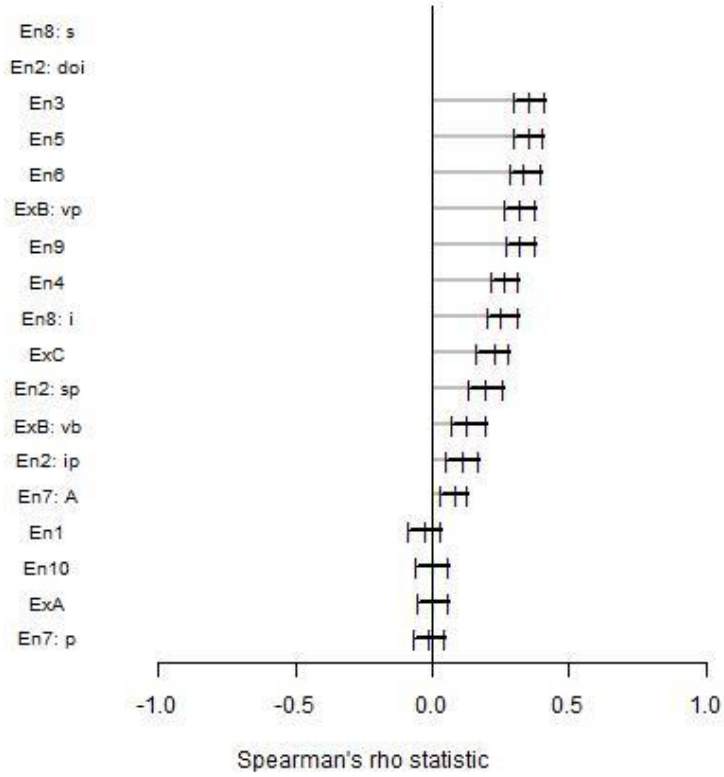


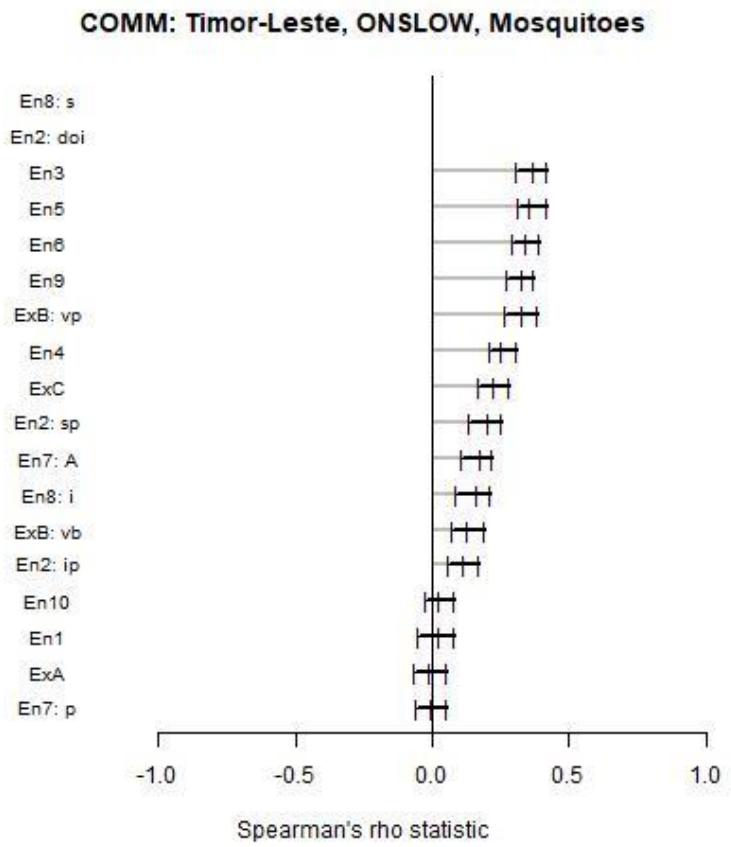


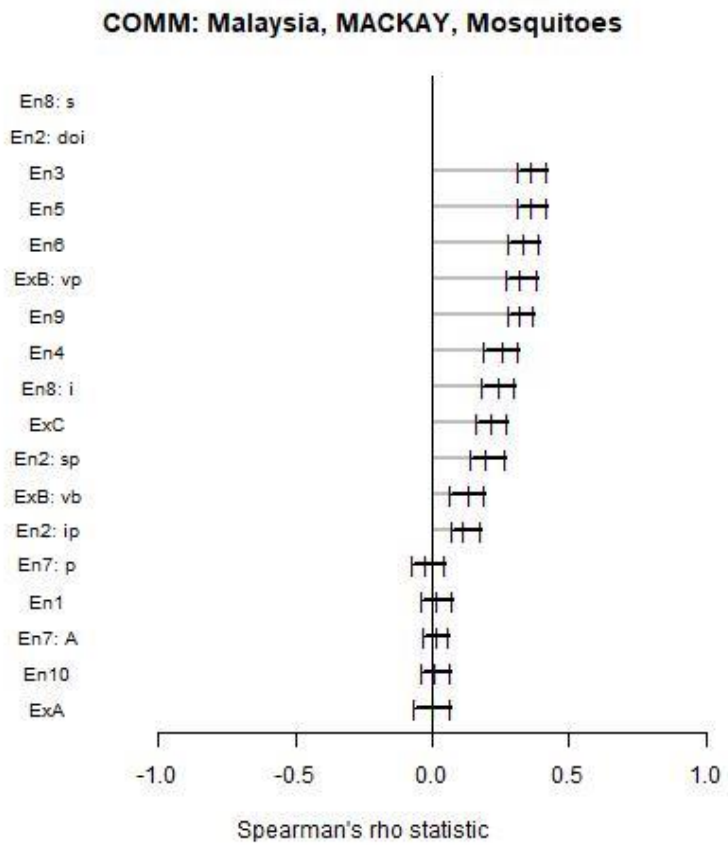
COMM: Central Indonesia, CAPE FLATTERY HARBOR, Mosquitoes

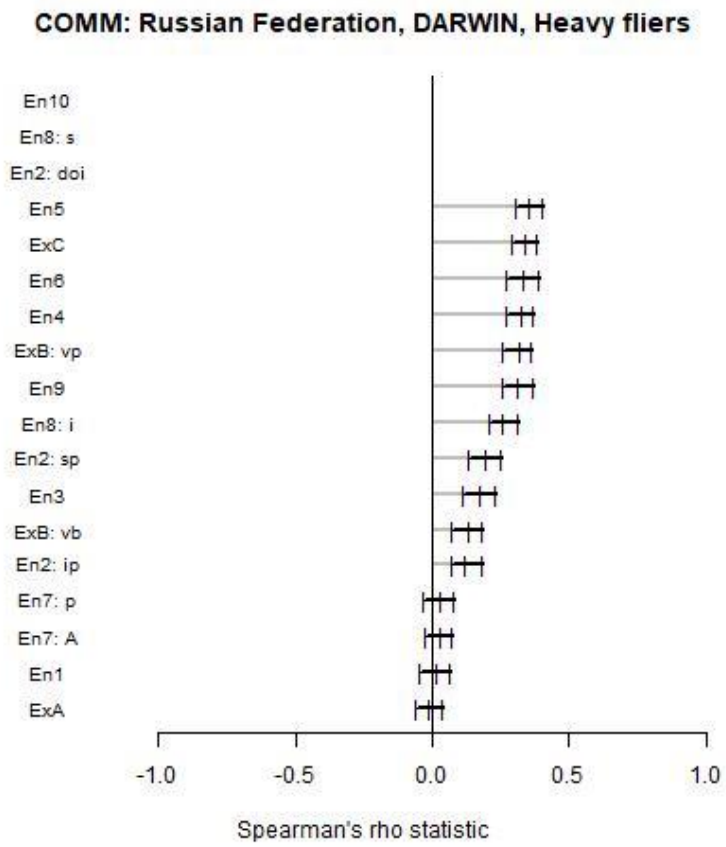


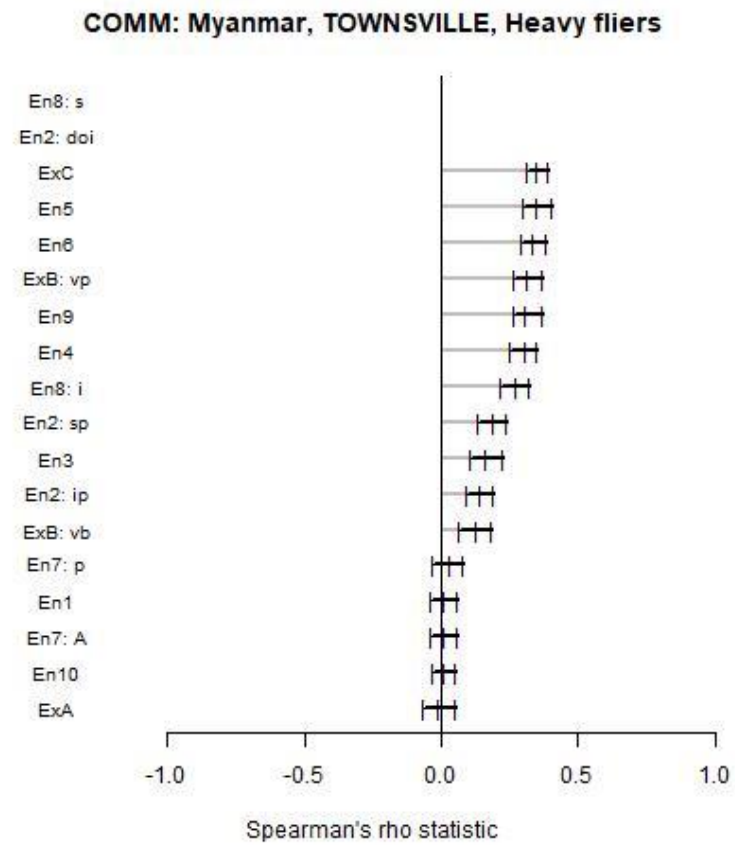
COMM: Korea, Republic of, WEIPA, Mosquitoes

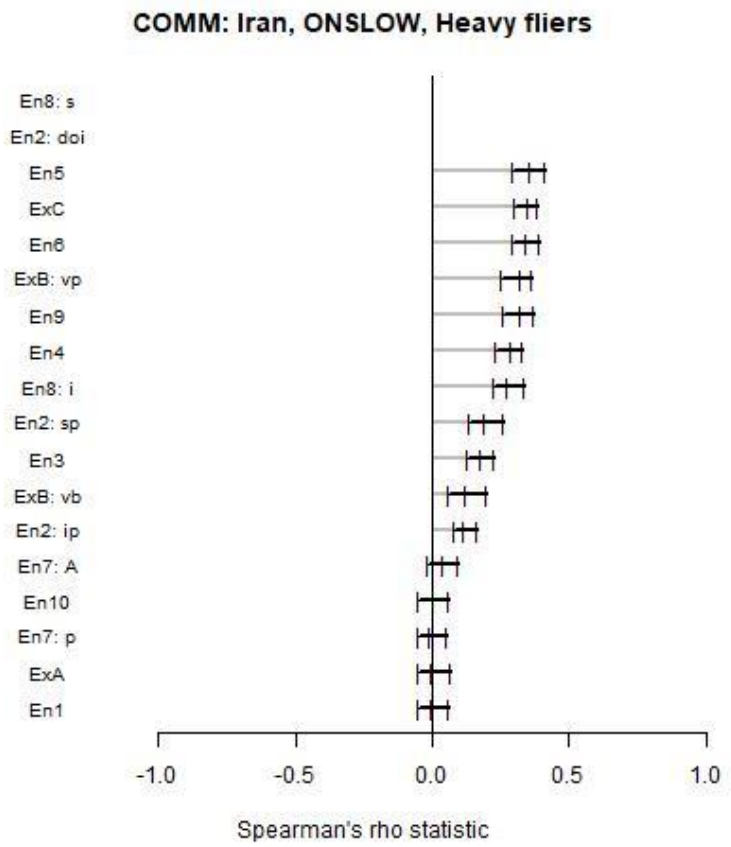


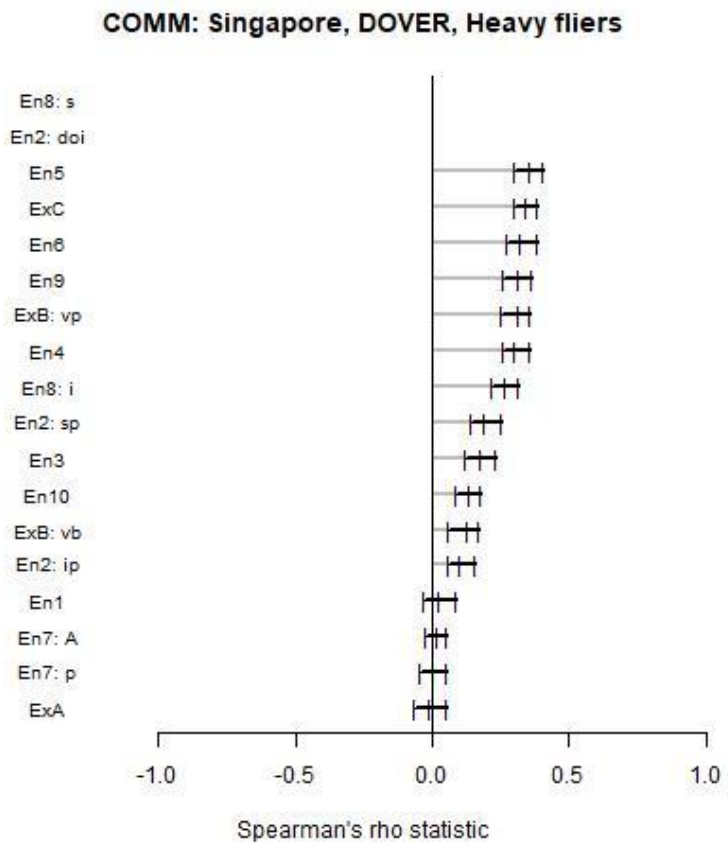

COMM: Timor-Leste, ONSLOW, Mosquitoes
En8: s
En2: doi
En3
En5
En6
En9
ExB: vp
En4
ExC
En2: sp
En7: A
En8: i
ExB: vb
En2: ip
En10
En1
ExA
En7: p
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


COMM: Malaysia, MACKAY, Mosquitoes
En8: s
En2: doi
En3
En5
En6
ExB: vp
En9
En4
En8: i
ExC
En2: sp
ExB: vb
En2: ip
En7: p
En1
En7: A
En10
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

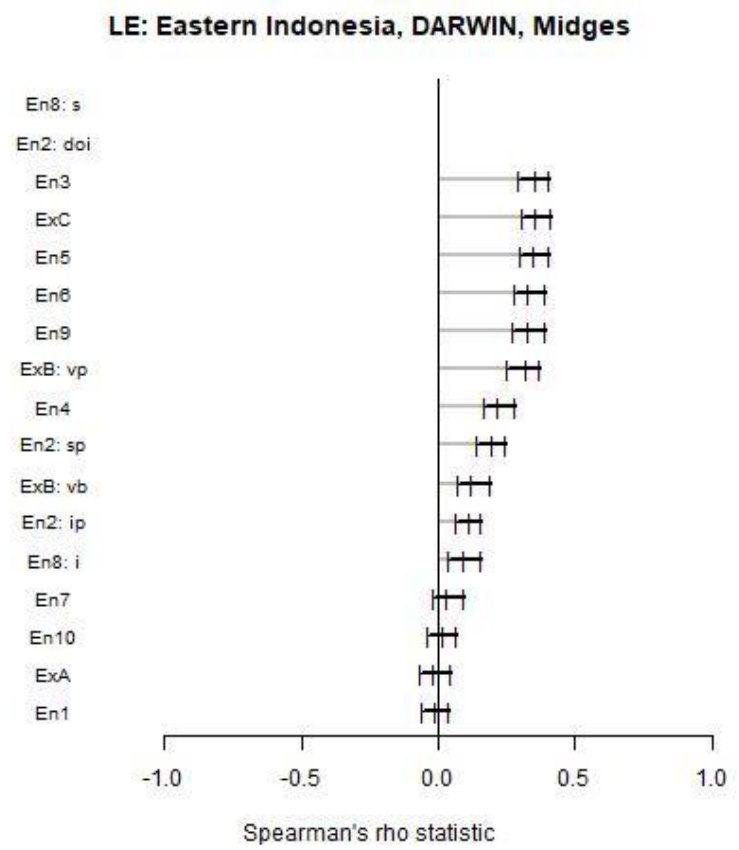

COMM: Russian Federation, DARWIN, Heavy fliers
En10
En8: s
En2: doi
En5
ExC
En6
En4
ExB: vp
En9
En8: i
En2: sp
En3
ExB: vb
En2: ip
En7: p
En7: A
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

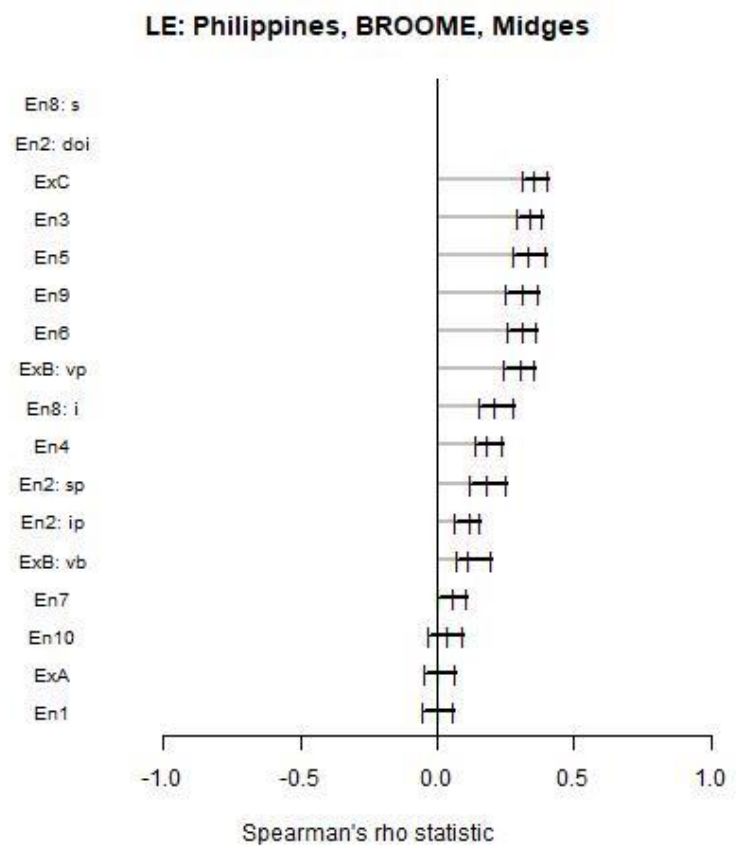

COMM: Myanmar, TOWNSVILLE, Heavy fliers
En8: s
En2: doi
ExC
En5
En6
ExB: vp
En9
En4
En8: i
En2: sp
En3
En2: ip
ExB: vb
En7: p
En1
En7: A
En10
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

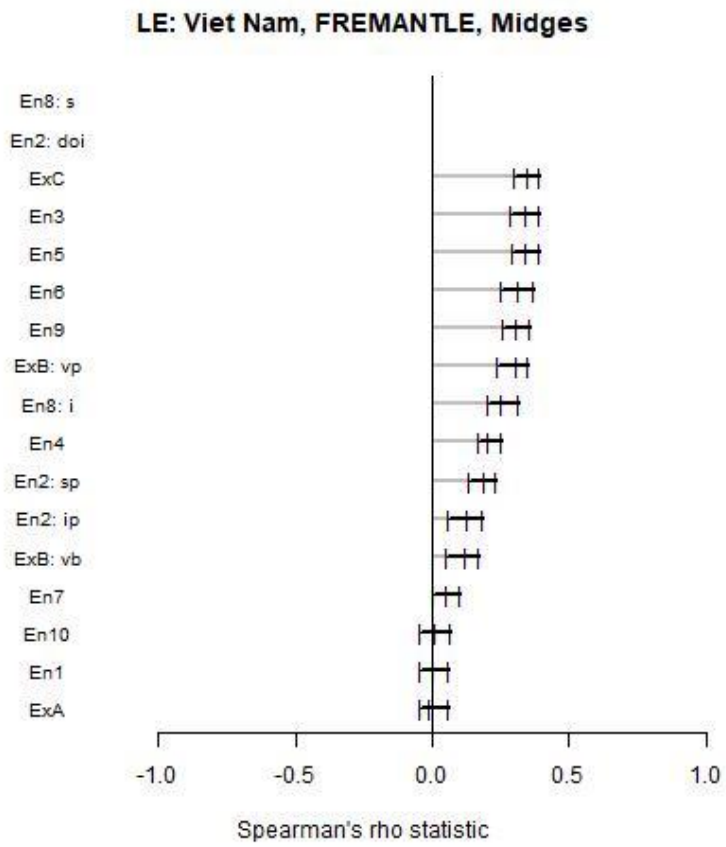

COMM: Iran, ONSLOW, Heavy fliers
En8: s
En2: doi
En5
ExC
En6
ExB: vp
En9
En4
En8: i
En2: sp
En3
ExB: vb
En2: ip
En7: A
En10
En7: p
ExA
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

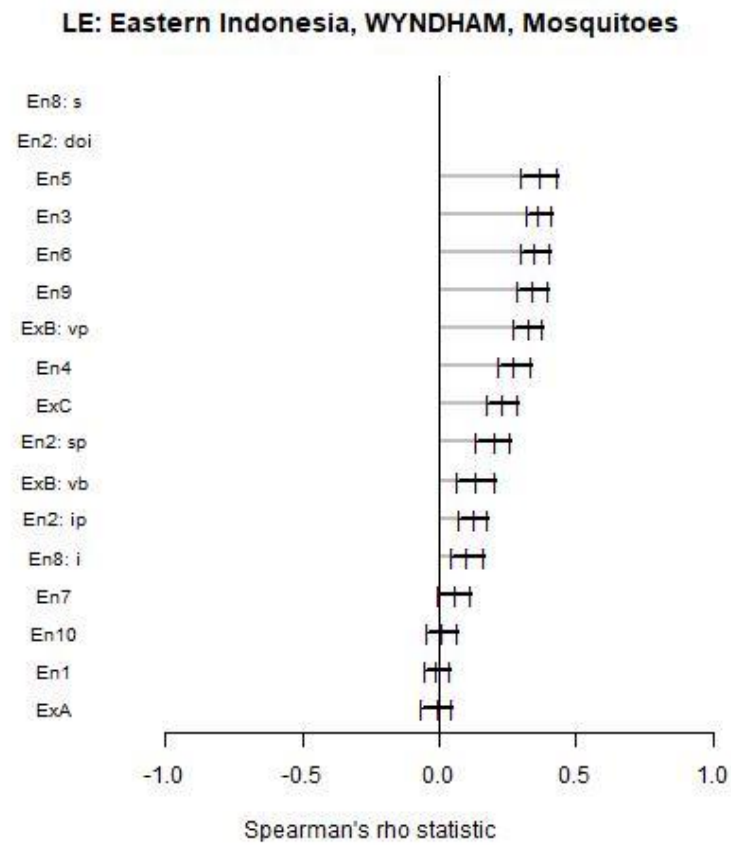

COMM: Singapore, DOVER, Heavy fliers
En8: s
En2: doi
En5
ExC
En6
En9
ExB: vp
En4
En8: i
En2: sp
En3
En10
ExB: vb
En2: ip
En1
En7: A
En7: p
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

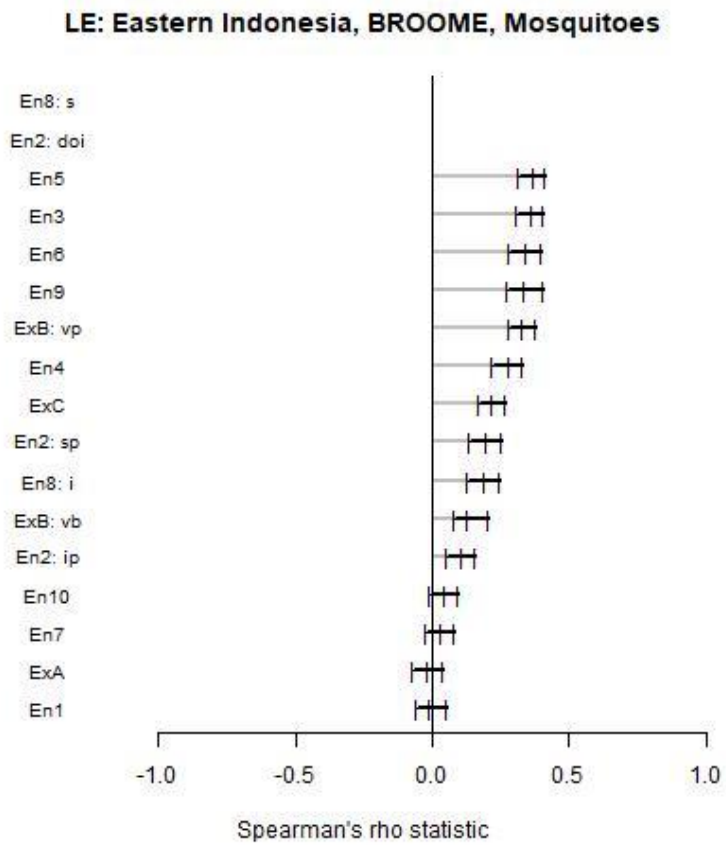
Returning live export vessels

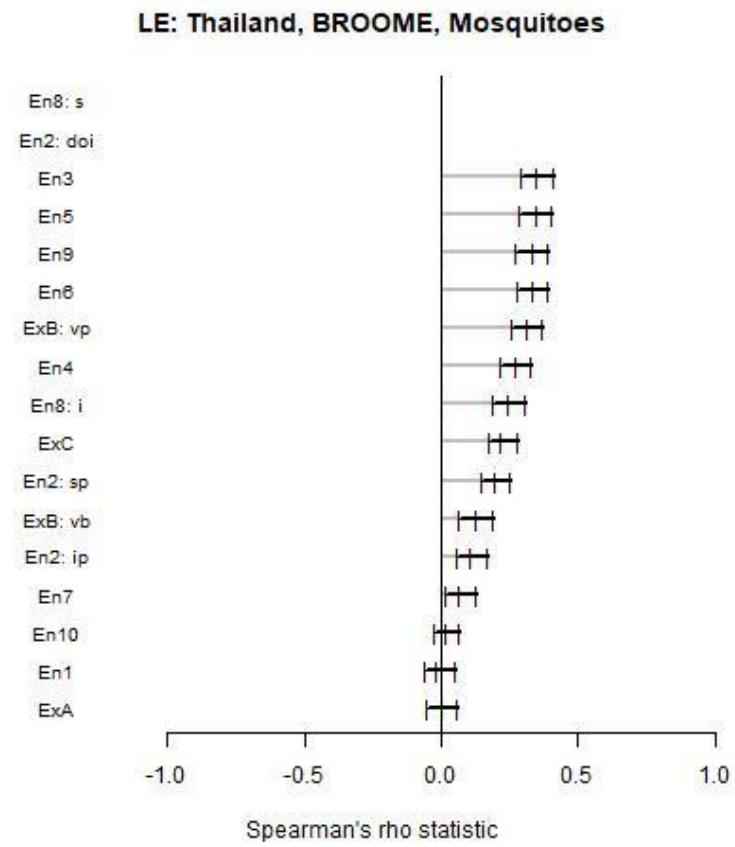


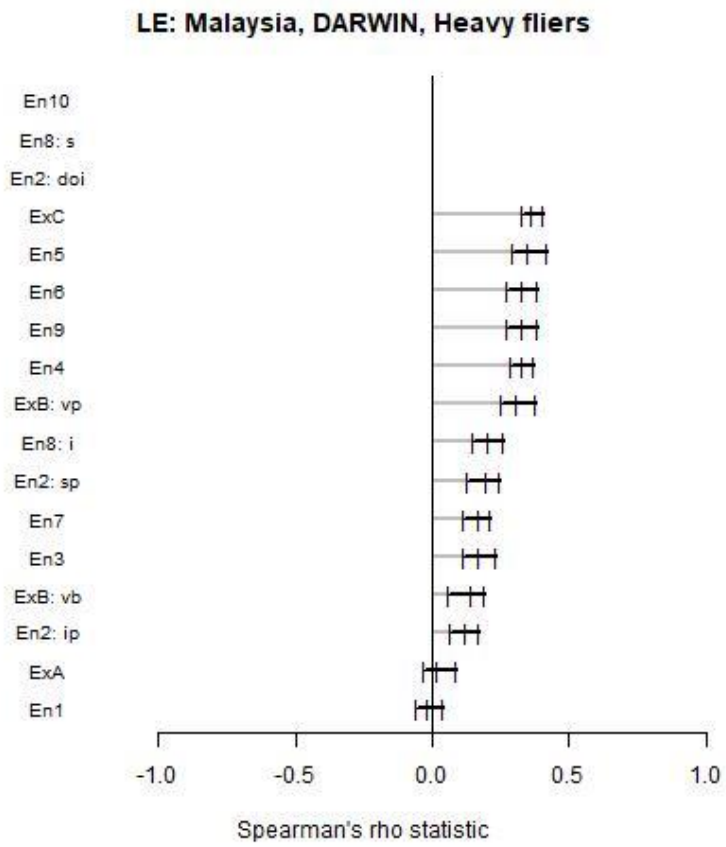


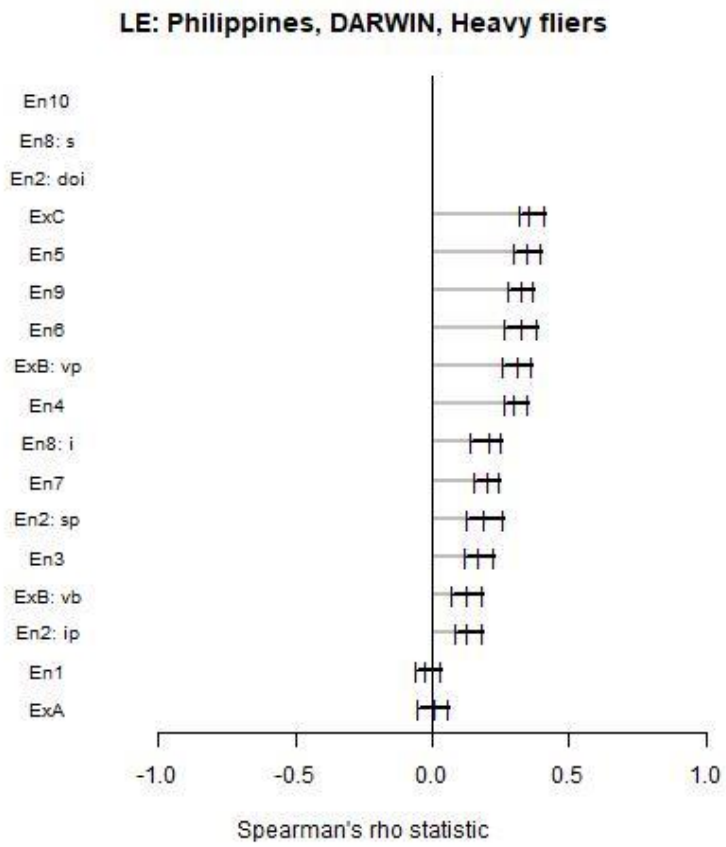

LE: Viet Nam, FREMANTLE, Midges
En8: s
En2: doi
ExC
En3
En5
En6
En9
ExB: vp
En8: i
En4
En2: sp
En2: ip
ExB: vb
En7
En10
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

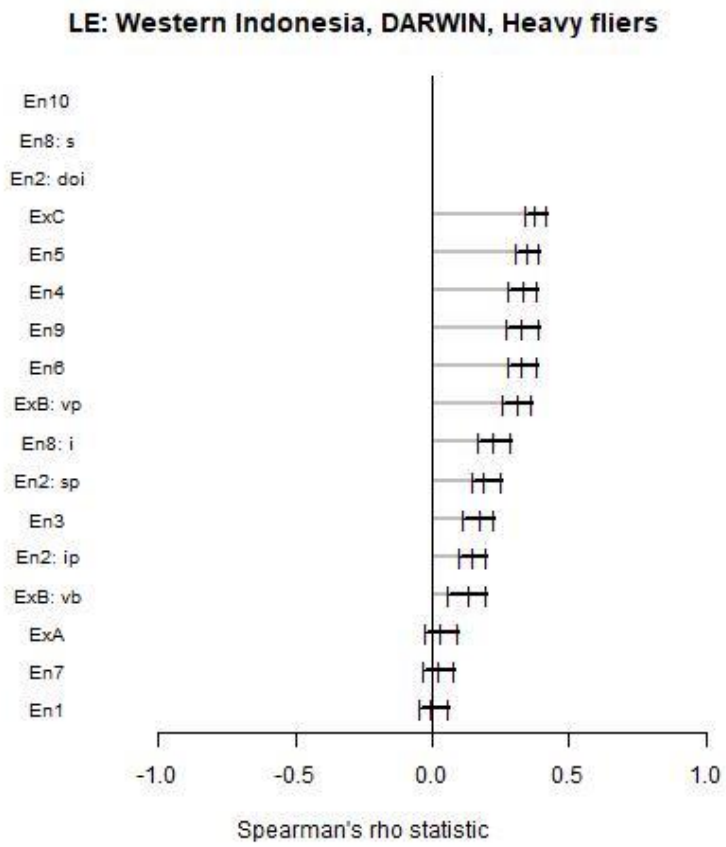

LE: Eastern Indonesia, WYNDHAM, Mosquitoes
En8: s
En2: doi
En5
En3
En6
En9
ExB: vp
En4
ExC
En2: sp
ExB: vb
En2: ip
En8: i
En7
En10
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic

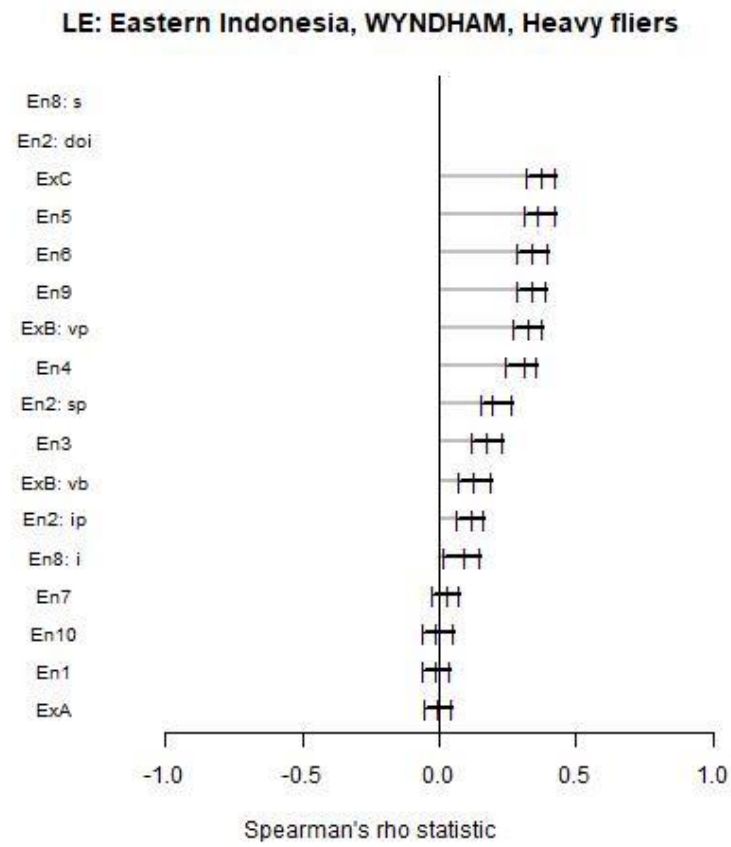

LE: Eastern Indonesia, BROOME, Mosquitoes
En8: s
En2: doi
En5
En3
En6
En9
ExB: vp
En4
ExC
En2: sp
En8: i
ExB: vb
En2: ip
En10
En7
ExA
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


LE: Thailand, BROOME, Mosquitoes
En8: s
En2: doi
En3
En5
En9
En6
ExB: vp
En4
En8: i
ExC
En2: sp
ExB: vb
En2: ip
En7
En10
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


LE: Malaysia, DARWIN, Heavy fliers
En10
En8: s
En2: doi
ExC
En5
En6
En9
En4
ExB: vp
En8: i
En2: sp
En7
En3
ExB: vb
En2: ip
ExA
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


LE: Philippines, DARWIN, Heavy fliers
En10
En8: s
En2: doi
ExC
En5
En9
En6
ExB: vp
En4
En8: i
En7
En2: sp
En3
ExB: vb
En2: ip
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


LE: Western Indonesia, DARWIN, Heavy fliers
En10
En8: s
En2: doi
ExC
En5
En4
En9
En6
ExB: vp
En8: i
En2: sp
En3
En2: ip
ExB: vb
ExA
En7
En1
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic


LE: Eastern Indonesia, WYNDHAM, Heavy fliers
En8: s
En2: doi
ExC
En5
En6
En9
ExB: vp
En4
En2: sp
En3
ExB: vb
En2: ip
En8: i
En7
En10
En1
ExA
-1.0
-0.5
0.0
0.5
1.0
Spearman's rho statistic