Import risk analysis: Specified members of the Order: Squamata from government-approved zoological collections in Australia

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Biosecurity New Zealand
Ministry of Agriculture and Forestry
Wellington
New Zealand

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1 Executive Summary

This risk analysis considers the disease risks associated with the importation of specified members within the Order Squamata (Class Reptilia) from government-approved zoological collections in Australia and eggs of these species from the same source.

1.1 LIVE SQUAMATA

From a preliminary list, those organisms considered to be potential hazards in the commodity were subjected to individual risk assessments. As a result of these, it was concluded that the risk in live Squamata was non-negligible for the following organisms:

- Pathogenic adenoviruses (Atadenoviruses)
- Exotic *Salmonella* spp.
- Gastro-intestinal nematodes
- Haemosporidian protozoa
- *Entamoeba invadens*
- Ectoparasites (ticks and mites).

Options for sanitary measures to effectively manage risks associated with these hazards are presented. These include requirements that imported animals are held in pre-export quarantine for 90 days and measures based on treatment, diagnostic testing, or veterinary certification.

1.2 EGGS OF SQUAMATA

Individual risk assessments for the importation of eggs of Squamata were carried out for those hazards identified in live Squamata. It was concluded that risks in eggs of Squamata are limited to pathogenic adenoviruses of lizards (Atadenoviruses) and exotic *Salmonella* spp.

Options for sanitary measures to effectively manage risks associated with these hazards are presented. These include requirements that eggs originate from premises where reptiles are under veterinary supervision and measures based on diagnostic testing and veterinary certification.

Disinfection of eggs is not considered appropriate and the reasons for this are discussed.

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2   Introduction

This risk analysis examines the disease risks posed by the importation of live animals and eggs of species within the Order Squamata (Class Reptilia) from government-approved zoological collections in Australia.

2.1   COMMODITY DEFINITION

The commodities covered in the risk analysis are clinically healthy live animals from defined species within the Order Squamata (Class Reptilia) (1) from Australia and eggs of those same species, also from Australia.

Only the following species are considered in this risk analysis:

Family: Agamidae	Frilled Lizard, *Chlamydosaurus kingii* Philippine Sail-finned Water Dragon, *Hydrosaurus pustulatus* Eastern Water Dragon, *Physignathus lesueurii lesueurii* Bearded Dragon, *Pogona* spp.
Family: Anguidae	Scheltopusik, *Pseudopus apodus*
Family: Chamaeleonidae	Veiled Chameleon, *Chamaeleo calyptratus*
Family: Gekkonidae	Madagascar Day Gecko, *Phelsuma madagascariensis grandis* Knob-tailed Gecko, *Nephrurus* spp.
Family: Iguanidae	Fijian Crested Iguana, *Brachylophus vitiensis* Common Iguana, *Iguana iguana*
Family: Scincidae	Cunningham's Skink, *Egernia cunninghami* Shingleback, *Trachydosaurus rugosus*
Family: Varanidae	Komodo Dragon, *Varanus komodoensis* Lace Monitor, *Varanus varius*

Individuals to be imported will have been resident in a government-approved zoological collection in Australia for at least twelve months prior to importation or will have been born in captivity in a government-approved zoological collection and remained there for their entire lives prior to importation.

Eggs to be imported will have been derived from individuals who have been resident in a government-approved zoological collection in Australia for at least twelve months or

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born in captivity in a government-approved zoological collection and remained there for their entire lives.

NOTE: The prevention or management of any adverse effects associated with a new lizard species entering New Zealand is a requirement of the Hazardous Substances and New Organisms (HSNO) Act 1996. Any application to import a new lizard species under Part V of the HSNO Act would need to be assessed under this Act by the Environmental Risk Management Authority (ERMA).

2.2 BACKGROUND

Curators of New Zealand zoological collections wish to import species from within the Order Squamata for the purposes of display and as part of a regional cooperative breeding programme for species conservation. Acquisition of some species through the importation of hatching eggs is considered feasible but that is not the case for all species, particularly those that are viviparous or oviviviparous, or some small species.

In general, disease surveillance in both wild and captive reptiles is poor (2). Availability of specialist veterinary services may be limited. Tentative diagnoses of diseases not previously diagnosed in a country may be made solely on the basis of clinical examination and, possibly, gross pathology. There is a need for specialist diagnostic services which are scarce or even unavailable in individual countries or particular localities (3). This situation is true of New Zealand, as recognised in the comprehensive review of disease surveillance in wildlife by McKenzie *et al*. (4), and this is almost certainly repeated in many other countries.
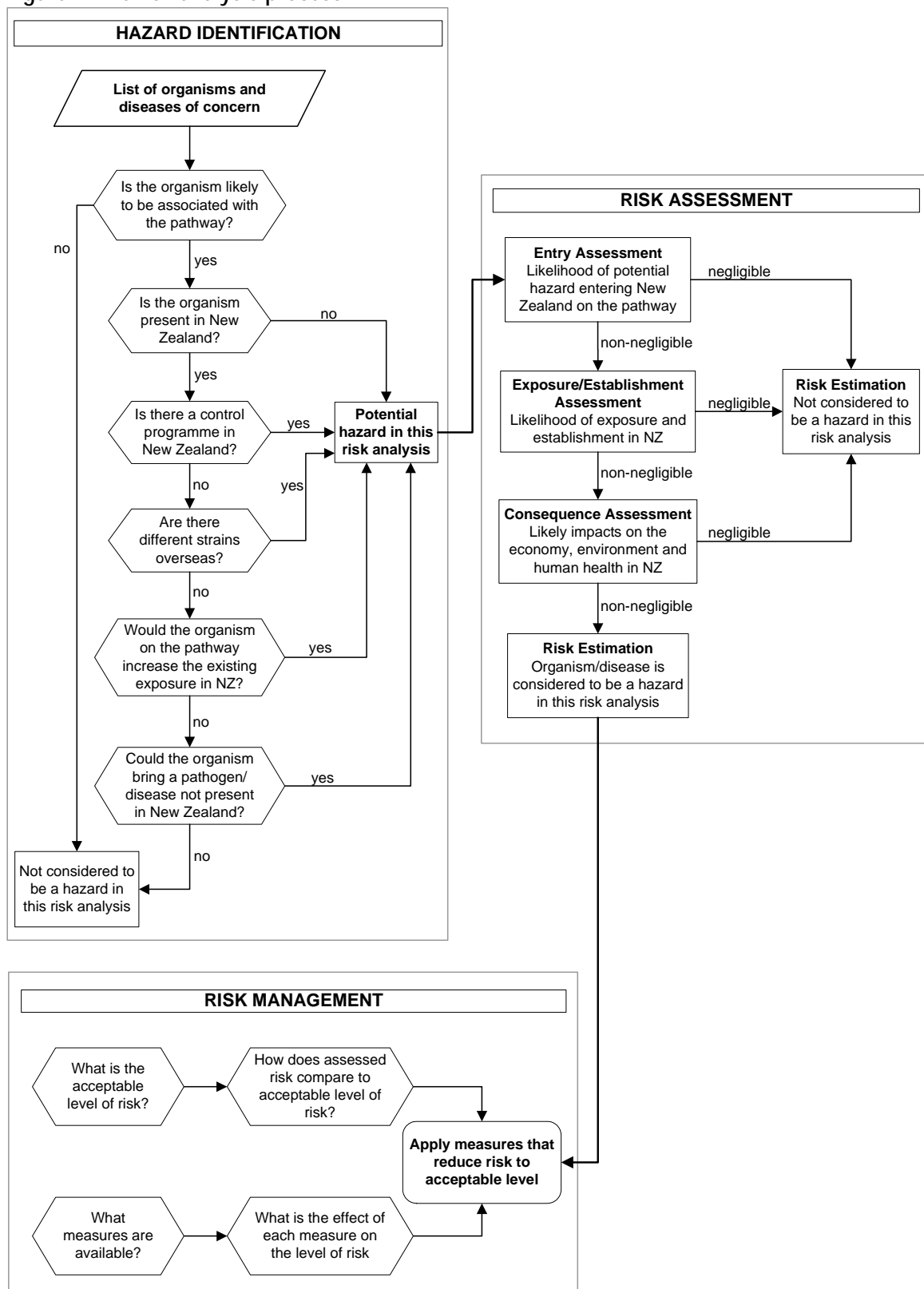
There are at least 59 endemic or native species from the Order Squamata in New Zealand. Twenty four of the species were classified by Daugherty *et al*. as rare and 22 were reported as mainly or entirely restricted to off-shore islands (5). It is vital that importation of Squamata does not put these endemic and native species at risk. In addition, lizard collections, including imported species, are held at a number of zoological and wildlife facilities, and others are held by professional or amateur herpetologists.

2.3 METHODOLOGY

The methodology used in this risk analysis follows the guidelines in Section 1.3 of the OIE *Terrestrial Animal Health Code* (6). In New Zealand, the OIE risk analysis framework is applied as described in *Risk Analysis Procedures – Version 1* (7).

The risk analysis process used by the MAF is summarised in Figure 1.

Figure 1. The risk analysis process.

HAZARD IDENTIFICATION

List of organisms and
diseases of concern

Is the organism likely
to be associated with
the pathway?

no

yes

Is the organism
present in New
Zealand?

no

yes

Is there a control
programme in
New Zealand?

yes

no

**Potential
hazard in this
risk analysis**

yes

Are there
different strains
overseas?

yes

no

Would the organism
on the pathway
increase the existing
exposure in NZ?

yes

no

Could the organism
bring a pathogen/
disease not present
in New Zealand?

yes

no

Not considered to
be a hazard in
this risk analysis

RISK ASSESSMENT

Entry Assessment
Likelihood of potential
hazard entering New
Zealand on the pathway

negligible

non-negligible

**Exposure/Establishment
Assessment**
Likelihood of exposure and
establishment in NZ

negligible

non-negligible

Consequence Assessment
Likely impacts on the
economy, environment and
human health in NZ

negligible

non-negligible

Risk Estimation
Not considered to
be a hazard in this
risk analysis

Risk Estimation
Organism/disease is
considered to be a hazard
in this risk analysis

RISK MANAGEMENT

What is the
acceptable
level of risk?

How does assessed
risk compare to
acceptable level of
risk?

**Apply measures that
reduce risk to
acceptable level**

What
measures are
available?

What is the effect of
each measure on
the level of risk

Preliminary hazard list

The hazard identification process begins with the collation of a list of organisms likely to be associated with the commodities. Table 1 shows these organisms, together with some of the key information considered for each organism. This list was compiled from those contagious diseases of lizards identified from the current edition (2006) of the multi-authored textbook *Reptile Medicine and Surgery* (8), other texts, key literature reviews, and electronic sources.

Table 1. Preliminary hazard list for species within the Order Squamata

Organism	Reported from lizards?*	Causes disease in lizards?	Disease in other Orders?**	Recognised as present in New Zealand?***	Requires further analysis?
Viruses					
Orthomyxovirus	No	N.A.	N.A.	N.A.	No
Paramyxovirus	Yes	Yes	Uncertain	No	Yes
Rhabdovirus	Yes	No	No	N.A.	No
Retrovirus	No	N.A.	N.A.	N.A.	No
Calicivirus	No	N.A.	N.A.	N.A.	No
Picornavirus	No	N.A.	N.A.	N.A.	No
Reovirus	Yes	Uncertain	Uncertain	No	Yes
Bunyavirus	No	N.A.	N.A.	N.A.	No
Togavirus	Yes	No	Yes	No	Yes
Flavivirus	Yes	No	Yes	No	Yes
Parvovirus	Yes	Yes	No	No	Yes
Iridovirus	Yes	Yes	Yes	No	Yes
Erythrocyticvirus	Yes	No?	Yes	No	Yes
Poxvirus	Yes	Yes	No	No	Yes
Herpesvirus	Yes	Yes	No	Uncertain	Yes
Adenovirus	Yes	Yes	No	No	Yes
Papillomavirus	Uncertain	Uncertain	No	No	Yes
Bacteria					
Chlamydophila spp. ****	Yes	Yes	Yes	Yes	No
Salmonella spp.	Yes	Yes	Yes	Yes/No	Yes
Escherichia coli ****	Yes	Yes	Yes	Yes	No
Neisseria iguanae	Yes	Yes	No	No	Yes
Mycobacterium spp.	Yes	Yes	Uncertain	Uncertain	Yes
Staphylococcus aureus ****	Yes	No	Yes	Yes	No
Pseudomonas spp. ****	Yes	Yes	Yes	Yes	No
Proteus spp. ****	Yes	Yes	Yes	Yes	No
Edwardsiella spp. ****	Yes	Yes	Yes	Yes	No
Aeromonas spp. ****	Yes	Yes	Yes	Yes	No
Edwardsiella tarda	Yes	No	Yes	No	Yes
Pseudomonas reptilivorous	Yes	Yes	No	No	Yes
Campylobacter spp. ****	Yes	No	Yes	Yes	No
Dermatophilus congolensis ****	Yes	Yes	Yes	Yes	No

Table 1 (continued)

Organism	Reported from lizards?*	Causes disease in lizards?	Disease in other Orders?**	Recognised as present in New Zealand?***	Requires further analysis?
Bacteria (cont.)					
Borrelia burgdorferi	Yes	No	Yes	No	Yes
Coxiella burnetii	Yes	No	Yes	No	Yes
Fungi					
Fungi and Yeasts	Yes	Yes	Yes	Yes/No	Yes
Protozoa					
Blood borne protozoa	Yes	Yes?	No	Yes/No	Yes
Entamoeba invadens	Yes	Yes	No	No?	Yes
Cryptosporidium spp. ****	Yes	Yes	No	Yes	No
Helminth parasites					
Nematoda	Yes	Yes	No	No	Yes
Trematoda	Yes	No	No	No	No
Cestoda	Yes	Yes	No	No	Yes
Acanthocephala	No	N.A.	N.A.	N.A.	No
Arthropods					
Pentastoma	Yes	No?	Uncertain	No	Yes
Ectoparasites	Yes	Yes	Yes	Yes/No	Yes

* "Lizards" = species with the suborder Sauria of the Order Squamata
** This relates to disease being caused by species or strains of organisms identified in lizards.
*** Refers to species or strains potentially in the commodity.
**** Consideration of the potential for these organisms to present a hazard to human health has been given. It is not considered that the potential presence of these organisms in the commodity will result greater exposure of people than currently occurs.
N.A. = Not applicable.

2.3.1 Risk analysis for importation of live Squamata

In section 3 of this analysis, for each organism identified as requiring further consideration in Table 1, the epidemiology is discussed, including a consideration of the following questions:

1. Whether imported lizards could act as a vehicle for the introduction of the organism?
2. If the organism requires a vector, whether competent vectors might be present in New Zealand?
3. Whether the organism is exotic to New Zealand but likely to be present in exporting countries?
4. If it is present in New Zealand,

      i. whether it is "under official control", which could be by government departments, by national or regional pest management strategies or by a small-scale programme, or

     ii. whether more virulent strains are known to exist in other countries?

For any organism, if the answer to question one is "yes" (and the answer to question 2 is "yes" in the cases of organisms requiring a vector) and the answers to either questions three or four are 'yes', it is classified as a potential hazard requiring risk assessment.

Under this framework, which is based on international agreements on trade in agricultural products, organisms that are present in New Zealand cannot be considered as potential hazards unless there is evidence that strains with higher pathogenicity are likely to be present in the commodity to be imported. Therefore, although there may be potential for organisms to be present in the imported commodity, the risks to human or animal health are no different from risks resulting from the presence of the organism in this country already. In such situations, measures to limit negative impacts on the health of humans or animals in contact with the imported commodity, or subsequent progeny, should be those appropriate to good practice irrespective of the importation.

In line with the OIE and MAF Biosecurity New Zealand risk analysis methodologies, for each potential hazard requiring risk assessment the following analysis is carried out:

Risk Assessment

a) Entry assessment -   the likelihood of the organism being imported in the commodity.

b) Exposure assessment -   the likelihood of animals or humans in New Zealand being exposed to the potential hazard.

c) Consequence assessment -   the consequences of entry, establishment or spread of the organism.

d) Risk estimation -   a conclusion on the risk posed by the organism based on the release, exposure and consequence assessments. If the risk estimate is non-negligible, then the organism is classified as a hazard.

In assessing the likelihood of exposure to lizards in New Zealand, an assumption is made that there is potential for contact between imported animals and their offspring and lizards in the outside environment. Such contact might be direct through the walls of enclosures, indirect through transfer of fomites, movement of rodents, insects or other animals, or through escape or release of the imported lizards or their progeny.

It is important to note that all of the above steps may not be necessary in all risk assessments. The OIE and MAF Biosecurity New Zealand risk analysis methodologies make it clear that if the likelihood of entry is negligible for a potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises where the likelihood of entry is non-negligible but the exposure assessment concludes that the likelihood of exposure to susceptible species in the importing country is negligible, or where both entry and exposure are non-negligible but the consequences of introduction are concluded to be negligible.

2.3.2   Risk management

For each organism classified as a hazard, a risk management step is carried out, which identifies the options available for managing the risk. Where the *Code* lists recommendations for the management of a hazard, these are described alongside options of similar, lesser, or greater stringency where available. In addition to the options presented, unrestricted entry or prohibition may also be considered for all hazards. Recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when an import health standard (IHS) is drafted. As obliged under Article 3.1 of the WTO Agreement on Sanitary and Phytosanitary Measures (the SPS Agreement) the measures adopted in IHSs will be based on international standards, guidelines and recommendations where they exist, except as otherwise provided for under Article 3.3 (where measures providing a higher level of protection than international standards can be applied if there is scientific justification, or if there is a level of protection that the member country considers is more appropriate following a risk assessment).

2.3.3   Risk communication

This draft import risk analysis is issued for a six-week period of public consultation to verify the scientific basis of the risk assessment and to seek stakeholder comment on the risk management options presented. Stakeholders are also invited to present alternative risk management options they consider necessary or preferable.

Following this period of public consultation on this draft document, a review of submissions will be produced and a decision-making committee will determine whether any changes need to be made to this draft risk analysis.

Following this process of consultation and review, the Imports Standards team of MAF Biosecurity New Zealand will decide on the appropriate combination of sanitary measures to ensure the effective management of identified risks. These will be presented in a draft IHS which will also be released for a six-week period of stakeholder consultation. Stakeholder submissions in relation to the draft IHS will be reviewed before a final IHS is issued.

2.3.4   Risk analysis for importation of hatching eggs of Squamata

In section 4, for each organism regarded as a hazard following the considerations in section 3, the risk associated with the importation of hatching eggs, rather than hatched lizards, is considered following the same procedures as in section 3 but considering only additional information relating to the importation of eggs. Because of the scarcity of information relating to transovarial transmission of disease agents in lizards, information pertaining to the transovarial transmission of similar organisms both in other reptiles and in birds has been used to provide guidance.

3   Organism Risk Analyses – Live Squamata

3.1   VIRUSES

3.1.1   Paramyxoviruses of lizards

3.1.1.1   *Hazard identification*

Aetiological agent

Reptilian paramyxoviruses (PMVs) are serologically related to avian paramyxoviruses (APMVs), mainly APMV-7 but with some cross reacting with APMV-1 (9, 10). Antigenic relationships between reptilian isolates, irrespective of their source, are stronger than the relationships between reptilian and avian PMVs (Ahne, unpublished, Cited by (11)). The majority of PMV isolates from reptiles have come from snakes, with very few from lizards. Richter *et al*. (12) confirmed that characteristics of viruses isolated from three snakes were consistent with their classification as PMVs although they were antigenically distinct from other PMVs. RNA sequence analyses of 16 reptilian PMVs (one of which came from a lizard with the others from snakes) suggested that they fell into two species with strain differences within the groups. Groupings coincided, largely, with the source of the isolates (USA and Germany/Switzerland) (11). On the basis of a number of parameters, Franke *et al*. (13) concluded that PMVs from snakes differed from other groups of PMVs and suggested that they should be classified within a new genus within the Paramyxoviridae.

OIE list

Reptilian PMVs are not notifiable to the OIE.

New Zealand status

Reptilian PMVs are not included in the register of unwanted organisms and have not been recognised in New Zealand.

Epidemiology

There are few reports of PMV infections of lizards and only two reports associating disease with those infections have been located.

Serological testing has shown evidence of exposure to PMVs to be common in clinically healthy Iguana (*Ctenosaura* spp. and *Iguana iguana*) sampled on islands off the coast of Honduras (20 of 49 sampled) (14), wild-caught clinically healthy *Xenosaurus* and *Abronia* spp. captured in Mexico (nine of 23 sampled) (15), and a mixed collection of lizards at London Zoo (9). The testing of samples from London Zoo used two isolates from different reptilian sources. Results indicated differences between the two isolates but with considerable cross reactivity.

In the studies at London Zoo (9) PMVs were isolated from two of the serologically positive animals and from another two that had not been tested serologically. One

Rhinoceros Iguana had hind-leg paralysis but there was no information to link that condition with PMV infection. All other animals tested were clinically healthy. Ahne and Neubert (16) (cited by (17)) isolated a PMV-like agent from a healthy Teju (*Callopistes maculatus*).

Jacobson *et al*. (18, 19) reported that on three occasions, from late 1998 to early 1999, Caiman Lizards (*Dracaena guianensis*) that had been imported from Peru to the United States of America became affected by respiratory disease with proliferative pneumonia. Viruses consistent with Paramyxoviridae were observed in tissues and isolated in tissue culture. Surviving animals from the first disease incident were found to have haemagglutinating antibodies to the isolated virus.

Ritchie (20) refers to a case in which a PMV-like virus was identified by electron microscopy in ascitic fluid from a Bearded Dragon with hepatitis. The relationship of this finding to the disease is unclear as hepatitis is not a feature of PMV-related disease reported from other reptiles or other animals.

There are numerous reports of PMV infections associated with high mortality in captive snake colonies. In snakes it is considered that excretion via the respiratory route is the main source of infectious virus. Following infection, serological responses might not be measurable for up to eight weeks and virus shedding may be prolonged. Whether vertical transmission occurs is not known (20). The evidence from London Zoo (9) suggests that there are limitations to the host range of specific PMVs but the range of PMVs infecting lizards and the degree of host-specificity of these viruses is unknown. No reports on means of PMV spread within lizard colonies have been located

Apart from the epidemic in snakes described by Hoser (2), which was initially diagnosed as due to paramyxovirus, and later attributed to a reovirus, the only reference located referring to evidence of reptilian paramyxoviruses in Australia is one commenting on positive serology to PMV-1 and PMV-2 in captive snakes (3).

Hazard identification conclusion
PMVs of lizards are considered to be a potential hazard in the commodity.

3.1.1.2   *Risk assessment*

Entry assessment
Although there is neither evidence of PMVs in lizards in Australia nor evidence of PMV-related disease in Australian snakes, the results of surveys of healthy animals in other countries (9, 14, 15) suggests that the likelihood of PMVs in lizards in Australia cannot be excluded.

The entry assessment is considered to be non-negligible.

Exposure assessment

The prevalence of PMV seropositive animals in populations studied suggests that PMVs in lizards act as contagious organisms within their host range (9, 14, 15). There is no evidence that PMVs infecting lizards may be transmitted to non-lizard species. The likelihood of spread from infected lizards to other lizards of the same species with which they are housed is considered high. The likelihood of spread to other species is unknown, although there is evidence that individual virus strains have restricted host ranges.

The exposure assessment is considered to be non-negligible.

Consequence assessment

Reports of disease in lizards, which may be attributable to PMV infection, have come from only one species (Caiman Lizards) imported to the United States of America from South America over a short period of time (18, 19). However, as no other descriptions of clinical disease in lizards due to PMVs have been located, and published surveys (9, 14, 15) have demonstrated no clinical effects in lizards infected with PMVs, the likelihood of disease arising from PMVs infecting lizards imported to New Zealand is considered to be negligible.

The consequence assessment is considered to be negligible.

Risk estimation

Since the consequence assessment is negligible, the risk estimate is considered to be negligible and PMVs are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.1.2 Herpesviruses of lizards

3.1.2.1 *Hazard identification*

Aetiological agent
Herpesviruses are enveloped viruses with a double stranded DNA core. The viruses fall into three Sub-Families, Alpha- Beta- and Gammaherpesvirinae. Viruses in this Family tend to have a high degree of host-specificity, commonly cause latent infections, and are labile in the environment.

OIE list
Reptilian herpesviruses are not notifiable to the OIE.

New Zealand status
Reptilian herpesviruses are not included in the register of unwanted organisms and have not been reported in New Zealand. No reports of virological examination of the disease known as "Mouth rot" (21) which is seen in New Zealand reptiles have been discovered but it seems reasonable to suggest that this condition might be similar to the conditions reported from *Gerrhosaurus* spp. and *Varanus prasinus* (22, 23) from which herpesviruses were identified (even though their roles in the aetiology of these conditions were not confirmed).

Epidemiology
The only reports located of herpesviruses from lizards are:

- Isolation and electron microscopic examination of a herpesvirus from cultured cells of *Iguana iguana* (24, 25). Experimental studies showed no evidence of pathogenicity. Although the authors named this virus "Iguana virus", it is now tentatively named Iguanid herpesvirus 1.

- Wellehan *et al*. (26) reported a novel herpesvirus from a San Estaban Chuckwalla (*Sauromalus varius*) which had died without previous signs. They considered that this virus was an alphaherpesvirus and applied the name Iguanid herpesvirus 2.

- Identification of viral particles morphologically similar to herpes-, reo- or papovaviruses from papillomas on the skin of laboratory-housed Green Lizards (*Lacerta viridis*) (27). This virus has now been tentatively named Lacertid herpesvirus.

- Identification of three novel herpesviruses from two Sudan Plated Lizards (*Gerrhosaurus major*) and one Black-lined Plated Lizard (*Gerrhosaurus nigrolineatus*) all with clinical signs of glossitis (23). The authors named these viruses Gerrhosaurus herpesviruses-1 to -3.

- Herpesvirus was identified from the three of four Green Tree Monitors (*Varanus prasinus*), with proliferative stomatitis, tested using PCR. DNA *in-situ* hybridisation was positive in tissues from the oral mucosa of all three animals tested and in the brains of two of them (22). The authors applied the name Varanid herpesvirus 1 to this virus.

Although classification of herpesviruses from lizards (and, more broadly, reptiles) is not complete, all authors have suggested that they fall within the Alphaherpesvirinae. Taxonomic studies have identified those viruses evaluated (Gerrhosaurid HV1, 2, and 3, Iguanid HV2, and Varanid HV1) as within an evolutionary branch separate from those of avian and mammalian alphaherpesviruses and suggest that they may have evolved with their hosts over 200 – 400 million years (22, 28, 29). This is consistent with the apparent close host-virus relationship of most herpesviruses with their hosts that contributes to pathogenicity being expressed only rarely.

Hazard identification conclusion
Herpesviruses of lizards have not been described in New Zealand and are considered to be potential hazards in the commodity.

3.1.2.2 *Risk assessment*

Entry assessment
These viruses commonly remain latent and, for many, expression of pathogenicity is exceptional. Uncommon expression of pathogenicity is consistent with the suggestion by McGeoch and Gatherer (28) that undetected herpesviruses may well be present in reptiles.

The entry assessment is considered to be non-negligible.

Exposure assessment
Although means of transmission of herpesviruses of lizards have not been examined, both vertical and horizontal transmission of other herpesviruses is reported with horizontal transmission requiring close contact because of the labile nature of these viruses.

The exposure assessment is considered to be non-negligible.

Consequence assessment
As with other herpesviruses, the herpesviruses of lizards appear highly host specific and very few incidents of disease have been reported. Should herpesvirus infections of imported lizards result in disease, this is likely to be restricted to the imported species. Based on reports (see above) morbidity is likely to be low and mortality unusual.

The consequence of herpesviruses in the commodity is considered negligible.

Risk estimation

Since the consequence assessment is negligible, the risk estimate is considered to be negligible and herpesviruses are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.1.3   Togaviruses in lizards

3.1.3.1   *Hazard identification*

Aetiological agent
The only reports of Togaviruses in lizards discovered refer to Eastern Equine
Encephalitis Virus (EEEV).

OIE list
EEEV is included in the OIE list of notifiable diseases.

New Zealand status
EEEV is included in the register of unwanted organisms.

Epidemiology
EEEV has been identified in Cuban Iguanas and other reptiles (Garter Snakes and Spotted
Turtles) but not other lizards. No reports associating clinical disease or pathology with
EEEV in reptilian species have been discovered. Infection of reptiles results in viraemia
lasting from 36 days to in excess of six months (17, 30).

EEEV is endemic in passerine birds in eastern North America and in parts of South
America. It is transmitted by *Culicoides* and *Aedes* mosquitoes and causes disease in
horses, humans, and pheasants (31).

Hazard identification conclusion
EEEV is considered to be a potential hazard in the commodity.

3.1.3.2   *Risk assessment*

Entry assessment
The limited geographic distribution of EEEV (parts of the Americas) (31), together with
the limited reports of EEEV in lizards, means that the likelihood of infection in lizards
(iguanas) from Australia is negligible.

The entry assessment is considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible
and EEEV is not classified as a hazard in the commodity. Therefore, risk management
measures are not justified.

3.1.4 Flaviviruses in lizards

3.1.4.1 *Hazard identification*

Aetiological agent
The flaviviruses that have been reported from lizards are West Nile Virus (WNV) in Green Iguana and Japanese Encephalitis Virus (JEV) in skinks (17, 30).

OIE list
Both WNV and JEV are included in the OIE list of notifiable diseases.

New Zealand status
JEV is included in the register of unwanted organisms. WNV is not included in the register of unwanted organisms.

New Zealand is considered to be free of both JEV and WNV.

Epidemiology
The only reports of JEV infection in lizards located were those by Doi, Oya, and others from Japan in 1983 (32, 33). They reported that *Takydromus tachydromoiodes* (Lacertid Lizards) and *Eumeces* spp. (skinks) (but not *Gekko japonicus*) became infected after intraperitoneal injection of JEV and after being fed infected mosquitoes. They also demonstrated that JEV infection could be established in the susceptible genera through the bites of infected mosquitoes and that the virus could be transferred to mice following the feeding of mosquitoes (*Culex* spp.) on infected lizards. 14.3% of *E. latiscutatus* and 4.0% of *T. tachydromoiodes* caught wild were serologically positive to JEV but attempts at virus isolation from blood were unsuccessful. A number of *Culex*, *Aedes,* and *Anopheles* spp. are competent vectors (34).

Birds, particularly passerines, are the reservoir hosts of WNV. The main vectors for the virus are mosquitoes. Disease has been reported from humans and horses, and in some locations (especially North America) there have been large numbers of deaths in birds. Green Iguanas (*Iguana iguana*) have been infected with WNV by inoculation with the virus. Some of the animals developed low levels of viraemia and virus was detectable in oral swabs, cloacal swabs, and organs of animals killed (35).

Hazard identification conclusion
It is concluded that JEV and WNV are considered to be potential hazards in the commodity.

3.1.4.2 *Risk assessment*

Entry assessment
JEV is present through temperate and tropical Asia. The virus has spread through Indonesia to Papua/New Guinea and islands in Torres Strait. In Australia, recognition of

the virus has been restricted to the northern tip and west coast of Cape York Peninsula (36) and it appears that the virus may not be established, but periodically introduced from Torres Strait islands or New Guinea (37). No reports suggesting that lizards play a significant role in the epidemiology of JEV have been located. The likelihood of JEV infection in the commodity is negligible.

Experimental infections of lizards with WNV have produced only low levels of viraemia. No suggestions that lizards play an important role in the epidemiology of the virus have been located. The geographic distribution of WNV is restricted to Africa, the Middle East, Europe, and North America. The likelihood of WNV infection in the commodity is negligible.

The entry assessments for both JEV and WNV are considered to be negligible.

Risk estimation

Since the entry assessments are negligible, the risk estimate is negligible and JEV and WNV are not classified as hazards in the commodity. Therefore, risk management measures are not justified.

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3.1.5 Adenoviruses of lizards

3.1.5.1 *Hazard identification*

Aetiological agent
Adenoviruses are non-enveloped, double-stranded DNA viruses (31). Four genera are identified by the International Committee on the Taxonomy of Viruses (38), although a fifth genus has been proposed (39). All reptilian adenoviruses characterised fall within the genus *Atadenovirus* (40).

OIE list
No members of the *Atadenovirus* genus are included in the OIE list of notifiable diseases.

New Zealand status
No members of the *Atadenovirus* genus are included in the register of unwanted organisms.

There are reports of adenoviruses from chickens, pigeons, and a number of mammalian species in New Zealand. Positive serology to adenoviruses is common in both chickens and pigeons (see release assessment).

The first report of adenoviral infection of a Bearded Dragon (*Amphibolurus barbatus*) was from an animal that died in quarantine in New Zealand following confiscation as an illegal importation (41). There is no evidence that infection spread beyond the quarantine facility.

Epidemiology
Individual adenovirus species have high levels of host-specificity confined to specific or closely-related host species. Most adenoviruses are able to survive for long periods in latent or inapparent forms in the absence of disease (42). A large proportion of adenovirus species exhibit no pathogenicity, others show pathogenicity only under conditions leading to increased susceptibility of the host species while a small proportion of species are pathogenic, regularly producing disease (31, 43).

Essbauer and Ahne (17) identified reports of adenoviruses in Bearded Dragons (*Amphibolurus barbatus* now *Pogona vitticeps*), Rankin's Dragons (*Pogona henrylawsoni*), a Jackson's Chameleon (*Chamaeleo jacksoni*), a Mountain Chameleon (*Chamaeleo montium*), and Savannah Monitors (*Varanus exanthemicus*). Wellehan *et al.* (23) used adenovirus material from Fat-tailed Geckos (*Hemitheconyx caudicinctus*), Leopard Geckos (*Eublepharis macularius*), a Tokay Gecko (*Gekko gecko*), a Gila Monster (*Heloderma suspectum*), a Blue-tongued Skink (*Tiliqua scincoides intermedia*), a Bearded Dragon, and a Mountain Chameleon in their phylogenetic studies.

Information on the sources of the material used by Wellehan *et al.* (23) is not complete but it appears that the only species from which there have been multiple reports of disease incidents is the Bearded Dragon (40, 41, 44, 45). Lay literature available on the internet indicates that adenovirus related disease is common and severe in this species held in captivity and that hobbyists commonly believe that individual breeders with infected colonies are the source of infected stock. Other reports have come from single disease incidents with several animals involved in each. No reports of investigations of the means of transmission of adenoviruses in lizards have been located but there is evidence of both vertical and horizontal transmission of adenoviruses in birds (46, 47).

Wellehan *et al.* (23) considered that the six lizard atadenoviruses examined by them, each from a different host species, were sufficiently different to be regarded as separate species. This is consistent with the general pattern of host-specificity of adenoviruses.

Hazard identification conclusion

It is concluded that atadenoviruses are considered to be a potential hazard in the commodity.

3.1.5.2   *Risk assessment*

Entry assessment

The extent of *Atadenovirus* infection of lizards is unknown. In other host species, the more investigations that are carried out, the more adenoviruses are found. This is illustrated by the recognition of at least 51 species of adenoviruses in humans, the development of antibody titres to a single strain of adenovirus in all lambs sampled on three farms in New Zealand without any established association with disease (48), the recognition of four serotypes of adenoviruses in chickens in New Zealand, mostly isolated from healthy birds (49), and the finding that 40% of New Zealand cattle tested were serologically positive to adenovirus (50) although associated disease is not common (51). There are very few reports of prospective investigations for the presence of viral agents in lizards and, with most infections likely to be sub-clinical, it is highly likely that adenovirus infections are more common than the scarce literature might suggest.

The entry assessment is considered to be non-negligible.

Exposure assessment

Atadenoviruses are infectious agents and, in classes other than reptilia, are known to be transmitted both vertically and horizontally. Even if infection at the time of importation were latent, transmission to other animals of the same, or closely related, species would be highly likely.

The exposure assessment is considered to be non-negligible.

Consequence assessment

The consequence of the introduction of *Atadenovirus* strains of nil, or low, pathogenicity is considered to be negligible.

Because of the host-specificity of adenoviruses, it is highly likely that any spread of higher pathogenicity *Atadenovirus* strains will be restricted to other animals of the same, or closely related, species. Based on the information available, it appears unlikely that disease, other than a small number of incidents in which individual animals might be affected, will occur in species other than Bearded Dragons. Nevertheless, because of the degree of uncertainty, the consequence is regarded as non-negligible.

The consequence assessment of the introduction of *Atadenovirus* or strains of high potential pathogenicity is considered to be low but non-negligible.

Risk estimation

Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and atadenoviruses are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

3.1.5.3   *Risk management*

Options

Serological tests for atadenoviruses of lizards are not available and culture of samples from respiratory or digestive tracts are of unknown sensitivity. The most reliable evidence of the absence of pathogenic atadenoviruses from imported lizards is considered to be the disease history of the source collection.

One or a combination of the following sanitary measures could be considered in order to effectively manage the risk:

1. Imported animals could originate from premises approved by the relevant government, or government approved agency, for holding reptiles.

2. All animals of the species to be exported to New Zealand could have been resident in the premises for at least 90 days prior to the commencement of a pre-export quarantine period or since birth/hatching.

3. The premises of origin could be under veterinary supervision and the health of the animal(s) monitored so that incidents of disease and death are identified promptly and Atadenoviruses excluded as the cause of illness or death within the past 12 months affecting any animals of the genus to be exported.

Options 1 and 2 are requirements of the commodity definition of this risk analysis. These commodity requirements will therefore provide some management of the risk associated with this hazard. However, if these measures are not considered to provide effective management of this risk, the inclusion of option 3 would be likely to significantly reduce any residual risk.

NOTE: The application of specific laboratory diagnostic procedures is not, necessarily, a requirement for the exclusion of atadenovirus-induced disease (or many other diseases). Commonly, specific pathogens can be excluded on clinical or pathological grounds or on the basis of the diagnosis of an alternative cause of disease. Certification required is from a veterinarian who must meet professional ethical standards in any certification. If he/she is not able to provide that certification then the animals will not be permitted to enter New Zealand. This statement applies to all conditions in the risk analysis where the exclusion of a particular aetiology of disease is desirable.

3.1.6 Poxviruses of lizards

3.1.6.1 *Hazard identification*

Aetiological agent

Poxviridae are enveloped DNA viruses. There are two Sub-Families, with all of the poxviruses of vertebrates being in the Chordopoxviridae. Genetic recombination of viral DNA may occur between viruses within the same genera, resulting in serological cross-reactions and cross-protection (52). No poxviruses of lizards appear in the International Committee on the Taxonomy of Viruses index of viruses (38) although poxviruses from Spectacled Caiman and from Nile Crocodiles are listed as non-assigned viruses within the Chordopoxviridae.

OIE list

Several poxviruses of mammals are included in the OIE list of notifiable diseases but no poxviruses of reptiles are included.

New Zealand status

No poxviruses of reptiles are included in the register of unwanted organsims.

A number of poxviruses of mammals are present in New Zealand as is fowlpox virus and poxviruses infecting a number of other avian species. No reports of poxviruses in reptiles in New Zealand have been located.

Epidemiology

Poxviruses are stable in dry environments and can be transmitted by aerosols, direct contact, fomites, or biting insects. Although there are suggestions by some authors that latent infections may occur and that these may be reactivated during times of stress (52) others, including Deem *et al*. (53), have doubted that latency occurs. Deem *et al*. (53) stated that latency has not been confirmed in any avian species and an extensive search of the literature has not identified confirmation that latency of poxviruses occurs. Recurrence of infections in individual animals and maintenance of infection in populations can be explained by the stability of poxviruses in the environment and the partial immunity developed by some animals in response to infections (54). These factors, together with differences in intensity of surveillance of the health status of different species, creates a situation in which absence of diagnoses of poxvirus disease in particular species has doubtful relevance as an indication of absence of infection. The clinical presentation of poxvirus infections is often mild to negligible. Bolte *et al*. (55) commented that although avipoxviruses have been reported from only 232 of the, approximately 9000 species of birds, "it is likely that many more birds are susceptible to avipoxviruses". Most poxviruses infect specific or closely-related species.

Reports of poxvirus (or pox-like virus) infections in lizards include:

- Virus associated with papillomatous lesions of the skin of a Green Lizard, (*Lacerta-viridis*) in France (27).

- Papular dermatitis in a captive Tegu Lizard (*Tupinambis teguexin*), which resolved over three to four months in the United States of America (56).

- Pox-like inclusions in circulating monocytes of a Flap-necked Chameleon (*Chamaeleo dilepis*) in Tanzania that had died after being held in captivity (57).

- A case of nodular dermatitis associated with a poxvirus in Emerald Swifts (*Sceloporus malachiticus*) held in a Hungarian zoo (58).

Although reports in the scientific literature are scarce, lay publications suggest that lesions attributed to poxvirus may be more common. Kaplan (59) identifies poxvirus as a cause of "bumps, lesions, and other dermatitis" of lizards and "Dr. Gecko" (60) suggests that poxvirus is a common viral infection of Leopard Geckos (*Eublepharis macularius*).

None of the viruses considered to be poxviruses or pox-like viruses in lizards has been characterised. Whether they fit within a recognised genus, or should be classified separately, is not known. Given the low level of surveillance of diseases in lizards and the unspectacular nature of disease reported to date, the range of poxviruses and their lizard hosts is likely to be much wider than documented.

Hazard identification conclusion
On the basis of the known host range for poxviruses in lizards and the high likelihood of viruses within this family infecting a wider range of hosts than currently recognised, it is concluded that poxviruses are considered to be a potential hazard in the commodity.

3.1.6.2   *Risk assessment*

Entry assessment
Given the low level of disease surveillance in lizards and the lack of clinical signs in infected animals, it cannot be assumed that poxviruses of lizards do not occur in Australia. The entry assessment for poxviruses in the commodity is considered to be non-negligible.

Exposure assessment
Introduction of poxvirus-infected animals to a colony previously free of the virus can be expected to result in direct spread to susceptible animals, contamination of the environment, and transfer of virus through human contact or through fomites to other sites. Infection and clinical disease are likely to be restricted to animals of the species originally infected or species closely related.

The exposure assessment is considered to be non-negligible.

Consequence assessment

Based on reports available, it is highly likely that disease will be restricted to one species although it may include other closely related species. Disease is likely to be restricted to skin lesions and to resolve with only minor, or no, intervention within several weeks.

The consequence of poxviruses in the commodity is considered to be negligible.

Risk estimation

Since the consequence assessment is negligible, the risk estimate is considered to be negligible and poxviruses are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.1.7   Iridoviridae of lizards

3.1.7.1   *Hazard identification*

Aetiological agent

The family Iridoviridae consists of large viruses with double stranded DNA. There are four genera within the family. Iridoviruses and chloriridoviruses mainly infect invertebrates, particularly insects. Lymphocystiviruses and ranaviruses infect ectothermic vertebrates and fish species. Ranaviruses also infect amphibians and reptiles (38, 61, 62).

OIE list

Epizootic haematopoietic necrosis virus and Red sea bream iridovirus (both unassigned members of the *Ranaviru*s genus) are included in the OIE list of notifiable diseases.

New Zealand status

Epizootic haematopoietic necrosis virus and Iridovirus of bivalve molluscs are listed in the unwanted organisms register.

Iridoviruses have been identified infecting larvae of *Wisneana cervinata* (Porina) (63, 64), *Eudonia* sp. (sod web worms) (63), *Costelytra zealandica* (grass grub) (64), and *Opogonia sp*. (scarabeid beetles) (65) in New Zealand.

Epidemiology

The phylogeny of Iridoviridae is uncertain with initial nomenclature being largely based on the host species from which the virus was first identified. Later work on characterisation used DNA techniques to produce different pictures of genetic diversity or homology (38, 62, 66). The ability of specific viral strains to infect multiple host species has been demonstrated (61, 67). Both Webby *et al.* (67) and Hyatt *et al.* (66) reported phylogenetic patterns corresponding with the broad geographic sources of virus isolates used in their studies. They suggested that individual isolates not fitting this pattern might have been moved between continents with host species.

Two iridoviruses, one from each of Australia and New Zealand, were shown to have close homologies in their nucleotide sequences (67). Experimental transmission of ranaviruses has been accomplished by oral and intraperitoneal routes (38) but the importance of these, or other, routes in natural transmission is not known.

There is a large number of reports of ranaviruses in fish (17, 38, 62, 66) and a considerable number from amphibians and testudines (turtles and tortoises) (17, 61, 62, 66). Reports of ranaviruses from lizards and snakes are rare.

Reports of a total of six *Iridovirus* infections of lizards have been located (all from Germany) (61, 68). These included infections of two Bearded Dragons (*Pogona vitticeps*), a chameleon (*Chamaeleo quadicornis*), and a Frill-necked Lizard (*Chamydosaurus kingii*) from different sources between 1998 and 2000. The affected

lizards had pox-like lesions on their skin, became emaciated and died. All viral isolates were characterised and identified as identical to *Gryllus bimaculatus* iridovirus (GbIV) (68, 69). *Gryllus bimaculatus* is a species of cricket, produced commercially and used as food for captive lizards.

The only report located of a *Ranavirus* being isolated from a lizard was also from Germany. That was of a virus isolated from a gecko (*Uroplatus fimbriatus*) and characterised as being related to frog virus 3 (FV3), the type species for the *Ranavirus* genus (70). This animal had developed an ulcerative glossitis with bacterial infection in the tongue and a bacterial focal hepatic necrosis. Inclusion bodies characteristic of infection with *Iridoviridae* were not identified. The gecko was housed in a private collection with one amphibian and several other reptiles. It was the only animal affected and the relationship between the virus and disease was uncertain.

Hazard identification conclusion
The incidents in which iridoviruses of insects, bred as food for lizards, became pathogenic to lizards are not considered to represent hazards in the commodity as the infections in lizard colonies appear to have been self-limiting and maintenance of the iridovirus was dependent upon the cricket colonies.

It is concluded that iridoviruses are not considered to be a potential hazard in the commodity.

The identification of a single lizard, housed with an amphibian and other reptiles, becoming infected with a *Ranavirus* of uncertain pathogenicity is not considered to represent a hazard.

It is concluded that ranaviruses are not considered to be a potential hazard in the commodity.

3.1.8   Erythrocytic virus of lizards

3.1.8.1   *Hazard identification*

Aetiological agent

Structures observed in erythrocytes of lizards and other ectothermic animals, previously considered to be protozoa and named *Pirhemocyton* or *Toddia*, are now recognised as due to viruses termed erythrocytic viruses (EVs) (71-75). The term *Toddia* has been used most commonly for EVs in frogs, erythrocytic necrosis virus and *Immanoplasma* for EVs in fish, and Pirhemocyton for EVs in reptiles and frogs.

Although EVs are commonly assumed to be in the *Iridovirus* genus, there is insufficient information available to allow classification (71-73, 76). In blood smears EVs appear as an acidophilic area up to 4μm diameter with, at times, a central heavily staining area. Electron microscopy reveals numerous polygonal structures (mostly hexagonal) approximately 0.20 – 0.24 μm wide with the appearance of viral particles (71). On the basis of size, structure and positive Feulgen staining (77) the structures are considered to be DNA viruses.

In this risk analysis the term erythrocytic virus (EV) will be used in place of other terms unless there is a particular reason not to do so.

OIE list

EV is not included in the OIE list of notifiable diseases.

New Zealand status

Erythrocytic necrosis virus is listed in the register of unwanted organisms. In the scientific literature, the use of this term is restricted to EVs in fish.

Epidemiology

Reports of lizard EVs have been from *Gehyra variegata* collected in New South Wales (71), a further 11 species of lizards in northern Queensland and South Australia (78), two species of Agamidae and one Scincidae examined in southern Queensland (79), three species of *Takydromus* in Japan and Thailand (and expected to be present in these species through most of their range from Japan to Indonesia and through much of eastern and south eastern Asia) (80, 81), two species of chameleon (*Chamaeleo dilepis* and *Bradypodion fischeri*) in Tanzania (82), *Iguana iguana* in Brazil (83), and two species of *Lacerta* in Portugal (84). EVs of reptiles and anurans have been reported from North, South, and Central America, Europe, Asia, Australia, Japan, and Pacific Islands (76).

Telford (81) described the co-infection of *Takydromus* spp. with EV and protozoal parasites as a symbiotic relationship while Davies and Johnston (76) commented on the large number of blood borne parasites of ectotherms (25 genera, including EV, in lizards) with very few negative effects on their hosts and contrasted that with mammals in which there are fewer blood borne parasites but with many of them causing serious disease. The

prevalence of infection detectable by examination of blood smears is variable: 4.3% in *Takydromus tachidromoides* in Japan (80), 10% of lizards sampled in northern Queensland and South Australia (78), 15% of chameleon sampled in Tanzania and 8% of all lizards sampled at a south east Queensland wildlife park (79).

The majority of reports of EVs in lizards have been from the sampling of free-living animals. No reports arising from investigations of disease or population die-offs, either in the wild or in zoological collections have been located. The only report traced in which the sampled animals had histories of injury or illness is that of Pierce and Adlard (79) where the animals had been brought to a wildlife hospital. They were sampled as part of a survey and there were no connections drawn between the viral infections and the reasons for their presentation.

Alves de Matos *et al*. (84) reported that, following experimental infection of *Lacerta monticola* and *L. schreiberi* with EVs derived from the same species as the experimental hosts, evidence of infection was limited to erythrocytes in most animals and recovery followed even in animals with up to 98% of erythrocytes showing evidence of viral infection. The death of five experimental *L. schreiberi* in which there was no evidence of infection in erythrocytes was unexplained.

Hazard identification conclusion

On the basis of evidence that EVs are endemic in many lizard populations and the lack of evidence that EVs cause disease in lizards except, possibly, following artificial infections, it is concluded that EVs are not considered to be a potential hazard in the commodity.

3.1.9   Papillomavirus of Lizards

3.1.9.1   *Hazard identification*

Aetiological agent
Papillomavirus is a genus within the family Papillomaviridae.

OIE list
Reptilian papillomaviruses are not included in the OIE list of notifiable diseases.

New Zealand status
Not listed in the unwanted organisms register.
No reports of papillomaviruses in lizards in New Zealand have been located.

Epidemiology
Jacobson (85) identified a number of reports of papilloma-like lesions in lizards, in none of which did there appear to be serious health consequences. The reports, most clearly suggestive of papillomas of lizards with *Papillomavirus* as their cause are those of Cooper (86) referring to viral particles in lesions on a Green Lizard (*Lacerta viridis*) and papillomatous growths around the eyes of iguanas ((87) cited by (20)).

Hazard identification conclusion
On the basis of the scarcity of reports, the relatively minor nature of lesions and the uncertainty of aetiological diagnoses, papillomaviruses are not considered to be a potential hazard in the commodity.

3.1.10 Parvoviruses of lizards

3.1.10.1 *Hazard identification*

Aetiological agent

The *Parvoviridae* family includes the genera *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Densovirus*, *Iteravirus,* and *Brevidensovirus*. The only reports located of *Parvoviridae* in lizards are of *Dependovirus*.

Parvoviridae are single stranded DNA viruses replicating within the nuclei of dividing cells. With the possible exception of goose parvovirus, which may be able to be classified as a *Dependovirus* (88), dependoviruses are considered of no clinical importance. Dependoviruses are usually dependent upon co-existence with adenoviruses.

OIE list

No members of the Parvoviridae are included in the OIE list of notifiable diseases.

New Zealand status

Goose Parvovirus is listed in the unwanted organisms register.
No reports of *Parvoviridae* infections of lizards in New Zealand have been located.

Epidemiology

Two reports of dependoviruses in lizards have been located. Both reports involved multiple cases of adenoviral disease in Bearded Dragons in which the adenovirus was sufficient to explain the disease (45, 89).

Hazard identification conclusion

Reported dependovirus infections of lizards appear consistent with those of other species in that they are dependent upon adenovirus infection and may be considered clinically irrelevant.

Dependoviruses are not considered to be a potential hazard in the commodity.

3.1.11   Reoviruses of lizards

3.1.11.1   *Hazard identification*

Aetiological agent
The *Reoviridae* family includes several genera including orthoreo- orbi- and rotaviruses which are the genera considered of veterinary importance. Reoviruses are non-enveloped viruses with double stranded DNA.

OIE list
Reptilian reoviruses are not included in the OIE list of notifiable diseases.

New Zealand status
Reptilian reoviruses are not listed in the unwanted organisms register and no reports of their identification in New Zealand have been located.

Epidemiology
Serological evidence of reoviral infection was found in 23 of 49 serum samples from healthy iguanas (*Ctenosaurus* and *Iguana* spp.) wild caught on islands off the Honduras coast (14) and in three of 23 healthy *Xenosaurus* and *Abronia* spp. wild caught in Mexico (90). Reoviruses were not isolated from the tissues collected from animals in either of these investigations.

Drury *et al*. (91) identified reovirus and *Salmonella* infection along with large numbers of eggs and larvae thought to be those of oxyurid nematodes in a group of *Uromastyx hardwickii* imported from Pakistan to the United Kingdom, all of which became ill and died. The authors made no interpretations as to the pathogenic role of any of these infectious agents. Drury (unpublished, cited in (91)) identified reovirus in faeces from a chameleon. There is no comment on the clinical condition of the chameleon.

Reoviruses isolated from iguanas and serotyped by Blahak *et al*. (92) were classified into serotypes, one of which was shared with at least two isolates from snakes. The six reptilian reoviruses included in this study were all considered to be distinct from avian and mammalian reoviruses.

Hazard identification conclusion
On the basis that serological evidence indicates that reovirus infections are common in healthy lizards, at least in some localities, and that the only available evidence that reoviruses might contribute to disease comes from one incident in which other known pathogens were present, these viruses are not considered to be a potential hazard in the commodity.

3.2 BACTERIA

3.2.1 Salmonellae

3.2.1.1 *Hazard identification*

Aetiological agent

The *Salmonella* genus contains over 2,400 serotypes within two species; *S. enterica*, which contains most Salmonellae of veterinary or human interest, and *S. borgori*. *S. enterica* is further divided into subspecies *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), *bongori* (V), and *indica* (VI). Over 2,300 of the serotypes fall within the *S. enterica enterica* subspecies. The commonly used names (e.g. *Salmonella* Typhimurium) identify serotypes within the *Salmonella enterica enterica* sub-species. Some of these serotypes are further partitioned on the basis of phage type. *Salmonella enterica arizonae* contains over 300 serotypes (93, 94).

OIE list

Salmonella serotypes other than *S*. Gallinarum and *S*. Pullorum are not included in the OIE list of notifiable diseases.

New Zealand status

S. Gallinarum, *S*. Pullorum, *S*. Abortusovis, *S. arizonae*, *S*. Dublin, *S*. Typhimurium DT 104, *S*. Typhimurium DT 44, *S*. Enteritidis pt 4, and *Salmonella* spp. (exotic, affecting animals) are included in the register of unwanted organisms.

S. Gallinarum has not been diagnosed in New Zealand and, following an extensive eradication programme operated within the commercial poultry industries, *S*. Pullorum was last diagnosed in 1985.

S. Typhimurium DT 104 is isolated from humans and non-human sources in New Zealand relatively infrequently. *S*. Enteritidis phage type 4 is the second most common *S*. Enteritidis phage type isolated from humans in New Zealand and isolations from animal sources have been infrequent.

In New Zealand, over the period 2003 to March 2006, 21 *Salmonella* isolates from reptiles were typed at the Enteric Reference Laboratory of the Institute of Environmental Science and Research Ltd (ESR). Nineteen of those isolates were submitted to ESR during 2005 with ten of the isolates (*S*. Mount Pleasant, *S*. Onderstepoort and *S*. Biljmer) coming from one property. The 21 isolates, together with data on the same serotypes from other sources, are listed in Table 2.

Table 2. New Zealand reptile-associated Salmonella isolates, 2003-06

Salmonella serotype	Number of isolates from reptiles	Isolates from other non-human sources	Isolates from human sources
S. Adelaide	2	1-environmental	0
S. Bijilmer	6	0	0
S. Mississippi	1	0	56
S. Mount Pleasant	1	0	0
S. Muenchen	4	1-source not specified	0
S. Onderstepoort	3	0	0
S. Saintpaul	1	1-feed 1-canine 5-bovine	148
Subspecies I	1	0	0
Group R:-:-	1	0	0
Group P 38:-:1,5	1	0	0

At Auckland Zoo, from 1985 to 2002, eleven salmonellae were isolated from reptiles. Nine serovars of *S. enterica enterica* were identified along with two isolates of *S. enterica arizonae* (which were not serotyped further). Six of these isolates came from lizards and four (two from lizards) represented first records of the serotype in New Zealand.

McInnes (95) reported that *S.* Saintpaul had been isolated from approximately 10% of lizards sampled in Central Otago prior to 1968. However, *S.* Saintpaul was not recovered from any of 35 lizards (*Hoplodactylus pacificus* and *Leiolopisma zelandica*) sampled from an Otago farm on which sheep were infected with the organism during that year.

Over the period from 1999 to 2005 *Salmonella* isolates from humans yielded over 140 serotypes/phage types. During the same period, typing of isolates from animals, their feeds, and their environment yielded over 80 serotypes/phage types. The frequency with which specific types were isolated each year varied greatly and many of the serotypes/phage types were isolated from human or non-human sources on only one occasion. Each year, three to five serovars or phage types not previously identified in New Zealand were reported. Most were from humans, most of whom were travellers or immigrants (96, 97).

As many *Salmonella* infections are subclinical, the full range of serovars and phage types present in New Zealand and the extent of introductions to the country are unknown. The extent to which the range of salmonellae in New Zealand may be understated is illustrated by an incident investigated by Biosecurity New Zealand in 2005, in which three previously unrecorded serotypes (*S.* Mountpleasant, *S.* Onderstepoort and *S.* Biljmer) were identified in lizards on the one property.

Epidemiology

The epidemiology of different *Salmonella* serotypes follows broadly similar patterns. Spread within and between susceptible species is mainly via the faecal-oral route, with

bacteria passed by infected animals able to survive for varying periods of time in different environmental niches. Host-specificity or host preference varies between *Salmonella* serotypes.

There are numerous reports in published literature reporting the recovery of multiple serotypes of *S. enterica enterica* and *S. enterica arizonae* associated with both free-living and captive lizard populations (98-103). A great many healthy lizards harbour salmonellae and Burnham *et al*. (103) suggested that all iguanas and probably most reptiles may be infected with *Salmonella*. The intermittent shedding of salmonellae demonstrated by Burnham *et al*. will have resulted in an underestimate of the prevalence of infection in most surveys. It appears that salmonellae are part of the normal gut flora of healthy lizards although at times they may act as opportunist pathogens.

The potential host ranges of salmonellae found in lizards are not known. Some have been isolated from domestic animals but many more have been isolated from humans in association with disease. Weiss *et al*. (104) reported on 858 *S. arizonae* isolates examined at the United States Center for Disease Control (CDC) between 1967 and 1976. These isolates fell into 143 serotypes, seven of which were recovered from lizards (reptiles other than snakes or turtles). One of these serotypes was also recorded as infecting humans and another had been isolated from humans, sheep, snakes, and turtles. One of the serotypes identified by Weiss *et al*. only from lizards was recovered from a human infection in the United Kingdom (105). Examination of more recent data from the CDC (106) reveals that the majority of *S. enterica enterica* serotypes identified from reptiles have also been isolated from humans but that applies to only a smaller proportion of *S. enterica arizonae* serotypes. The extent to which that reflects a lesser ability of the *S. enterica arizonae* serotypes to infect humans, or a lower level of human exposure to the organisms, is not known.

Pasmans *et al*. (107), in Belgium, examined a wide range of salmonellae, from four subspecies, isolated from lizards and concluded on the basis of phenotypic and genotypic characteristics that all strains were capable of infecting humans. Whether such infections arise will be influenced by contact between the infected lizard and humans and on the hygiene precautions taken.

Reptiles, including lizards, are a source of *Salmonella* infection in humans. The role of lizards as a source of human *Salmonella* infections is illustrated by case reports, predominantly from the United States of America but also from Canada, Europe, and elsewhere (108-117). Mermin *et al*. (118), based on a case-control study involving five States, calculated that exposure to reptiles or amphibians contributed approximately 74,000 human cases of salmonellosis (or 6% of sporadic cases) in the United States each year.

Hazard identification conclusion
Salmonellae are considered to be a potential hazard in the commodity.

3.2.1.2   *Risk assessment*

Entry assessment

High proportions of reptiles, including lizards, are infected with salmonellae. It should be assumed that any group of lizards destined for importation to New Zealand is infected unless there is very good evidence to the contrary. Reliable evidence would require sampling and testing of animals on several occasions over a period of weeks or months.

The entry assessment is considered to be non-negligible.

Exposure assessment

Salmonellae are contagious organisms. Strains introduced with the commodity may infect other lizards and it is likely that they will have the potential to infect humans who come in contact with them and do not take appropriate hygiene precautions. Some strains may have the potential to infect other species but for them to do so will require contact between that other species and the lizard, its faeces, contaminated fomites, or humans carrying infection from the lizards.

The exposure assessment is considered to be non-negligible.

Consequence assessment

Given that many lizards currently in New Zealand can be expected to be infected with salmonellae, any changes in the level of risk to humans will arise mainly from changes in the level of exposure of the human population to lizards. Reptile (lizard)-associated salmonellosis can be expect to vary with the number of households owning reptiles and the degree of direct contact between reptiles (lizards) and people (118).

It is anticipated that approval from ERMA for the importation of lizards in the category of "new organisms" will require that they be held in containment. This will limit contact to people approved to enter the containment facility who should be well briefed on precautions to avoid zoonotic infections. Any increase in likelihood of human infections will arise from any increase in numbers of staff required to enter containment facilities. Occupational safety and health legislation will apply to establishments managing containment facilities and systems will be required to ensure that the hazards to staff are minimised.

Lizards not in the category of "new organisms" are those species recognised by ERMA as having been present in New Zealand (outside containment) prior to 29 July 1998. It is anticipated that these species may be legally released from containment when biosecurity requirements have been met. Any increased likelihood of human (or animal) infection arising will arise from increased numbers and/or broader distribution of members of the imported species. It is considered very unlikely that importation of members of a species that has been present in New Zealand for at least eight years will result in such increases.

When viewed in the context of the ongoing infection of humans and other species in New Zealand and the range of pathways available for entry of salmonellae (119), it could be

argued that any salmonellae which might be present in lizards will not result in exposure of humans or other animal species that is much greater than that which currently occurs.

However, given that imported lizards could potentially be harbouring Salmonella serotypes and phage types that are not known to be present in New Zealand, and imported lizards that are not "new organisms" could be sold as pets, the Ministry of Health have indicated that they consider there to be a non-negligible likelihood of humans being exposed and consequently infected with exotic serotypes/phage types of Salmonella and the consequence assessment should therefore be considered to be non-negligible.

Risk estimation

Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and exotic *Salmonella* spp. are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

3.2.1.3   *Risk management*

Options

Mitchell (8) compared the use of microbiological enrichment culture, a commercial ELISA, and PCR (University of Georgia) to detect *Salmonella* spp. in Green Iguanas. He concluded that the PCR assay was considerably more sensitive than either the ELISA or culture, whilst the specificity of culture was higher than the PCR and ELISA tests. It was suggested that parallel testing with both the PCR assay and microbiological culture could be used to further increase the overall sensitivity and specificity of the testing methods.

Mitchell went on to suggest that where access to tests other than microbiological culture is limited, then a minimum of five cloacal/faecal samples should be collected over a 30-day period to determine the *Salmonella* status of a reptile.

It is suggested that one or a combination of the following sanitary measures could be considered in order to effectively manage the risk:

1.   Animals to be imported could be required to be clinically healthy and in particular not to have diarrhoea.

2.   Faecal/cloacal samples could be cultured for *Salmonella* spp. All *Salmonella* spp. isolated could be serotyped (and, where appropriate, phage typed) and the results reported to MAF. Where exotic *Salmonella* spp. are isolated, importation could be prohibited.

3.   Five faecal/cloacal samples could be collected over a 30-day period, consistent with the advice of Mitchell (8). Alternatively, parallel testing of faecal/cloacal samples with both the PCR assay and microbiological culture could be used to further increase the overall sensitivity and specificity of the testing methods.

3.2.2   *Neisseria* spp.

3.2.2.1   *Hazard identification*

Aetiological agent
Neisseria spp. are aerobic, Gram-negative, diplococci. They normally inhabit mucosal surfaces and require a moist environment to survive.

OIE list
Neisseria spp. are not included on the OIE list of notifiable diseases.

New Zealand status
Neisseria catarrhalis has been reported in New Zealand, particularly in association with pneumonic lesions in lambs.

Epidemiology
A *Neisseria* sp. was isolated from both healthy and diseased iguanas at the National Zoological Park in Washington, D.C. Associated disease included septicaemia and chronic abscesses. 50% of healthy iguanas were found to be carrying the organism (which was of an unidentified species) in their mouths (120). This organism was subsequently characterised and named *Neisseria iguanae* (121).

Hazard identification conclusion
Based on the single geographic location from which this organism has been reported and the lack of other reports of *Neisseria* spp. from lizards, *N. iguanae* is not considered to be a potential hazard in the commodity.

3.2.3 *Mycobacterium* spp.

3.2.3.1 *Hazard identification*

Aetiological agent

Mycobacterium spp. are non-motile, aerobic, rod-shaped bacteria with lipid-rich ("acid-fast") cell walls. Although *Mycobacterium* spp. are well known as pathogens affecting humans, other mammals and birds, the majority of mycobacteria survive in soil and water, rarely, if ever, causing disease.

OIE list

M. tuberculosis is not included in the OIE list of notifiable diseases.

Bovine tuberculosis due to *M. bovis* and paratuberculosis due to *M. avium* subsp. *paratuberculosis* are both included in the OIE list of notifiable diseases.

New Zealand status

M. tuberculosis is not listed in the Ministry of Agriculture and Forestry unwanted organisms register. It is endemic in New Zealand and predominantly a disease of humans. It is a notifiable disease under the provisions of the Tuberculosis Act 1948 administered by the Ministry of Health.

M. bovis is listed in the unwanted organisms register as a reportable organism. *Mycobacterium* spp. (exotic strains) are listed in the unwanted organisms register as an "other exotic organism".

Epidemiology

No reports of *M. tuberculosis* or *M. bovis* infecting lizards have been discovered.

Soldati *et al*. (122) comment that "mycobacterial infections have been reported frequently in a wide variety of reptiles, including snakes, turtles, lizards, and crocodiles". However, references provided by Soldati *et al*. are text books unavailable to this author. It might be that reports of *Mycobacterium* spp. infecting lizards formed only a small proportion of those encountered by Solidati *et al*. as reports specifically relating to mycobacterial infections of lizards are not identifiable from the text Reptile Medicine and Surgery (8). Friend and Russell (123) reported a case of *M. intracellulare* in a Water Monitor and Solidati *et al*. (122) identified mycobacterial DNA in archived formalin-fixed tissue from three of 15 lizards with granulomatous lesions. The techniques used required extraction of DNA, PCR testing, and subsequent sequencing. Two of the mycobacteria were identified as "other than *Mycobacterium tuberculosis* complex" and the other as having a 97% sequence homology with *M. agri*.

The "mycobacteria other than *M. tuberculosis* complex" includes a very large number of mycobacteria, most of which do not cause disease or contribute to disease only rarely as adventitious invaders. *M. avium-intracellulare* is present in New Zealand.

Hazard identification conclusion
Based on the scarcity of reports of infections in lizards with mycobacteria not known to be present in New Zealand, and the lack of evidence that these are contagious infections, it is concluded that *Mycobacterium* spp. are not considered to be a potential hazard in the commodity.

3.2.4 Borrelia burgdorferi

3.2.4.1 *Hazard identification*

Aetiological agent

Borrelia burgdorferi is a large spirochaete, labile in the environment, and sensitive to desiccation. As with other *Borrelia* spp., it is transmitted by arthropod vectors. This organism is the cause of Lyme disease. A number of genotypes (genospecies) have been identified in the United States of America and Europe (124).

OIE list

B. burgdorferi is not included in the OIE list of notifiable diseases.

New Zealand status

Borrelia burgdorferi is listed in the register of unwanted organisms and has not been identified in New Zealand.

Epidemiology

Lyme disease, caused by *Borrelia burgdorferi*, affects dogs, horses, cattle, and humans. These species are incidental hosts to an organism that normally cycles between reservoir hosts (predominantly small mammals) and tick vectors (generally of the *Ixodes* genus). The maintenance hosts for adult ticks are larger mammals which are not reservoir hosts for *Borrelia*. In Europe, the main vector is *Ixodes ricinus*, in the eastern United States it is *I. scapularis*, in the western United States it is *I. pacificus,* and in Eurasia it is *I. persulcatus* (124). The distribution of *Ixodes* spp. ticks that are able to transmit the agent of Lyme disease spreads in a broad band across North America, Europe, and northern Asia (125).

Because of the importance of *I. scapularis* as a vector for *B. burgdorferi* in the United States, and its parasitism of lizards, there have been a number of investigations of the potential role of lizards as sources of infection for the ticks. Although reports from the western United States (126-131) do not support the hypothesis that lizards play a role in epidemiology of the spirochaete, investigations in the south eastern United States indicated that ticks feeding on artificially infected *Eumeces inexpectatus* (Five-lined Skink) and *Anolis carolinensis* (Green Anole) could become infected with *B. burgdorferi* for at least eight weeks (131) and Clark *et al*. (132), using a *B. burgdorferi*-specific PCR, were able to identify flagellin gene in ten of eleven species of lizard collected from Florida and South Carolina. The authors suggested that failure to culture *B. burgdorferi* may have been due to the culture medium used being selective for specific genotypes, or the number of spirochaetes being so low as to be beyond the sensitivity of the test method.

Hazard identification conclusion

B. burgdorferi is considered to be a potential hazard in the commodity.

3.2.4.2   *Risk assessment*

Entry assessment

Although there have been suspect human cases of Lyme disease in Australia none has been confirmed (133). Work to determine whether *B. burgdorferi* is present in Australia has included assessment of the vector competence of *Ixodes holocyclus* (the most widespread ixodid tick in Australia) using a United States strain of the spirochaete (134), serological testing of dogs from Brisbane (where exposure of dogs to *I. holocyclus* and other ticks is high) (135), and the examination of approximately 12,000 ticks for spirochaetes (including testing of more than 1,000 ticks by PCR) (136) all with negative results.

The entry assessment for *B. burgdorferi* is considered to be negligible.

Risk estimation

Since the entry assessment is negligible, the risk estimate is considered to be negligible and *B. burgdorferi* is not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

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3.2.5 Edwardsiella tarda.

3.2.5.1 *Hazard identification*

Aetiological agent
Edwardsiella spp. are members of the Enterobacteriaceae.

OIE list
Edwardsiella tarda is not included on the OIE list of notifiable diseases.

New Zealand status
E. tarda has not been recorded in New Zealand. It is not included in the register of unwanted organisms.

Epidemiology
E. tarda is considered to be an opportunist pathogen mainly affecting fish. It is one of the major diseases in aquaculture systems in Japan, affecting a variety of species (137) and a significant pathogen of Channel Catfish (138), Largemouth Bass (*Micropterus salmoides*) (139), and other fish species. *E. tarda* is a relatively infrequent cause of human infections, most commonly causing gastro-intestinal disease. It is uncommon outside tropical and subtropical regions, and fish and water contaminated by fish are considered the most common sources of human infections (140, 141). *E. tarda* has been reported from lizards in Germany (142) and in Singapore (143). This organism was also isolated from lizards in Togo (144) and an Australian Skink (*Teliqua scincoides*) in the USA (145).

The great majority of reports of *E. tarda* infection are from fish. Other animals from aquatic environments reported as infected with *E. tarda* include chelonians, crocodilians, and marine mammals. Reports of *E. tarda* in terrestrial animals are rare (146-148).

There is a growing literature on the strain-differentiation of *E. tarda* from different habitats and with differing levels of pathogenicity (149-151) but these systems have not been developed sufficiently to allow differentiation of strains of *E. tarda* for biosecurity purposes.

Hazard identification conclusion
E. tarda is considered a potential hazard in the commodity.

3.2.5.2 *Risk assessment*

Entry assessment
In Australia, *E. tarda* has been reported from a diseased native eel (*Anguilla reinhardtii*) (152), diseased, stressed Rainbow Trout (*Oncorynchus mykiss*) (153), and samples from a farmed Golden Tiger Barb (*Barbus tetrazona*), and one other piscine sample (154). Buenviaje *et al.* (155) reported three isolates of *Edwardsiella* sp. from crocodiles with

bacterial hepatitis / septicaemia on farms in northern Australia. Whether the Australian crocodile isolates were *E. tarda* is not known.

E. tarda is, almost exclusively, an organism associated with aquatic environments and reports of the organism in Australia are consistent with that. It is concluded that it is extremely unlikely that lizards in Australia and meeting the commodity definition will be infected with *E. tarda*.

The entry assessment is considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and *E. tarda* is not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.2.6   Pseudomonas reptilivorous

3.2.6.1   *Hazard identification*

Aetiological agent
Pseudomonas spp. are members of the Enterobacteriaceae.

OIE list
Pseudomonas reptilivorous (*P. reptilivorous*) is not included on the OIE list of notifiable diseases.

New Zealand status
P. reptilivorous has not been recorded in New Zealand. It is not included in the register of unwanted organisms.

Epidemiology
P. reptilivorous was isolated from diseased Horned Lizards (*Phrynosoma solare*), Gila Monsters (*Heloderma suspectum*) and Chuckawallas (*Sauromalus ater*) previously captured from the semi-arid areas around Tucson, Arizona. Pathogenicity was confirmed following experimental inoculation of the same species and of guinea pigs and rabbits (156). The only other report discovered of the isolation of an organism meeting the criteria for *P. reptilivorous* is that by Mayne from cottonseed harvested on the Southern United States of America (157).

Although Liu differentiated pseudomonads, including *P. reptilivorous*, on the basis of antigenicity of extracellular toxins (158), Lysenko (159) did not include *P. reptilivorous* in his reclassification of *Pseudomonas* spp. *P. reptilivorous* is not recognised in the List of Prokaryotic names with Standing in Nomenclature (160).

Based on the paucity of reports, their restricted geographic sources and the lack of formal recognition for this species, *P. reptilivorous* is not considered to be a potential hazard in the commodity.

Hazard identification conclusion
P. reptilivorous is not considered a potential hazard in the commodity.

3.2.7 Coxiella burnetii

3.2.7.1 *Hazard identification*

Aetiological agent
The rickesttsia *Coxiella burnetii* is the cause of the zoonotic disease, Q fever.

OIE list
Q fever is included in the OIE list.

New Zealand status
Coxiella burnetii is exotic to New Zealand and is listed in the unwanted organisms register as a notifiable organism.

Epidemiology
Q fever is widely distributed throughout the world and found in many species of mammals and birds. Q fever has been associated with a large number of species of ticks from several genera. However, the exact role that ticks play in transmission is unclear and it has been suggested that the disease is more likely to be spread by inhaling dust contaminated with the agent derived from placentas of animals that have aborted. Others have suggested tick faeces in dust as a source of infection. Infection can induce abortion in cows, ewes, and goats. In humans, it causes a febrile influenza-like condition, pneumonia, hepatitis, and endocarditis. Humans at most risk are those in occupational groups working with animals including those in slaughter plants (161-163).

There have been occasional suggestions that lizards may play a role in the epidemiology of *Coxiella* infections but literature searches have not recovered reports confirming this. Reports that reptile-related ticks may have been responsible for human cases of Q fever have been reviewed by Burridge (164) and he concluded that any association between *Aponomma exornatum* and Q fever was tenuous while the possible role of *Amblyomma nuttalli* required further investigation.

Hazard identification conclusion
Coxiella burnetii is considered to be a potential hazard in the commodity.

3.2.7.2 *Risk assessment*

Entry assessment
The single report located of *Coxiella burnetii* infection of a lizard (*Varanus indicus*) came from India (165). Both of the reptile-related ticks that have been suggested as possible vectors of Q fever are ticks restricted in distribution to Africa.

The likelihood of *Coxiella* infection being transmitted through the importation of lizards from Australia is negligible. Therefore, the entry assessment is considered to be negligible.

Risk estimation

Since the entry assessment is negligible, the risk estimate is considered to be negligible and *Coxiella burnetii* is not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.3   FUNGI AND YEASTS

3.3.1   Fungi and yeasts of lizards

3.3.1.1   *Hazard identification*

Aetiological agent

A wide range of fungi is present on the skin and in the intestines of healthy lizards with *Aspergillus* spp., *Candida* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and *Paecilomyces* spp. being the most common (166, 167). These fungi are common environmental contaminants capable of causing disease under conditions of poor husbandry, poor sanitation, overcrowding or failure to have environmental conditions controlled appropriately (168).

There are a number of reports of fatal disease in reptiles, including lizards, caused by the *Chrysosporium* anamorph of *Nannizziopsis vriessi* (CANV).

OIE list

Epizootic lymphangitis (which is a disease of horses caused by *Histoplasma capsulatum* var. *farciminosum*) is included in the OIE list.

New Zealand status

Histoplasma farciminosum is listed in the register of unwanted organisms. This is the same organism as *Histoplasma capsulatum* var. *farciminosum* included in the OIE list.

Reports of the identification, in New Zealand, of CANV or its telemorph counterpart have not been located.

Epidemiology

Aspergillus spp., *Candida* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., and *Paecilomyces* spp. are present in New Zealand and it is not considered that their possible presence on imported lizards will significantly increase the exposure of humans or other animal species. These organisms are not considered to be hazards in the commodity.

CANV was first reported as a cause of disease in reptiles in 1997, with isolation from skin lesions on three species of captive chameleon from two collections in Canada (169). The same fungal species has since been associated with fatal skin diseases in captive snakes in the United States (170), hatchling salt-water crocodiles in captivity in northern Australia (171), and freshwater captive-bred snakes in Canada (172). Pare (173) stated that CANV is under-diagnosed due to mis-identification as *Trichophyton* sp., *Trichosporon* sp., *Geotrichum* sp., non-speciated *Chrysosporium,* or left as an unknown fungus.

Chrysosporium are keratinophilic filamentous fungi commonly found in soil, plant material, dung, and birds (174), and only rarely recoverable from the skin of healthy snakes (175). Examination of skin samples from 36 healthy lizards and 91 snakes from

zoological and veterinary institutions produced only one culture of CANV, that from an African Rock Snake (166, 175). Experimental exposure of Veiled Chameleons (*Chamaeleo calyptratus*) to CANV established that the organism is a primary pathogen of reptiles requiring direct contact with the organism and that it behaves as a contagious disease within colonies (168, 176).

Hazard identification conclusion
It is concluded that CANV is considered to be a potential hazard in the commodity.

3.3.1.2   *Risk assessment*

Entry assessment
The culture of CANV from the skin of only one of 91 healthy snakes and none of 36 healthy lizards suggests that that the organism is rare on healthy animals. Reports of CANV infection of lizards come only from North America. As lizards for importation will be required to be clinically healthy, the likelihood of infection is considered negligible.

The entry assessment is considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and CANV is not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.4 HELMINTH PARASITES

3.4.1 Gastro-intestinal nematodes

3.4.1.1 *Hazard identification*

Aetiological agent
The section covers all gastro-intestinal nematode parasites considered to be relevant to the commodity.

OIE list
There are no gastro-intestinal nematodes of lizards in the OIE list.

New Zealand status
12 nematodes (either species or genera) are listed in the register of unwanted organisms. None of these have been reported from lizards.

Gastro-intestinal nematodes reported from lizards in New Zealand are:

- *Capillaria* sp. – This genus is widely spread geographically and in different hosts but this record seems likely to be a New Zealand species as it was identified from a skink on Stephens Island (177).

- *Hedrusis minuta* – a species endemic to New Zealand.

- *Parathelandros* sp. – Nematodes of this genus have been reported from Australia (178), the United States (179, 180), and other countries, most commonly in amphibians. With confusion over nomenclature, this parasite may be *Skrjabinodon* sp. (177).

- *Pharyngodon* sp. – a genus represented in Australia (181), the Pacific Islands (182), Asia (183), and the Americas (179, 184). It has been suggested that this record may be incorrect and that the parasite might be *Skrjabinodon* sp. (177).

- *Skrjabinodon poicilandri* and *S. trimorphi* – species reported only from New Zealand (185, 186).

- *Skrjabinodon* spp. – Six further species beyond *S. poicilandri* and *S. trimorphi* have been identified in New Zealand. Members of this genus are host specific to either skinks or geckos (177).

Epidemiology
A scan of literature databases and texts (75, 184, 187-195) reveals a large number of gastro-intestinal nematode parasites of genera and species not recorded in New Zealand but present in lizards in Australia and elsewhere. Neither the detailed epidemiology of most nematode parasites of lizards, nor their effects on the health of their hosts, is well

described. The lifecycle of gastro-intestinal nematodes of vertebrates involves the adult worm living in the gastro-intestinal tract. Eggs are laid and passed in faeces then development of larvae proceeds to a point where they are infective to the host. Most nematode species reinfect the host through the oral route but direct tissue penetration or other means of infection occur with some species.

Under most circumstances, nematodes have relatively little effect on their host but, under conditions of crowding or stress, negative effects may occur.

Hazard identification conclusion
Exotic gastro-intestinal nematodes of lizards are considered to be a potential hazard in the commodity.

3.4.1.2   *Risk assessment*

Entry assessment
Lizard/nematode relationships are part of the normal host-parasite combinations that have developed through evolution. Gastro-intestinal nematodes will be present in most lizard populations unless intensive control measures have removed them from closed groups in captivity.

The entry assessment is considered to be non-negligible.

Exposure assessment
Highly host-adapted parasite species are unlikely to infect other species of lizard. The likelihood of gastro-intestinal nematode species that are not highly host-adapted infecting other imported species in New Zealand or species endemic to this country will be affected by the suitability of the external environment for larval hatching and survival.

There has been a wide range of lizard species imported to New Zealand in the past, many of which are held by hobbyist herpetologists, and no reports of exotic nematodes in endemic or native species have been located. Although details of the timing and conditions under which importations took place are not known, it appears unlikely that transmission to native species will take place. Nevertheless, the likelihood of introduced populations sharing environments with endemic species and gaining nematode infections is considered to be non-negligible.

The exposure assessment is considered to be non-negligible.

Consequence assessment
Gastro-intestinal nematode parasites of lizards generally live in balance with their hosts with minimal, if any, negative effects on the host in the wild. However, in captivity, with higher host densities and greater environmental contamination, nematode populations can increase and cause disease in their hosts.

The consequence assessment is considered to be non-negligible.

Risk estimation

Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and gastro-intestinal nematodes are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

3.4.1.3   *Risk management*

Options

No reports of scientific trials assessing the efficacy of anthelmintic treatment of lizards for nematode infections have been located and the use of anthelmintics in non-mammalian species is not without risk, especially for reptiles and fish where the therapeutic level may be close to the toxic level (196).

Textbooks (197-200) and other sources do, however, provide recommendations and guidance. Fenbendazole (25 to 50 mg/kg by mouth for four days then repeated in 10 days), albendazole (a single does of 50mg/kg), and ivermectin (0.2 mg/kg intramuscularly every two weeks for a minimum of two treatments) are alternatives proposed by Diaz-Figueroa (197). Recommendations from Frye (200), the Merck Veterinary Manual (199) and Klingenberg (201) are similar but with some variations in dose rate and dosing regime.

One or both of the following measures could be considered in order to effectively manage the risk:

1. Imported animals could be subject to an anthelmintic treatment regime recognised amongst herpetologists and veterinarians experienced in herpetological medicine as effective for the removal of gastro-intestinal nematodes from lizards. The regime used could be documented.

2. During the treatment regime, lizard accommodation could be cleaned of all faecal material regularly and quarantine measures maintained to prevent exposure to sources of reinfection. Section 3.6.2.3 describes twice weekly cleaning of cages to control ectoparasites. It is suggested that this interval would also be appropriate for the control of gastro-intestinal nematodes.

3.4.2 Filariid nematodes

3.4.2.1 *Hazard identification*

Aetiological agent
The section covers the nematode parasites of lizards in the family Filariidae, the adults of which live in the blood stream or in tissues and which produce microfilaria into the blood of the their hosts.

OIE list
There are no filariid nematodes of lizards in the OIE list.

New Zealand status
No reports of filariid nematode parasites of Squamata in New Zealand have been located.

Epidemiology
Adult filariid nematodes live in the blood stream or in tissues of their hosts. Completion of their life-cycle is dependent upon haematophagous arthropods feeding on the blood of the host and passing larvae (microfilaria) to another host at a later feeding.

A scan of literature databases and texts (75, 187, 191, 192, 198, 200, 202) reveals a considerable number of filariid nematode parasites of reptiles, including a number present in lizards in Australia (203-206). The detailed epidemiology of most filariid parasites of lizards is not well described and reports defining the intermediate hosts of most have not been located. For most filariid parasites of Squamata the effects on the well-being of the host are not described but where descriptions are available there is not a consistent pattern. Varying degrees of host-specificity are apparent.

Hazard identification conclusion
Exotic filariid nematodes of lizards are considered to be a potential hazard in the commodity.

3.4.2.2 *Risk assessment*

Entry assessment
The great majority of reports of filariid nematodes in Squamata are based on observations in populations of free-living hosts. Information on the status of captive collections is scant. While some collections may have been removed from the environment of the vectors of filariid nematodes, there is no basis for assuming this. Filariid nematodes may be present in captive collections.

The entry assessment is considered to be non-negligible.

Exposure assessment
The extent of transmission of filariid nematodes from imported infected individuals will be dependent upon the availability of vectors and the host-specificity of the nematode

species. An epidemiological study in Italy found that although four of 23 "pet" wild-caught chameleons were infected with *Foleyella* spp., infection was not detected in any of 33 chameleons bred in captivity. There has been a wide range of lizard species imported to New Zealand in the past, many of which are held by hobbyist herpetologists, and no reports of exotic nematodes in native species have been located. Although details of the timing and conditions under which importations took place are not known, it appears unlikely that transmission to native species will take place. Nevertheless, the likelihood of exposure to exotic species already present is higher and is considered non-negligible.

The exposure assessment is considered to be non-negligible.

Consequence assessment

Few reports of investigations of the effects of filariid nematodes on their free-living hosts have been located but those obtained do not show adverse effects. Christian and Bedford (205), in Australia, found a higher prevalence *of Oswaldofilaria chlamydosauri* in larger *Chlamydosaurus kingii* but no relationship to body condition. Concentrations of microfilaria in blood samples were not related to measured physiological parameters. They concluded that the infection had no adverse effects.

Both Frye (200) and Greiner and Mader (207) identify very small numbers of reports of pathology associated with filariid nematodes in snakes but they make no reference to diseases in lizards and extensive literature searches have failed to identify such reports.

Based on the lack of evidence that filariid nematodes cause adverse effects in Squamata the consequences of these parasites in the commodity are concluded to be negligible.

The consequence assessment is considered to be negligible.

Risk estimation

Since the consequence assessment is negligible, the risk estimate is considered to be negligible and filariid nematodes are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.4.3 Cestoda

3.4.3.1 *Hazard identification*

Aetiological agent
This section covers all cestode parasites relevant to the importation of the commodity.

OIE list
There are no cestodes of lizards in the OIE list.

New Zealand status
Five species or genera of cestodes are listed in the register of unwanted organisms.

In his review, McKenna (2003) identified three species of cestodes in New Zealand lizards. Little is known about the prevalence and distribution of the two *Baerietta* spp. while *Oochoristica novaezealandicae* appears to be common in skinks on Banks Peninsula and has been recorded on Stephens Island. It is thought that the intermediate host of *Oochoristica novaezealandicae* is likely to be a beetle but information on the intermediate hosts of the other cestodes is lacking (177).

Epidemiology
Reports of cestodes in lizards are relatively scarce. Textbook sections on reptilian cestodes by Greiner and Mader (207), Hernandez-Divers (198), and Frye (200) are similar; reporting several genera of cestodes as having Squamata, including varanids (monitors) and smaller lizards, as their definitive hosts. Authors commented that the cestodes had no adverse effects so long as the hosts were receiving adequate nutrition. These comments are supported by searches of literature databases.

Both Frye (200) and Hernandez-Divers (198) record that the tetrathyridium cysts (an intermediate stage) of *Mesocestoides* spp. are found in snakes and iguana, and that these Mesocestoididae are zoonotic. *Mesocestoides lineatus* is the species occasionally transmitted to humans through ingestion of snake liver or other tissues. The majority of reports of *Mesocestoides* spp. in Squamata do not identify the species of *Mesocestoides*. Although there was one very early report proposing that a specimen from a snake was of a *Mesocestoides* sp., that is no longer accepted and Australian authorities claim that Australia is free of *Mesocestoides* spp. (208, 209).

Many digenetic cestodes have restricted host ranges in both the definitive and intermediate stages of their lifecycles. If lizards were to carry cestode infections internationally, it is highly unlikely they would establish lifecycles in their new location.

Hazard identification conclusion
Cestodes are not considered to be a potential hazard in the commodity.

3.5 PROTOZOA

3.5.1 Blood-borne Protozoa of Lizards

3.5.1.1 *Hazard identification*

Aetiological agent

Intraerythrocytic protozoa (IP) from two Phyla, three Classes, four Orders and 18 Genera have been reported as infecting lizards (see Figure 1). With Schall (210) identifying reports of 77 species of *Plasmodium* from lizards, the total number of species of IP in lizards is very large.

Figure 2. Intraerythrocytic protozoa identified in lizards.

```
PHYLUM: EUGLENOZOA
        CLASS: KINETOPLASTIDEA
                Order: Trypanosomatida
                        Family: Trypanosomatidae
                                Genus: Sauroleishmania


PHYLUM: APICOMPLEXA (SPOROZOA)
        CLASS: COCCIDEA
                Order: Eimeriida
                        Family: Haemogregarinidae
                                Genus: Haemogregarina
                                Genus: Hemolivia
                                Genus: Hepatozoon
                                Genus: Karyolysus
                        Family: Lankesterellidae
                                Genus: Laisonia
                                Genus: Lankesterella
                                Genus: Schellackia
                        Family: Dactylosomatidae
                                Genus: Babesiona
                                Genus: Dactylosoma


        CLASS: HAEMATOZOEA
                Order: Haemosporida
                        Family: Garnidae
                                Genus: Garnia
                                Genus: Saurocytozoon
                        Family: Plasmodidae
                                Genus: Billbraya
                                Genus: Plasmodium
                        Family: Haemoprotidae
                                Genus: Haemocystidium
                                Genus: Haemoproteus


                Order: Piroplasmida
                                Genus: Sauroplasma
                                Genus: Theileria
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DRAFT

OIE list
No IP of lizards are included in the OIE list of notifiable diseases.

New Zealand status
No IP of lizards are included in the unwanted organisms register.

In his annotated analysis of past records of parasites of New Zealand reptiles, McKenna (177) identified the following haemoparasites as having been reported from lizards in New Zealand.

Table 3. Haemoparasites identified in New Zealand lizards

Parasite	Recorded hosts(s)	Vector
Plasmodium lygosomae	Moko Skink (*Oligosoma moco*)	Unknown
Haemogregarina sp.	Common Gecko (*Hoplodactylus maculatus*)	Unknown
	Duvacel's Gecko (*H. duvaucelii*)	
	Pacific Gecko (*H. pacificus*)	
	Common Skink (*O. nigraplantare*)	
	Speckled Skink (*O. infrapuntatum*)	
	Spotted Skink (*O. nigraplantare*)	
Hepatozoon lygosomarum	Common Skink (*O. nigraplantare*)	*Ophionyssus scincorum*
	Moko Skink (*O. moco*)	

Epidemiology
This section draws heavily on the review of intraerythrocytic parasites of ectothermic animals by Davies and Johnston (76) with other sources referred to as required.

The general patterns of lifecycles of all IP are similar, with lizards being infected by trophozoites through either ingestion of invertebrates or through being bitten by the invertebrate host (arthropods). Variations occur with different stages of the lifecycle taking place in different tissues of the lizard host and with some variations in stages that take place in the invertebrate host. For example, Lankasterellidae pass through all stages of the lifecycle in the lizard host with sporozoites being ingested by invertebrate hosts (mites, mosquitoes, or biting flies) and then, without further development of the sporozoites, infection is passed to new hosts through ingestion of the arthropod by a lizard. Lankasterellidae may infect an intermediate vertebrate host with infection being passed on through predation. In this way, lizards may act as sources of *Hemolivia*, *Hepatozoon*, *Lankasterella,* and *Schellackia* infection of snakes or saurophagus (lizard-eating) lizards. Leeches (*Hirudinea*), as well as arthropods, may contribute to transmission of some *Karyolysus* species, and *Hepatozoon* species may, variously, include sucking lice (Anoplura), fleas (Siphonaptera), mosquitoes (*Culex*, *Aedes* and *Anopheles* spp.), sand flies (Phlebotominae), ixodid and argasid ticks, mites (Acarina), and other arthropods as invertebrate hosts. Sand flies, mosquitoes, and midges are the main groups involved in transmission of *Plasmodium* spp. For many IP, vertebrate host ranges are not clearly defined and, for many more, the invertebrate hosts are not known.

In his review of the *Hepatozoon* genus, Smith (211) states that host-specificity of some species is low for both vertebrate and invertebrate hosts whereas *Plasmodium* spp. infecting lizards have restricted host and geographic ranges (212).

There is limited information on the persistence of haemoparasite infections but the information that has been reported on *Billbraya australis* in the South Australian Gecko (213), *Schellackia aganae* in the Starred Lizard (*Agama stellio*) (214), a haemogregarine in the Common Lizard (*Lacterta vivipara*) (215), and *Hepatozoon hinuliae* in *Eulamprus quoyii* (216) indicates that infection may persist for long periods and that self cure may not occur.

Davies and Johnston (76) contrasted the frank pathogenicity of many IP in mammals with the absence of reports of disease attributed to IP in ectotherms.

Hazard identification conclusion
IP are considered to be a potential hazard in the commodity.

3.5.1.2   *Risk assessment*

Entry assessment
Mackerras (217), in 1961, listed 14 named species of *Haemogregarina*, one *Haemocystidium* sp, two *Trypanosoma* spp., and one *Plasmodium* sp. as having been described from four species of geckos, two species of agamid lizards, six species of skinks, and four species of goanna in Australia. There have been additions since that time including *Billbraya australis*, described infecting the South Australian Gecko (*Phyllodactylus marmoratus*) (213), *Hemolivia mariae* which infects the Australian Sleepy Lizard (*Tiliqua rugosa*) and has *Amblyomma limbatum* and *Aponomma hydrosauri* as its invertebrate hosts (218-220), *Hepatozoon hinuliae*, which infects the Australian Eastern Water Skink (*Eulamprus quoyii*) (216), *Plasmodium mackerrasae* from two species of skinks (221), and *P. circularis* from a further species of skink (222). All of these reports are from reptiles in their natural habitat. The only species for which invertebrate hosts appear to be defined is *Hemolivia mariae*.

Given the ranges of lizard species in Australia, it seems inevitable that there are many species of haemoparasites yet to be identified.

Lizards, not native to the area defined in the commodity definition, are relevant to this risk analysis because they may be imported under an import health standard developed from it. Imported lizards, like those endemic to Australia or the Pacific Islands, commonly carry infections of haemoparasites. What is less certain is whether haemoparasites have entered Australia with imported lizards and, if so, whether they have found competent intermediate hosts.

In wild-captured lizards infected with *Plasmodium* spp., parasitaemia drops markedly following introduction to a laboratory environment (212) but no reports on whether that affects the ongoing status of infection have been located. Apart from articles on

laboratory studies, literature searches have failed to identify reports of haemoparasites in captive lizards. This may suggest that any effects of parasitaemia are minimal but the likelihood of infection cannot be excluded.

The entry assessment is considered to be non-negligible.

Exposure assessment

If haemoparasites enter New Zealand with imported lizards, transmission will depend upon contact with competent intermediate hosts. For those species reliant on ectoparasites (mites and ticks) for completion of their life-cycle, transmission will be limited by the risk management provisions in the ectoparasite section of this risk analysis, but only for so long as the hosts do not regain contact with competent intermediate hosts. For those using mosquitoes, midges and sand flies as intermediate hosts, the forecasting of the likelihood of transmission is difficult because of the lack of knowledge of specific requirements of the parasites. The speciation of haemoparasites of lizards (and reptiles generally) remains poorly defined, with Smith (211) proposing, ten years ago, that the *Hepatozoon* genus be expanded to include all members of the *Haemogregarina* genus infecting reptiles and other groups of animals and ongoing lack of clarity of the basis of speciation of haemoparasites reported in the literature. Many species continue to be accepted as distinct on the basis of morphology, host, and geographic location. This makes objective assessment of host-specificity, and potential for transmission to new hosts, difficult.

Smith *et al*. (223) reported that both *Culex* and *Culiseta* mosquitoes were competent definitive hosts for *H. sipedon*, that oocyst development could occur in a range of frog and toad species, and that merozoites and gametocytes could develop in at least four species of snakes. This is consistent with the broader view expressed by Smith in his general paper on the genus *Hepatozoon* (211), that host (both primary and intermediate) specificity of some *Hepatozoon* spp. is low. Investigations of *Plasmodium* and *Haemoproteus* spp. of birds, using molecular biological techniques, have shown substantial host-family specificity of *Haemoproteus* but weaker host-family specificity of *Plasmodium* (224). Szymanski and Lovette (225), however, considered that there was a high degree of host sharing by *Haemoproteus* spp. infecting birds in the New York area. These observations relate to the biology of the parasites within established ecosystems where there has been opportunity for adaptation of the host-parasite relationship over many years. In the laboratory, Schall (210) was able to infect the North American Fence Lizard (*Sceloporus occidentalis*) with *P. giganteum* and *P. agamae*, both naturally parasites of the African Rainbow Lizard (*Agama agama*).

Haemoparasites of birds may offer a model of behaviour of comparable parasites in lizards. Ishtiaq *et al*. (226) investigated haematozoan parasites of populations of native birds and populations of Common Myna (*Acridotheres tristis*) established in Hawaii, Australia, New Zealand, South Africa, and the Cook Island following introductions during the period 1862 to 1900. They considered there was some evidence that *Plasmodium* spp. and *Haemoproteus* spp. had moved to those locations, established and spread to infect native bird species. The authors could not exclude the possibility that the

parasite lineages being investigated had been present in the native birds prior to introduction of the mynas and they expressed the view that acquisition of local parasites by the introduced host was, generally, more likely than establishment of introduced parasites in endemic bird populations.

Investigating the behaviour of *Hepatozoon* spp. in unnatural host species, Wozniak and Telford (227) reported liver pathology in lizards to which mosquitoes had been fed after the insects had fed on snakes infected with *Hepatozoon* spp. Up to 40% of mosquitoes feeding on one of the snakes died within 48 hours, and the authors concluded that the hepatic pathology may have prevented the release of schizonts, thus contributing to minimisation of parasitaemia and the transitory nature of infection. That particular host – parasite – host relationship seems unlikely to be sustainable.

Although evidence of geographic relocation of haemoparasites of lizards has not been found, and the evidence presented by Ishtiaq *et al* (226) relating to the geographic translocation of haemoparasites of birds is inconclusive, assurance that haemoparasites of lizards imported to New Zealand would not establish cannot be given.

The exposure assessment is considered to be non-negligible.

Consequence assessment

- *Plasmodium* **spp**. – Schall (210, 212), in papers reviewing earlier work by him and his students, reported that *Plasmodium* spp. in lizards cause a decrease in haemoglobin levels, decreased running stamina (but not speed in sprint running), and decreased ability to defend territories. He also reported reduced lipid stores, reduced testicular size and decreased egg clutch size. These effects are variable between species. On the Caribbean island of St Maarten, *P. azurophilum* (occurring in localised areas) affects the relative sizes of populations of two *Anolis* spp. as a result of differences in susceptibility to the parasite.

- **Haemogregarinid** species - Smallridge and Bull (220) reported that male Australian Sleepy Lizards (*Tiliqua rugosa*) infected with *Hemolivia mariae* had lower body condition than their uninfected counterparts. Examination of this paper, however, shows that this difference existed only early during their period of observation. They were unable to determine the basis of the relationship and considered a number of possible explanations including one that lizards in poorer body condition might be more susceptible to parasite infection. Amo *et al*. (228) found that Iberian Rock Lizards (*Lacerta monticola*) infected with haemogregarinid parasites lost more weight over the breeding season than uninfected lizards but no such relationship was found in *Lacterta lepida* (229) and, in *Podarcis muralis*, the individuals with higher burdens of Haemogregarine parasites were in better body condition than those with lower burdens (230).

- Reports of disease or other effects of other haemoparasites of lizards have not been located.

Based on these observations, the consequence assessment for *Plasmodium* spp. and other Haemosporida, in the commodity is considered to be non-negligible.

Based on the lack of evidence from either wild or captured lizards that haemoparasites other than *Plasmodium* spp. and other Haemosporidia cause disease or other negative effects, the consequence assessment for haemogregarinid and other haemoparasites in the commodity is considered to be negligible.

Risk estimation

Since the entry, exposure, and consequence assessments for *Plasmodium* spp. and other Haemosporida are non-negligible, the risk estimate is considered to be non-negligible and these parasites are classified as hazards in the commodity. Therefore, risk management measures can be justified.

Since the consequence assessment for Haemogregarine and other haemoparasites is negligible, the risk estimate is considered to be negligible and these parasites are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.5.1.3   *Risk management*

Options

Reports of reliable therapeutics for Haemosporida infections of lizards have not been located.

It does not appear that PCR primers are available for routine testing and, certainly not covering the range of organisms that would need to be tested for.

Haemosporida may be detected in Geimsa-stained (or other Romanowsky-stained) air-dried smears of peripheral blood of infected lizards. Perkins *et al*. (231) compared examination of smears with PCR as means of detecting *P. mexicanum* in Western Fence Lizards. Based on the prevalence as detected by PCR, examinations of 10,000 red blood cells per animals had a sensitivity of detecting infected lizards of 91% in a "high prevalence" population and 50% in a "low prevalence" population.

Examination of blood smears during periods of lizard hibernation has lower sensitivity but parasitaemia increases rapidly early in the spring.

Parasitaemia develops over a period of about one month after infection (212). For that reason, detection early after infection will have lower sensitivity.

One or a combination of the following measures could be considered in order to effectively manage the risk:

1. On two occasions, at least 14 days apart, during a pre-export quarantine period, air-dried, alcohol-fixed, Geimsa stained smears of peripheral blood from each animal to be imported could be examined with at least 10,000 erythrocytes examined per animal. Only animals with negative test results for *Plasmodium* sp. and other Haemosporida could be eligible for importation to New Zealand.

2. Testing of lizards could be limited to after the end of any hibernation period,

3. Testing could take place only after removal of lizards from potential vectors for approximately one month. That is, lizards must be free of ectoparasites and in an environment protected from mosquitoes, biting flies, and sand flies.

3.5.2 Entamoeba invadens

3.5.2.1 *Hazard identification*

Aetiological agent
The genus *Entamoeba* is within the phylum Sarcomastigophora and the Kingdom Protozoa.

OIE list
Entamoeba invadens is not included in the OIE list of notifiable diseases.

New Zealand status
 E. invadens is not included in the register of unwanted organisms.

An *Entamoeba* sp. has been identified in the Common Gecko (*Hoplodactylus maculatus*) in New Zealand. This organism was not classified as *E. invadens* and its species identity is unknown (177).

Epidemiology
E. invadens, like other *Entamoeba* spp., has a direct life cycle with cysts being ingested by the host, development and reproduction of trophozoites in the intestinal tract with some forming cysts and being passed in faeces. Trophozoites may invade the mucosa, causing damage and allowing bacterial invasion. Trophozoites may also locate in the liver, kidney, or lung (207). Text books (75, 187, 198, 201, 207, 232), and introductions to articles, identify *E. invadens* as infecting a wide range of reptiles and causing epidemics of disease and mortality in lizards, snakes, and tortoises, particularly those in captive collections (198, 207, 232). Although reports of disease episodes in snakes (233-235), and in tortoises (236-238), are relatively common, only two reports of disease incidents associated with *E. invadens* in lizards have been located. These were of a single Blue-tongued Skink (*Tiliqua scincoides*) housed near an integrated display of reptiles within which 15 of 16 snakes died with *E. invadens* infection (234) and multiple deaths of *Varanus salvator* shortly after their being shifted from Switzerland to Koln zoo (239).

Meerovitch (240, 241) studied host-parasite relationships of *E. invadens* and concluded that the parasite is a commensal in those turtles using ingested plant polysaccharides as a significant source of nutrients. She further concluded that pathogenicity arose very commonly in snakes because they are carnivores and, in the absence of plant polysaccharides, the organism obtained its polysaccharide requirements from mucous secretions in the gut, thus rendering the mucosa susceptible to bacterial invasion. Meerovitch considered that this explanation was consistent with the general pattern of *Entamoeba* spp. being commensals in strictly herbivorous mammals but pathogenic in carnivores, and with a pattern of *E. invadens* infections causing disease in carnivorous lizards but being subclinical in herbivorous species. This proposition is consistent with the report of multiple deaths in *Varanus salvator* (239) which are carnivores. The fatal infection of a *Tiliqua scincoides* (234), which is an omnivore, is not inconsistent with Meerovitch's observation that *E. invadens* is a commensal in strict herbivores.

Although others suggest that there are species of lizards that are susceptible to *E. invadens* disease and others that are not susceptible, none of the articles found list any specific species in these categories. Species that are not susceptible to *E. invadens* associated disease, however, may carry commensal infections.

Hazard identification conclusion
It is concluded that *E. invadens* is considered to be a potential hazard in the commodity.

3.5.2.2   *Risk assessment*

Entry assessment
E. invadens infects a number of lizard species with commensal infections of herbivores being most likely. *E. invadens* has been reported in captive reptiles in Australian zoos (242). On this basis there is a potential for *E. invadens* to be present in the commodity.

The entry assessment is considered to be non-negligible.

Exposure assessment
E. invadens is a contagious organism transmitted via the faecal-oral route. Reports identify omnivorous or herbivorous turtles as the principle reservoir host and the likely source of infection for other species. They also, however, consider that *E. invadens* can infect herbivorous lizards as commensals, in which case, spread from healthy animals is likely. Sound biosecurity measures can limit spread of *E. invadens* between lizard enclosures but the likelihood of such dissemination cannot be excluded.

The exposure assessment is considered to be non-negligible.

Consequence assessment
No reports of *E. invadens* causing disease in free-living reptiles have been located and the organism is considered to be a pathogen of reptiles in captive collections. Negative consequences of *E. invadens* appear likely to be limited to captive collections, and within those collections disease is likely to be limited to carnivorous (and, possibly, omnivorous) species. The effects of disease in susceptible species can be limited by early diagnosis and treatment. Although few reports of specific disease incidents have been located, those reports and general comments in textbooks and elsewhere indicate that mortalities are likely.

The consequence assessment is considered to be non-negligible.

Risk estimation
Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and *E. invadens* is classified as a hazard in the commodity. Therefore, risk management measures can be justified.

3.5.2.3 *Risk management*

Options

One or a combination of the following options could be considered in order to effectively manage the risk:

1. Animals to be imported could come from establishments that have no known history of *E. invadens* infection. The premises of origin could be under veterinary supervision and the health of the animal(s) could be monitored so that incidents of disease and death are identified promptly and *E. invadens* excluded as the cause of gastrointestinal disease in lizards, snakes, or testudines during the preceding 12 months

2. Faecal samples or cloacal washings could be examined for cysts of *E. invadens*. As other *Entamoeba* spp. can infect lizards with no deleterious effects, care must be taken in the identification of *E. invadens* cysts. The sensitivity of such examinations is less than 100% and repeat examinations are required if a high level of confidence in negative results obtained on individual animals is required.

3. Greater confidence that animals to be imported come from collections free of *E. invadens* could be gained through sampling of herbivorous reptile species that may have some degree of contact with the species to be imported. This could include turtles and herbivorous lizards that are cared for by the same staff, have utensils cleaned in shared facilities or are in enclosures with contact through drainage.

4. Treatment of infected animals with metronidazole reduces the pathogenic effects of infection in susceptible species but there is no evidence that it eliminates the excretion of cysts.

3.6 ARTHROPODS

3.6.1 Pentastomida

3.6.1.1 *Hazard identification*

Aetiological agent
The subclass Pentastomida is within the phylum Arthropoda, subphylum Crustacean. Pentastomids parasitic in lizards are within the Family Cephalobaenida, Genus *Raillietiella*, and Family Porocephalida, Genera *Sombonia* and *Elenia*.

OIE list
No Pentastomida of lizards are included in the OIE list of notifiable diseases.

New Zealand status
No members of the Pentastomida are included in the unwanted organisms register.

Linguatula serrata, a parasite primarily of mammalian carnivores, and of other species including humans and other mammals has been identified in New Zealand in dogs, a brown hare, European rabbit, cat, and sheep (243, 244). In his review of ecto- and endoparasites of New Zealand reptiles, McKenna (177) did not identify any reports of pentastomids.

Epidemiology
Adult pentastomids are, normally, located in the lungs or other parts of the respiratory system. They lay eggs that are coughed up, swallowed, and excreted in faeces. After ingestion by an intermediate host and larval development, the larvae are infective to the primary host. Following ingestion by the primary host, larvae penetrate the intestinal wall and undergo a period of tissue migration before entering the respiratory tract, usually the lungs (207).

Raillietiella spp. have been reported from a variety of Squamata, mostly from snakes and lizards. In their review of the genus, Ali *et al*. (245) grouped species on the basis of size and host.
- Groups I and II are small to medium in size (6 – 44 mm) and infect predominantly insectivorous lizards, particularly geckos, skinks, and agamids. Insects are the most likely candidates as intermediate hosts for the parasites in this group.
- Group III *Raillietiella* spp. infect varanid lizards, which are carnivorous, eating a wide variety of animals dead or alive. Ali *et al*. (245) suggested that their intermediate hosts may not be insects.
- Groups IV, V, and VI infect amphisbaenians, toads, and snakes respectively.

Reports of *Elenia* spp. are limited to one from an unknown host and one from *Varanus varius* (Lace Monitor) in Queensland, and one from a Scale-footed Lizard (*Lialis jicari*) in, what is now, Irian Jaya Barat. Experimental infection of frogs and small laboratory animals with eggs of *Elenia* leads to the development of infective larvae (246).

The only reports located of *Sambonia lohrmanni* were from Komodo Dragons (*Varanus komodoensis*), Bosc's Monitor Lizard (*V. exanthematicus*), and Nile Monitor (*V. niloticus*) (247). Both adult and intermediate stages of *S. lohrmanni* have been found in *V. komodoensis* raising the possibility that the parasite has a direct life cycle in this host (Fain and Mortelmans (248) cited by Flach *et al*. (247)).

Although little information is available on the pentastomids of reptiles, the wide host range of *Linguatula serrata*, illustrated by the range of hosts known in New Zealand, appears to be consistent with the behaviour of the parasites of lizards. Goldberg and Bursey (249) examined the helminth parasites of the Brown Anole (*Anolis sagrei*) in Hawaii, an island group on which all lizards are introduced species. *Raillietiella frenatus*, a species first reported from *Hemidactylus frenatus* in Malaysia, was found in that species plus *A. sagrei* and *Lepidodactylus lugubris*. *R. frenatus* has not been reported from *A. carolinensis* or *H. garnoti* in Hawaii (249) but it has been reported from *H. turcicus* (a lizard introduced from the Mediterranean) in Louisiana USA (250), and from *Cosymbotus platyurus* and *Gehyra mutilata* in Indonesia (251). This geographic spread of the parasite suggests flexibility in requirements for intermediate hosts. Also, individual species of lizards may be hosts to more than one species of *Raillietiella* as illustrated by both *R. frenatus* and *R. teagueselfi* being found in *H. turcicus* in Louisiana ((252) cited by (246)).

Hazard identification conclusion
It is concluded that pentastomids are considered to be a potential hazard in the commodity.

3.6.1.2   *Risk assessment*

Entry assessment
R. amphiboluri was described from a Bearded Dragon (*Amphibolurus barbatus*) in 1954 (Mahon (252) cited by Riley *et al*. (246)) and another specimen from the same host, thought to have come from the environs of Sydney is held in the South Australian Museum (246). No other reports of this parasite in Australia have been located.

The only other *Raillietiella* species reported from Australia is *R. scincoides* from an Eastern Blue-tongued Lizard (*Tiliqua scincoides*) (246).

Identified reports of both *Elenia* spp. and of *Sambonia lohrmanni* are limited to those specified in the epidemiology section above.

Although there are very few reports of pentastomids from lizards in Australia, Riley *et al*. (246) comment that it is likely that more species have yet to be identified. Pentastomid infections commonly cause no clinical signs (207, 247), and this means that recognition of infection is, most commonly, as a result of a chance discovery. No information on pentastomids in lizards in captivity in Australia has been located.

Based on the available evidence, the likelihood of pentastomid infection in the commodity is very low but not negligible. Hence the entry assessment is considered to be non-negligible.

Exposure assessment

The ability of pentastomids to establish in new environments is illustrated by the example of *Raillietiella frenatus* provided in the epidemiology section above (249). The discovery of a *Sambonia* sp. in two of four Bosc's Monitor Lizards imported to the United Kingdom from West Africa and held at a wild animal park for three years demonstrates the potential for persistence of infection in the absence of suitable intermediate hosts (247). The information on *R. frenatus* in Hawaii suggests that even when that parasite is present in an area, along with competent primary hosts, infection may not extend to all species even within the same genus (249). Intermediate hosts for many species of pentastomids infecting lizards are not known, therefore the availability of suitable intermediate hosts in New Zealand cannot be predicted. It can be forecast, however, that transmission will require that both the parasite larvae must be able to establish in the host, and the intermediate host must be available to (and acceptable as food for) any potential primary host. Information to enable a reliable forecast of exposure assessment has not been located. On that basis the likelihood of spread of infection cannot be classified as negligible.

The exposure assessment is considered to be non-negligible.

Consequence assessment

The consequences of establishment of a pentastomid parasite of lizards in New Zealand are expected to be small. Flach *et al*. (247) comment on the frequency of subclinical pentastomid infections in free-living reptiles and suggest that pentastomids may behave aberrantly in captive reptiles, presumably because of stress. This is consistent with the view of Greiner and Mader (207) that "most infections with pentastomids, especially in wild reptiles, are asymptomatic".

There are only a limited number of reports of disease associated with pentastomid infections in reptiles and reports of disease from Squamata are rare. Greiner and Mader (207) referred, particularly, to disease in crocodiles and alligators. Flach *et al*. (247) reported fatal respiratory disease in one Bosc's Monitor Lizard and respiratory disease that responded to treatment in another. Both cases were considered to be due to infection with both adult and nymphal *Sambonia sp*. The authors suggested that both auto-infection and development of disease occurred after four imported animals, that had been held in separate vivaria, were placed in one enclosure.

Although pentastomids are said to be capable of causing disease in humans, no records of *Raillietiella* spp., *Sambonia* spp., or *Elenia* spp. infecting humans or other non-reptilian species, except intermediate hosts, have been located.

The consequence assessment is considered to be negligible.

Risk estimation

Since the consequence assessment is negligible, the risk estimate is considered to be negligible and pentastomids are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.6.2 Ectoparasites (Ticks and mites)

3.6.2.1 *Hazard identification*

Aetiological agent
Most reports of ectoparasites on lizards are of trombiculid mites (Order Prostigmata; Family Trombiculidae) with a lesser number of reports of Pterygostomid mites (Order Prostigmata; Family Pterygostomadae). *Ophionyssus* spp. (Order Mesostigmata; Family Macronyssidae) are also reported.

Reports of ticks of the Family Ixodidae (Order Ixodida), predominantly *Ixodes* spp., are relatively common while those of *Amblyomma* spp. (Family Amblyommidae) and of argasid ticks (Order Ixodida; Family Argasidae) are less frequent.

OIE list
No ectoparasites of lizards are included in the OIE list of notifiable diseases.

New Zealand status
The ticks *Amblyomma* spp., *Boophilus* spp., *Ixodes* spp., *Rhipicephalus* spp*.,* and *Dermacantor* spp. are listed in the unwanted organisms register along with the rabbit flea (*Spilopsyllus cuniculi.*), *Psoroptes ovis* (a mite that parasitises sheep) and four mites that parasitise bees (*Euvarroa sinhai*, *Varroa destructor*, *Varroa underwoodi,* and *Acarapis woodi*). None of the mites listed is likely to be found on lizards, nor is the rabbit flea. A number of species of ticks infect lizards.

In his annotated list of past records of parasites of New Zealand reptiles, McKenna (177) identified the following ectoparasite host records (see Table 4). All of the listed species are mites.

Ticks reported from New Zealand are nine *Ixodes* species, one *Ornithodorus* species, one unidentified tick from the *Argasidae,* and *Haemaphysalis longicornis* (253). Of these, all but *H. longicornis* are parasites of birds.

Table 4. Ectoparasites identified on New Zealand reptiles

Mite	Recorded hosts(s)
Acomatacarus lygosomae	Brown Skink (*Oligosoma zelandicum*)
	Common Skink (*O. nigraplantare*)
	Grand Skink *(O. grande)*
	Spotted Skink (*O. lineoocellatum*)
Microtrombicula hoplodactyla	Common Gecko (*Hoplodactylus maculatus*)
Neotrombicula naultini	Common Gecko (*H. maculatus*)
	Duvaucel's Gecko (*H. duvaucelii*)
	Jewelled Gecko (*Naultinus gemmeus*)
	Common Skink (*O. nigraplantare*)
	Otago Skink (*O. otagense*)
	Tuatara (*Sphenodon punctatus*)
Neotrombicula sphenodonti	Brown Skink (*O. zelandicum*)
	Common Skink (*O. nigriplantare*)
	Speckled Skink (*O. infrapunctatum*)
	Spotted Skink (*O. lineoocellatum*)
	Tuatara (*S. punctatus*)
Geckobia hoplodactyli	Common Gecko (*H. maculatus*)
	Duvaucel's Gecko (*H. duvaucelii*)
	Pacific Gecko (*H. pacificus*)
Geckobia naultina	Common Green Gecko (*N. elegans*)
Ophionyssus galeotes	Common Gecko (*H. maculatus*)
	Duvaucel's Gecko (*H. duvaucelii*)
Ophionyssus scincorum	Common Skink (*O. nigraplantare*)
	Moko Skink (*O. moco*)
	Otago Skink (*O. otagense*)
	Spotted Skink (*O. lineoocellatum*)
Ophionyssus sp.	Duvaucel's Gecko (*H. duvaucelii*)
	Pacific Gecko (*H. pacificus*)
	Egg-laying Skink (*O. suteri*)
	Marbled Skink (*Cyclodina oliveri*)
	Ornate Skink (*C. ornata*)
Ophionyssus natricis *	Bluetongue Skink (*Tiliqua scincoides*)
Pterygosoma sp. *	Indian Blood-sucker Lizard (*Colotes versicolour*)

* The species were associated with imported lizards and appear not to have established in New Zealand.

Epidemiology

Ticks: The life-cycles of ticks have a general form of eggs being laid on a host, larvae hatching and feeding on blood of the host before falling to the ground, larvae moulting, progressing to adults and regaining access to the primary host on which mating takes place. Ticks are grouped as one host, two host and three host ticks on the basis of the number of hosts parasitised in the completion of their life-cycles. One-host ticks (e.g. *Boophilus* spp.) have larvae falling from the primary host, moulting, developing to adults and regaining access to animals of the primary host species. The larvae of two-host ticks (e.g. *Rhipicephalus* spp.) gain access to a species different from the primary host, feed, fall back to the ground, complete development, and then regain access to the primary host. Larvae of three-host ticks (e.g. *Ixodes* spp., *Amblyomma* spp., and *Dermacentor*

spp.) feed on alternative hosts on two occasions during their development before regaining access to their primary host.

Most reports of tick infestations on lizards are of larval forms of three-host ticks. There are a number of reports from California, USA, where work has been done on the effect of the Western Fence Lizard (*Sceloporus occidentalis*) in suppressing *Borrelia burgdorferi* infections in the *Ixodes pacificus* larvae (129, 130, 254-256). The geographic distribution of *I. pacificus* is restricted to the western parts of the United States where, in some locations, the prevalence of infestation of *S. occidentalis* can be high (88%) (256). A number of mammalian species are hosts of adult *I. scapularis*. Amongst other ticks found on lizards have been *I. ricinus* (257), *Amblyomma vikirri* (258), *A. limbatum* (259), *Aponomma hydrosauri* (258, 259), *A. komodoense* (260), and *Hyalomma aegyptium* (261). Burridge *et al.* (262) examined reptiles at the premises of importers, breeders, zoos, pet stores, and others in Florida for the presence of exotic ticks. They found *Amblyomma excoriatum* (monitor lizard tick), *Aponomma flavomaculatum* (yellow spotted monitor lizard tick), and *A. varanenensis* (Asia monitor tick), mainly on various species of monitor lizards, and *Amblyomma nutalli* (small reptile tick) and *Aponomma latum* (snake tick), predominantly on snakes but with small numbers on monitor lizards.

Ticks may have negative effects on lizards through direct damage to the skin, enabling bacterial infection to enter through the skin, and through removal of blood during feeding. They may be vectors of numerous diseases of livestock and have potential to transmit human diseases including Lyme disease (125) and, possibly, West Nile fever (35). The spread of ticks on reptiles through international trade and their potential role in the spread of diseases have been reviewed by Burridge (164).

- *I. ricinus* is a three host tick established in Europe, North Africa, and parts of Asia. Adults are found only on mammals but larvae include reptiles in their host range (263).

- *A. vikirri* has a host range restricted to two lizards (*Egernia stokesii* and *Tiliqua rugosa*) and a restricted geographic range in South Australia (258, 264, 265).

- Both *Amblyomma limbatum* and *Aponomma hydrosauri* are parasites of lizards with known geographic distributions restricted to central and southern Australia (266-268).

- *A. komodoense* is a parasite of Komodo Dragons (*Varanus komodoensis*) in Indonesia. The tick was imported to a zoo in Miami, USA, and established within the Komodo Dragon enclosure (260).

- The geographic range of *Hyalomma aegyptium*, another three-host tick, extends through Europe, Africa, and parts of Asia. The adults parasitise tortoises while immature stages are found on small mammals, birds and the lizard *Agama stellio* (261, 269, 270).

- *Amblyomma loculosum* is a three-host tick with sea birds as its main host. It has been reported from the areas of the Indian Ocean and the Coral Sea. There is a report of its being found on a single lizard on the Tanzanian coast (271).

- The *Amblyomma* and *Aponomma* species identified on imported reptiles in Florida by Burridge *et al*. (164, 262) have sub-tropical Africa, Asia, and Central America as their normal home ranges and reptiles as their usual hosts.

Trombiculid mites: Chiggers (trombiculid mites) have most of their life-cycle within soil, compost, or other inanimate material, with only their larval stages parasitising animals. Domrow and Lester (272) listed *Eutrombicula*, *Herpetocarus*, *Schoengastia*, *Neotrombicula*, A*scoschoengastia,* and *Trombicula* genera in Australia with lizards as hosts. Many of these mites have a wide host range with *E. hirsti* having been found on skinks, marsupials, rats, and humans.

Non-trombiculid mites: Most of these mites complete their lifecycle on the host. Spread between hosts is either at times of direct contact or by attaching to a new host within days of leaving a previous one. Within the Pterygosamidae, genera show strong preferences for particular host families or genera; *Geckobia* spp. of mites on Gekkonidae; *Pterygosoma* spp. on Agamidae; *Geckobliella* occasionally on Iguanidae; and *Zonierobia, Scaphothrix,* and *Ixodiderma* preferentially on Zonuridae (273). Extensive searches of the literature have failed to locate reports of mites on members of the Varanidae (monitor lizards).

Domrow's review of mites found on Australian lizards lists four *Geckobia* species as parasitising six species of geckos from five genera (274). Most have been reported from only one host species but three gecko species from three genera have been identified as hosts of *Geckobia gymnodactylus*. Three species of *Odontocarus* mites have been named with each having been reported from a single, different, host species. Unnamed *Odontocarus* spp. have been reported from small skinks.

Hazard identification conclusion
Ticks and mites can cause irritation and skin lesions, suck blood from the host and transmit a range of diseases. Some ticks are vectors for diseases of humans and domesticated animals. Mites cause skin irritation and lesions that can lead to infection, some suck blood and some act as vectors of haemoparasites of lizards. The negative effects of trombiculid mites are thought to be restricted to skin irritation (198).

Both ticks and mites are considered to be potential hazards on the commodity.

3.6.2.2 *Risk assessment*

Entry assessment

Ticks: Ticks found on lizards in Australia (*Amblyomma vikirri*, *A. limbatum* and *Aponomma hydrosauri*) have restricted geographic distributions and specific habitat and/or host requirements. Reports of international movement of ticks with lizards appear to be predominantly associated with trade in wild-caught animals. The exception to that is the movement of *A. komodoense* with Komodo Dragons from London Zoo to Miami (260). Although tick infections of the commodity are unlikely, the likelihood is not negligible.

Mites: Mites are relatively common on captive lizards and are referred to on many web sites directed at hobbyist and breeder herpetologists. The likelihood of mite infestations on imported lizards is non-negligible. Following the example of the international movement of *A. komodoense*, the establishment of mites from other localities has a reasonably high likelihood, aided by the range of species and by their small size making detection more difficult. In the absence of discoverable reports of mites on Varanidae, the likelihood of infestation of species from that family is considered to be negligible.

The entry assessment for ticks is considered to be non-negligible. The entry assessment for mites on the commodity, other than Varanidae, is considered to be non-negligible. The entry assessment for mites on Varanidae is considered to be negligible.

Exposure assessment

The ability of mites to become established after international movement is illustrated by the recognition of *Ophionyssus natricis* (a mite more commonly parasitising snakes) on a Blue-tongued Skink (*Tiliqua scincoides*) at Wellington Zoo three years after importation of its parents (275) and detection of *Hirstiella diolii* on four species of iguana at Taronga Zoo suggesting that this mite had entered Australia with imported lizards and became established in the zoo environment (276).

Burridge (164) provided examples where reptilian ticks imported to Florida had established on new hosts. This extended host range, however, remained almost entirely restricted to reptiles. Only one of the tick species had become established in Florida, although Burridge considered that the environment was suitable for the establishment of the other seven. For species that spend part of their lifecycle on the ground, this consideration of environment must include availability of suitable hosts in sufficient numbers and suitable ground and atmospheric conditions. Such establishment in Florida has been possible for the iguana tick (*Amblyomma rotundatum*).

Environmental conditions limit the geographic distribution of ticks in their natural environment even in the presence of competent hosts (267, 277). Environmental conditions and host availability are likely to prevent the establishment of some species of ticks and mites in New Zealand – others, however, might establish and have the potential to become pests.

The exposure assessment is considered to be non-negligible.

Consequence assessment

Environmental conditions and access to suitable hosts would limit the consequences of the establishment of many ticks and mites if they gained access to New Zealand. This has been illustrated by both *A. komodoense* and *H. diolii* in Australia and by *Ophionyssus natricis* in New Zealand remaining in the vicinity of their hosts.

Establishment of ticks and mites that entered New Zealand with lizards would have initial impact on the imported lizard species on which, if they were not well managed, the parasites could be expected to cause skin lesions, which may lead to infections and anaemia through blood loss. If haemoparasites were present, mites and/or ticks could act as vectors for spread within the host range of both the ectoparasite and the haemoparasite. The likelihood of this latter event is relatively low as most ectoparasites and most haemoparasites have restricted host ranges.

Aponomma hydrosauri is a vector of rickettsial disease of humans on Flinders Island, South Australia, (278) and other ectoparasites also act as disease vectors.

The consequence assessment is considered to be non-negligible.

Risk estimation

Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate for ticks and mites on non-Varanidae is considered to be non-negligible and they are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

Since the entry assessment for mites on Varanidae is negligible, the risk estimate is considered to be negligible and mites on those species of the commodity are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

3.6.2.3   *Risk management*

Options

No reports of scientific trials assessing the efficacy of ectoparasiticide treatment of lizards for tick and/or mite infestations have been located. Peveling and Demba (279) tested the toxicity of fipronil in the Fringe-toed Lizard (*Acanthodactylus dumerili*) at 30 μg per g body weight and found it produced a high mortality rate and ongoing adverse effects. Fitzgerald (280) commented that a number of the agents used for treatment of Acarid infestations in the past are dangerous and should not be used.

Holt (281) considered that dichlorvos strips were the most useful tool for ectoparasite control in reptiles, while Frye (200) included ivermectin, repeated after two weeks, and "almost any flea spray safe for kittens and puppies" as alternatives.

Fitzgerald (280) provides an extensive commentary on an integrated approach to the treatment of mites and emphasises that a combination of quarantine, sanitation and cleaning, and treatment of the host species is required:

- **Quarantine** – Quarantine for a minimum of three months is recommended. Animal's cages and rooms should be examined daily, with extension of the quarantine period to at least two weeks beyond the last detection of mites. This quarantine should be in a room separate from other reptiles. 5% Sevin dust (carbaryl) treatment for 6 hours in a ventilated container is said to be safe. Each cage should be placed in a shallow dish containing water with detergent to prevent migration between animals.

- **Sanitation and cleaning** – Animal cages should have smooth surfaces with no cracks or joins. They should contain as little material as practicable and they should be emptied, scrubbed, and refurbished at least twice per week. Hide cages and substrate should be treated as disposable and replaced with new material at each cage cleaning. Waste products should be bagged, treated with insecticide and either incinerated or autoclaved.

- **Treatment** – Fitzgerald comments on a variety of pyrethrins/pyrethroids, organophosphates, ivermectin, fipronil, and dichlorvos strips as potential agents for the treatment of mite infestations on reptiles and in their immediate environment.

One or a combination of the following measures could be considered in order to effectively manage the risk:

1. During the pre-export quarantine period, animals could be subject to a treatment regime recognised amongst herpetologists and veterinarians experienced in herpetological medicine as effective for the removal of ectoparasites from lizards. The regime used could be documented.

2. During the treatment regime, lizard accommodation could be regularly cleaned of all bedding material and other potential mite habitats. Quarantine measures could be maintained to prevent exposure to sources of reinfection and transport of the parasites from the quarantine area; **and**

3. Effectiveness of ectoparasite removal could be confirmed as follows with inspections carried out by a veterinarian experienced in herpetological medicine:

   a. Squamata other than varanids - Effectiveness of ectoparasite removal could be confirmed by negative findings on two occasions at least 14 days apart with the first of those two inspections being at least 14 days after completion of the treatment regime, with inspection including the examination of mite pockets and the cloaca of each animal.

    b.   Varanids – Effectiveness of ectoparasite removal could be confirmed by negative findings on two occasions at least 14 days apart with the first of those two inspections being at least 14 days after completion of the treatment regime. Animals of these species could be inspected sufficiently closely to ensure detection of ticks.

If ectoparasites are detected during inspections required above, a treatment and ectoparasite-management regime could be reinstituted and the inspections repeated following its completion. Certification of negative findings at two successive inspections, the first at least 14 days after completion of the last period of treatment and the second at least 14 days after that, could be required.

4 Risk Analysis – Eggs Of Squamata

Only those organisms considered to be hazards in live Squamata are considered in this section. This is on the basis that if the organism is not a hazard in the live animal it will not be a hazard in eggs.

4.1 VIRUSES

4.1.1 Adenoviruses.

4.1.1.1 *Risk assessment*

Entry assessment
Some adenoviruses of poultry have been shown to be transmitted transovarially. Infection of eggs can arise from birds with latent infections reactivated at the time of egg laying (46, 47). Although no reports of adenoviruses in lizard eggs, or of the transovarial transmission of such viruses, have been located, the examples from birds suggest that the likelihood of virus infection of lizard eggs and the hatching of infected young cannot be excluded.

The entry assessment is considered to be non-negligible.

Exposure assessment
If importation of eggs results in the hatching of infected young, transmission to other animals of the same, or closely related, species would be highly likely.

The exposure assessment is considered to be non-negligible.

Consequence assessment
The consequence assessment of the introduction of *Atadenovirus* strains of nil, or low, pathogenicity is considered to be negligible.

The consequence assessment of the introduction of *Atadenovirus* strains of high potential pathogenicity is considered to be non-negligible.

Risk estimation
Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and atadenoviruses are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

4.1.1.2 *Risk management*

Options

Assurance of the health status of the lizard colony from which eggs will be collected could be required. It does not appear that serological tests for atadenoviruses of lizards are available and virological cultures of swabs from respiratory or digestive tracts of lizards are of unknown sensitivity. The most reliable evidence of the absence of pathogenic atadenoviruses from lizards producing eggs for supply to New Zealand is the disease history of the source collection.

One or a combination of the following measures could be considered in order to effectively manage the risk:

1. The individual animals from which eggs are to be collected could be required to be resident for at least twelve months in premises approved by the relevant government, or government approved agency, for holding reptiles.

2. All animals of the species to be exported could be required to have been resident on the premises for at least 90 days prior to the commencement of pre-export quarantine or since birth/hatching.

3. All reptiles in the premises of origin could be under veterinary supervision, and the health of the animals monitored so that incidents of disease and death are identified promptly and that atadenoviruses have been excluded as the cause of illness or death of any animals of the genus from which eggs are to be collected within the past 12 months.

Options 1 and 2 are requirements of the commodity definition of this risk analysis. These commodity requirements will therefore provide some management of the risk associated with this hazard. However, if these measures are not considered to provide effective management of this risk, the inclusion of option 3 would be likely to significantly reduce any residual risk.

4.2   BACTERIA

4.2.1   Salmonellae

4.2.1.1   Risk assessment

Entry assessment
Salmonellae have been shown to readily penetrate reptile eggs with contamination of the internal contents within one hour of exposure (282). Infection through transovarial transmission has also been demonstrated (283). The entry assessment is therefore considered to be non-negligible.

Exposure assessment
Salmonellae are contagious organisms. Strains introduced with the commodity may infect other lizards and it is likely that they will have the potential to infect humans who come in contact with them and do not take appropriate hygiene precautions. Some strains may have the potential to infect other species but for them to do so will require contact between that other species and the lizard, its faeces, contaminated fomites, or humans carrying infection from the lizards. The exposure assessment is considered to be non-negligible.

Consequence assessment
Given that imported lizard eggs could potentially be harbouring Salmonella serotypes and phage types that are not known to be present in New Zealand, and lizards derived from these eggs that are not "new organisms" could be sold as pets, the Ministry of Health have indicated that they consider there to be a non-negligible risk of humans being exposed and consequently infected with exotic serotypes/phage types of Salmonella and the consequence assessment should therefore be considered to be non-negligible.

Risk estimation
Since the entry, exposure, and consequence assessments are non-negligible, the risk estimate is considered to be non-negligible and exotic *Salmonella* spp. are classified as a hazard in the commodity. Therefore, risk management measures can be justified.

4.2.1.2   Risk management

Options
Assurance of the health status of the lizard colony from which eggs will be collected could be required. Testing methods described in section 3.2.1.3 could be used to demonstrate source colony freedom from exotic *Salmonella* spp.

It is suggested that one or a combination of the following sanitary measures could be considered in order to effectively manage the risk:

1. The animals from which eggs are to be collected could be required to be clinically healthy and in particular not to have diarrhoea.

2. Faecal/cloacal samples could be taken from the animals from which eggs are to be collected and cultured for *Salmonella* spp. All *Salmonella* spp. isolated could be serotyped (and, where appropriate, phage typed) and the results reported to MAF. Where exotic *Salmonella* spp. are isolated, importation could be prohibited.

3. Five faecal/cloacal samples could be collected over a 30-day period, consistent with the advice of Mitchell (8). Alternatively, parallel testing of faecal/cloacal samples with both the PCR assay and microbiological culture could be used to further increase the overall sensitivity and specificity of the testing methods.

4.3   HELMINTH PARASITES

4.3.1   Nematodes

Entry assessment
No reports suggesting egg borne transmission of nematodes in either reptiles or birds have been located. The entry assessment is therefore considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and nematodes are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

4.4   PROTOZOA

4.4.1   Haemosporidian protozoa

Entry assessment
A search of CAB Abstracts reveals a record of a paper presented to a conference in Brazil in 1979, which appears to propose the vertical transmission of *Plasmodium juxtanucleare* in chickens (*Gallus gallus*) (284). The original article is in Portuguese and is not available to the author. Whether this presentation referred to adult to offspring or transovarial transmission is unknown but, in the absence of discovery of any other reports suggesting transovarial transmission, it is concluded that such transmission plays no significant role in the epidemiology of *Plasmodium* spp. in either reptiles or birds.

The entry assessment for haemosporidian protozoa is therefore considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and haemosporidian protozoa are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

4.4.2   Entamoeba invadens

Entry assessment
No reports suggesting egg borne transmission of *Entamoeba* spp. of reptiles or birds have been located.

The entry assessment for *Entamoeba invadens* is therefore considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and *Entamoeba invadens* is not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

4.5   ARTHROPODS

4.5.1   Ectoparasites (Ticks and mites)

Entry assessment
No reports suggesting egg borne transmission of ticks or mites of reptiles or birds have been located.

The entry assessment for ticks and mites is therefore considered to be negligible.

Risk estimation
Since the entry assessment is negligible, the risk estimate is considered to be negligible and ticks and mites are not classified as a hazard in the commodity. Therefore, risk management measures are not justified.

4.6   DISINFECTION OF EGGS

Disinfection of reptile eggs is not generally recommended (285) and very few reports of such disinfection have been discovered. Apart from reports of treatment of eggs of turtles with gentamycin (286-288), sodium hypochlorite (288) and polyhexamethylene biguanid (288) with the objective of controlling salmonella infection in hatchlings, the only report of disinfection of reptile eggs discovered is that of Booth who treated eggs of both Forest Dragons (*Hypsilurus spinipes*) and Green Turtles (*Chelonia mydas*) by brief immersion in 1% iodine. Results from Booth's use of iodine were not reported.

Although reports of the use of formaldehyde fumigation as in the poultry industry (289) for disinfection of reptile eggs have not been located, it is considered likely that such use would have major effects on hatchability. The structure and composition of reptile eggs differ from those of bird eggs. There is also considerable variation between the eggs of different reptilian sub-orders and between eggs of different species within sub-orders. The shells of Squamata eggs are more porous and less rigid than those of birds. They also have greater conductance of water and gasses. Few, if any, species of Squamata have a layer of albumin between the shells of their eggs and the embryos (290). The lack of an albumin layer places Squamata embryos in closer proximity to the egg shell and such embryos are in a more advanced stage of development than is the case with poultry eggs prior to incubation (290). The higher conductance of reptile eggs, together with the lack (or small quantity) of an albumin layer between the shell and the embryo increases the likelihood of exposure of the embryo to chemicals in contact with the egg. It is known that formaldehyde penetrates the shells of chicken eggs (291) and that treatment of eggs in which embryos have undergone development during 24 to 96 hours of

incubation can result in embryo mortality (289). Booth (285) comments that turtle and crocodile eggs can be cleaned of dirt and vegetation with tap water but counsels against the use of soap or detergent. Given the differences in composition of eggs, it is likely that Squamata eggs will be more easily damaged than those of crocodiles or turtles and Squamata embryos are substantially more likely to be damaged than those of pre-incubation poultry eggs.

Given the lack of technical justification for the disinfection of eggs of Squamata, the absence of validated methods for such treatment and the likelihood that attempts at disinfection with result in decreased hatchability, the disinfection of such eggs should not be required.

5 References

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