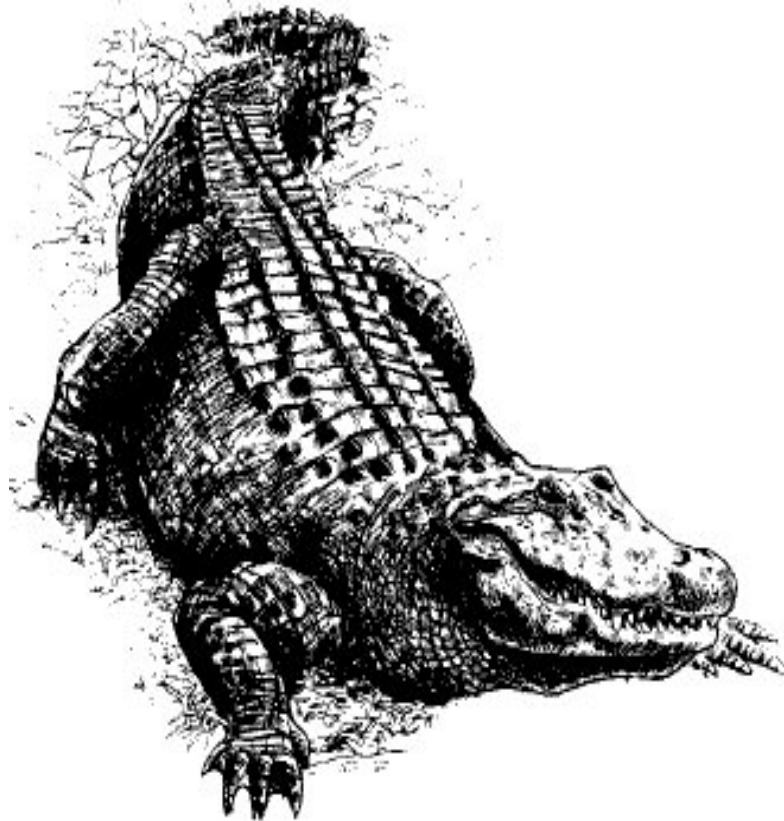


AQIS
Protecting our way of life!

**Import Risk Analysis Paper
for Live Crocodilians and their Eggs**

JANUARY 2000



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ABBREVIATIONS AND ACRONYMS

AFFA	Agriculture Fisheries and Forestry - Australia, Department of [formerly the Department of Primary Industries and Energy (DPIE)]
AQIS	Australian Quarantine and Inspection Service
ARAZPA	Australasian Regional Association of Zoological Parks and Aquaria
bp	base pair
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
cfu	colony forming units
CFT	complement fixation test
CVO	Chief Veterinary Officer
DNA	deoxyribonucleic acid
EA	Environment Australia
ELISA	enzyme linked immunosorbent assay
EEEV	eastern equine encephalomyelitis virus
EM	electron microscope
gm	gram (s)
IB	inclusion body; area with altered cytochemical staining properties in the nucleus and/or cytoplasm of an infected cell
ICTV	International Committee on Taxonomy of Viruses
IF	immunofluorescent (test)
IRA	import risk analysis
IRA Handbook	<i>The AQIS Risk Analysis Process Handbook</i> (1998)
kg	kilogram(s)
l	litre(s)
min	minute(s)
mg	milligram(s)
nm	nanometer(s)
OIE	Office International des Epizooties (World Organisation for Animal Health)(refer IRA handbook for further information)
PAQ	post-arrival quarantine
PEQ	pre-export quarantine
PCR	polymerase chain reaction
ppt	parts per thousand
ppm	parts per million
RAP	risk analysis panel
RNA	ribonucleic acid
SPS	sanitary and phytosanitary (SPS Agreement - refer IRA handbook)
TAG	taxon advisory group
TEM	transmission electron microscopy
WTO	World Trade Organization

DEFINITIONS¹

Code	means the OIE International Animal Health Code
Hazard	in the context of risk analysis, a biological agent which may have an adverse effect
Hazard identification	the process of identifying the biological agents which could potentially be introduced in the commodity considered for importation
Incubation period	the longest period which elapses between the introduction of the pathogen into the animal and the occurrence of the first clinical signs of the disease
Manual	means the OIE Manual of Standards for Diagnostic Tests and Vaccines

¹ Definitions are taken from the AQIS Handbook and the OIE International Animal Health Code

Risk	the integration of the likelihood of the occurrence and the magnitude of the consequences of an adverse event to animal or human health in the importing country
Risk assessment	the process of estimating the risk presented by a hazard, in qualitative or quantitative terms
Risk management	the process of selecting and implementing measures that can be applied to reduce the level of risk

Executive Summary

The conditions for the importation of crocodilians and their eggs from all countries are based on the final outcome of this risk analysis and replace the interim conditions, notified in 1997, as they apply to crocodilians.

Disease agents may be carried by live crocodilians and their eggs. This risk analysis has identified which of these are potential hazards and assessed the risk of establishment of these hazards in reptiles and other animals in this country. Risk management options have been considered for disease agents which pose a moderate to high risk of being introduced and becoming established. For each of these agents quarantine measures are recommended which are least trade restrictive and minimise the risk of establishment of unwanted organisms. Measures for each disease agent are consolidated into proposed quarantine conditions. It is concluded there is a negligible risk of introducing exotic diseases associated with crocodilians provided the quarantine conditions are met.

Risks are considered to be significantly different for the importation of eggs and live animals and consequently separate sets of conditions have been finalised.

1. Introduction

1.1 Scope of the import risk analysis

The disease risks associated with the importation of live animals in the order Crocodylia and their eggs are analysed in this paper. Disease agents carried by alligators, caimans, crocodiles and gharials are thus the subject of this import risk analysis (IRA). These animals will be referred to collectively in this paper as crocodylians. The IRA is generic, ie. disease risks potentially associated with all source countries are considered.

The disease risks associated with products derived from crocodylians, eg. meat and skins, and the public health implications of the importation of live crocodylians are not considered in this IRA.

Two species of crocodiles are native to Australia; the saltwater crocodile (*Crocodylus porosus*) and the freshwater crocodile (*Crocodylus johnstoni*). Both species are distributed along the coastal regions of northern and north-eastern Australia. *C. porosus* is widely distributed from Sri Lanka and the east coast of India in the west to the Caroline Islands in the east, from Burma and South-East Asia in the north to Australia in the south. *C. johnstoni* is endemic to Australia (Webb and Manolis 1993).

Crocodylians are held in a number of zoos and fauna parks in Australia. Native species, *C. porosus* and *C. johnstoni*, are usually held. Imported crocodylians are held at Taronga Zoo (*Alligator mississippiensis*) and the Australian zoo (*A. mississippiensis*), Broome (*Caiman crocodylus*, *A. mississippiensis*), Cairns (*Crocodylus novaeguineae*), Darwin crocodile park (*A. mississippiensis*), Crocodylus park (*A. mississippiensis*), Ballarat wildlife park (*A. mississippiensis*), and Melbourne zoo (*Crocodylus mindorensis*).

1.2 Animal quarantine policy

1.2.1 International framework

As a member of the World Trade Organisation (WTO), Australia has certain rights and obligations including those set out in the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement). Further information on the SPS Agreement may be found in the *AQIS Import Risk Analysis Process Handbook* (IRA Handbook). Copies of the IRA Handbook may be obtained from AQIS or viewed on AQIS's homepage on the Internet (<http://www.aqis.gov.au>).

The SPS Agreement recognises the Office International des Epizooties (OIE) as the international organisation that sets animal health standards, guidelines and recommendations relevant to international trade in animals and their products. Australia is a member of OIE and actively contributes to the process of standards development. The OIE publication relevant to this IRA is the 'International Animal Health Code' (hereinafter referred to as 'the Code'). The principal aim of the Code is to facilitate international trade in animals and their products by providing definitions of minimum health guarantees to be required of trading partners in order to avoid the risk of spreading animal diseases (through international trade).

Australia is a signatory to the international treaty *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES). International trade in all live crocodylians and their products is controlled through this treaty. Commonwealth legislation which allows obligations to CITES to be effected is the *Wildlife Protection (Regulation of Exports and Imports) Act 1982*. This act is administered by Environment Australia (EA).

1.2.2 Quarantine legislation in Australia

The *Quarantine Act 1908* and subordinate legislation, including Quarantine Proclamation 1998, regulates human, animal and plant quarantine in Australia. The scope of quarantine is defined in section 4 of the Act as follows:

'In this Act, Quarantine has relation to measures for the inspection, exclusion, detention, observation, segregation, isolation, protection, treatment, sanitary regulation, and disinfection of vessels, installations, persons, goods, things, animals, or plants, and having as their object the prevention of the introduction, establishment or spread of diseases or pests affecting human beings, animals, or plants.'

Quarantine Proclamation 1998 prohibits the importation of animals, plants and their products into Australia except under prescribed conditions or under a permit to import. Prescribed conditions and permit conditions to manage the quarantine risks posed by the imported animals are determined through an IRA process.

The IRA process provides the scientific and technical basis of quarantine policy and procedures. Quarantine Proclamation 1998 requires that the Director of Quarantine takes into account all relevant factors in considering the quarantine risk when making a decision on an import access request. The report of the IRA documents relevant information and makes recommendations for the Director of Quarantine to consider before making the final decision on an import access request.

The IRA process is described in detail in the IRA Handbook.

This IRA will provide the basis for future consideration of the matters outlined above in relation to the importation of live crocodylians from all countries. In keeping with the scope of the Quarantine Act, only those factors relevant to the evaluation of quarantine risk (ie the risk associated with the entry and establishment of unwanted pests and diseases) are considered in the IRA. Questions related to the potential economic consequences of importation (except for the economic impact of a disease outbreak, which is specifically considered) are not part of AQIS's process of evaluation. This approach allows Australia to meet its obligations as a WTO Member, in particular the disciplines imposed by the SPS Agreement.

1.2.3 Domestic policy environment

Quarantine conditions dated 1986 for the importation of reptiles into A and B class zoos were suspended in February 1997. In AQIS's judgement, these conditions exposed Australian animals to an undue risk of entry of unwanted pests and disease agents. Interim conditions for the entry of reptiles into quarantine premises approved by the Chief Quarantine Officer (Animals) ie. A class zoos, were promulgated, pending the outcome of a review of policy. These interim conditions specify that imported animals must remain in a registered A or B class zoo after release from post-arrival quarantine isolation unless otherwise agreed by the Director of Animal and Plant Quarantine (Australia).

In September 1997 AQIS advised stakeholders that the quarantine policy for the importation of reptiles was under review, and invited stakeholder comment. In December 1997 AQIS advised stakeholders that it proposed to use the 'routine' approach to the IRA and that separate risk analyses would be conducted for 5 groups within the class Reptilia. These groups will comprise the orders Crocodylia, Testudines, Rhynchocephalia and suborders Sauria and Serpentes within the order Squamata. This IRA is the first in the series.

1.2.4 Quarantine policy for the importation of live reptiles

The 1997 interim conditions for the importation of reptiles are at Appendix 1.

1.3 Health status of Australian crocodilians

The health of Australian crocodiles on farms in Queensland and the Northern Territory was investigated intensively during 1990. The major infectious and parasitic diseases recorded were bacterial hepatitis/septicaemia, superficial and deep mycoses, giant cell enteritis, pentostomiasis and coccidiosis (Buenviaje *et al* 1994). Investigation of the causes of skin disease was the focus of a more recent study (Buenviaje *et al* 1998) which concluded that *Dermatophilus* sp was the probably the most important skin disease in farmed crocodiles in Australia. Also, the prevalence of pox virus was found to have increased significantly since previous studies. Worm trails presumably caused by a species of *Paratrichosoma* were observed in crocodile skins from wild-caught animals in Australia (Buenviaje *et al* 1998; Webb and Manolis 1983).

Many farmed animals are captured from the wild and consequently records of disease in these animals also provide an indication of the health status of wild crocodiles. Manolis *et al* (1991) identified a number of species of *Salmonella* in farmed crocodiles in the Northern Territory. One intensive parasitological study of wild Northern Territory crocodiles found that just over one third of the animals examined contained nematode parasites in the stomach. These were identified as *Goezia fluvialis* and nematodes in the genera *Dujardinascaris*, *Eustrongylus*, *Contracaecum* and *Physaloptera* (Webb *et al* 1982).

2 Hazard Identification and Exposure Pathways

2.1 Hazard identification

AQIS has used a process of categorisation to identify disease agents requiring further consideration in this import risk analysis. Section 2.1.1 provides details of this scheme and its application to disease agents associated with crocodilians.

A summary list of disease agents is presented below; information on these disease agents may be found in Appendix 2.

2.1.1 Categorisation scheme

Identification of disease agents for further consideration in an import risk analysis

The following process of categorisation is used to determine which disease agents should be identified as potential hazards for further consideration in a quarantine import risk analysis (IRA) for reptiles. The identification of reptiles as potential pests, and hence hazards in environmental terms, is a separate process.

In this system, a disease agent is identified as a hazard and given further consideration in an IRA if it is:

- (i) found in association with crocodilians **and**
- (ii) infectious **and**
- (iii) (a) exotic to Australia **or**
 - (b) present in Australia but subject to official control **and**
- (iv) (a) OIE listed **and/or**

(b) likely to cause disease in a significant proportion of infected reptiles or other species or is associated with significant economic and/or ecological harm.

The categorisation of disease agents is based on the following criteria.

(i) The disease agent is found in association with crocodilians

The disease agents must be reported in association with crocodilians, not just generally reported in reptiles.

(ii) The disease agent is infectious

A putative disease agent must cause/be associated with a recognised disease and the disease must be shown to have an infectious aetiology.

The disease agent is transmissible to susceptible hosts and may have been isolated. Ideally Koch's² or Evan's (Thrusfield 1995) postulates have been satisfied. This excludes diseases caused by environmental (eg. toxicosis), genetic or nutritional factors.

(iii)a The disease agent is exotic to Australia

The disease agent is considered exotic in absence of any report of the disease or detection of the causal agent in Australia. The level of confidence which can be attributed to such a determination depends on factors such as the virulence of the organism, severity of the clinical disease and nature of targeted surveillance applied to the disease/agent in question.

Where a disease agent is present in Australia, but the strain(s) present in other countries is/are significantly more virulent, exotic strains of the disease agent meet this criterion.

(iii)b The disease agent is present in Australia but subject to official control.

A disease agent or disease occurs in Australia and one or more State/Territory Government(s) has enacted legislation to control or eradicate the disease/agent, ie. mandatory control measures are in place.

(iv)a The disease agent is OIE listed

The disease agent causes a disease as listed by the OIE. Animal diseases reported in crocodilians which are listed in the Code are:

List A³ Diseases notifiable to the OIE:

There are no list A diseases reported in crocodilians

List B⁴ Diseases

² Koch's postulates refer to the experimental evidence required to establish a relationship of causation between a microorganism and a disease. The conditions are: 1) the microorganism must be present in every case of the disease, 2) it must be isolated and cultivated in pure culture, 3) inoculation of such culture must produce the disease in susceptible animals, 4) it must be observed in, and recovered from, experimentally diseased animal.

³ List A means the List of transmissible diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence and which are of major importance in the international trade of animals and animal products. Reports are submitted to the OIE as often as necessary to comply with Articles 1.2.0.2. and 1.2.0.3. Diseases in List A are set out in Section 6.1. of the Code.

Dermatophilosis
Trichinellosis
Equine encephalomyelitis (Eastern and Western)

(iv)b The disease agent is likely to cause disease in a significant proportion of infected reptiles or other species or is associated with significant economic and/or ecological harm

The disease agent satisfies one or more of the following criteria:

- it would cause a distinct pathological effect in a significant proportion of an infected population;
- it would cause significant economic losses as a result of, for example, increased mortality, reduced growth rates, decreased product quality, loss of market access, increased management costs;
- it would cause significant harm to other species and/or the environment.

Some agents are included in the IRA for further consideration though they cause little or no harm to crocodilians harbouring them if they pose a threat to other animals. As an example, *Entamoeba invadens* is further considered in the IRA as it may be carried by apparently healthy crocodilians and can cause serious disease in other reptiles, especially snakes.

Where definitive data relevant to categorisation are lacking, AQIS makes conservative judgements which draw upon scientific knowledge and observations made in similar situations and any other relevant information.

2.1.2 Categorization of crocodilian disease agents

The process described above is applied in this section to the disease agents noted in the literature associated with crocodilians. Agents are categorised and identified for further consideration in the risk analysis and a summary list of these is given in section 2.1.3.

Viruses

Few diseases associated with viruses have been reported in crocodilians. Viral hepatitis and enteritis associated with an adenovirus-like agent has been included for further consideration in the risk analysis as it is considered to be a major, communicable disease of farmed crocodiles (Foggin 1992b) and has not been reported in crocodilians in Australia.

Outbreaks of disease attributed to pox-viruses have been reported in a range of crocodilian host species and in a number of countries, (Foggin 1992a) including Australia (Buenviaje *et al* 1992). The disease signs, host species affected and virion characteristics in these reports vary and this may indicate that separate viruses or strains of virus are responsible. This view is supported by the separate listing of spectacled caiman pox virus and Nile crocodile pox virus (Murphy *et al* 1995) and the reference of Jacobson *et al* (1989) to a separate “caiman pox”. The relationship between the viruses isolated from the various outbreaks is unclear and, for this reason, the pox-virus group will

⁴ List B means the List of transmissible diseases which are considered to be of socio-economic and/or public health importance within countries and which are significant in the international trade of animals and animal products. Reports are normally submitted once a year, although more frequent reporting may in some cases be necessary to comply with Articles 1.2.0.2. and 1.2.0.3. Diseases in List B are set out in Section 6.2. of the Code.

be included for further consideration in this IRA even though pox virus infections have been recorded in Australia.

EEEV has not been reported from reptiles in Australia, is OIE listed and has the potential to cause significant disease in humans and animals. Karstad (1961) found significant antibody titres to EEEV in 1 of 16 alligators (*A. mississippiensis*) and some other reptiles in the USA. This author concluded that, although this was evidence of natural infection with EEEV, establishment of the role of reptiles as reservoir hosts must await repeated isolations of virus from reptiles infected in nature, as well as demonstration of a suitable mechanism of virus transmission from infected reptiles into an epizootic cycle. Significantly, Fenner *et al* (1993) note that a possible role for reptiles as reservoirs of arboviruses has not been confirmed. Natural infection of alligators which is transmissible to susceptible hosts has not been reported. As a result EEEV is not considered further in the IRA.

Crocodiles fed Newcastle disease infected poultry seroconverted (Thompson 1972) but recovery of virus from infected animals has not been reported. Other paramyxoviruses as well as influenza viruses have been identified in the faeces of farmed crocodiles in South Africa (Huchzermeyer *et al* 1994). The association of these viruses with disease in crocodylians has not been established and they are not included in the IRA.

Bacteria

Mycoplasma spp. and *Chlamydia* spp. are associated with significant disease in crocodylians overseas (Mohan *et al* 1995) but have not been reported in crocodylians in Australia. These pathogens will be considered further in the IRA.

Salmonella spp., *Escherichia* spp., *Dermatophilus* sp., *Streptococcus* sp., *Mycobacteria* spp., *Aeromonas* spp., *Pseudomonas* spp., *Edwardsiella tarda*, *Pasteurella multocida*, *Providencia rettgeri*, and *Erysipelothrix insidiosa* have been recovered from crocodylians with and without signs of disease (Youngprapakorn *et al* 1994; Jacobson 1984; Jacobson 1989; White, 1984; Foggin 1987; Foggin 1992a; Ladds and Sims 1990; Mohan *et al* 1994; Migaki *et al* 1984; Ippen and Zwart 1996; Brownstein 1984; Buenviaje *et al* 1998). Bacterial disease is cited as the major cause of mortality of crocodiles from infectious disease in Zimbabwe (Foggin 1992a). However, bacterial infection is usually secondary to another cause (Foggin 1992a). Bacteria isolated from crocodiles may also be isolated from a wide range of other animals, including humans.

Dermatophilosis is an OIE list B diseases. *Dermatophilus* sp. has been reported in farm reared American alligators in Florida (Jacobson 1989), Zimbabwe (Phil Ladds, personal communication) and farmed *C porosus* in Australia (Buenviaje *et al* 1997; 1998).

With the exception of *Mycoplasma* and *Chlamydia* species reported in crocodylians, the bacteria noted above are not considered further in this IRA as they are apparently not host specific and have been reported from reptiles or other animals both overseas and in Australia. In addition, significantly more pathogenic variants (strain, pathotype, biotype, etc.) of any of these bacteria have not been described in association with diseases of crocodylians overseas.

Fungi

Reports of fungal disease in reptiles are rare compared to such reports in higher vertebrates (Migaki *et al* 1984). Fungi within genera *Paecilomyces*, *Beauveria*, *Candida*, *Metharhizium*, *Trichophyton*, *Mucor*, *Curvularia*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Penicillium*, *Trichosporon*, *Trichoderma* and *Cephalosporium* have been identified in crocodylians, either in association with disease or from asymptomatic animals in Australia and other countries (Jacobson 1989; Maslen *et al* 1988;

Buenviaje *et al* 1994; Fromtling *et al* 1985; Migaki *et al* 1984; Trevino 1972; Foreyt and Leathers 1985; Hibberd and Harrower 1993).

All the reports cited above identify fungi which are not host specific to crocodilians and can be isolated from a wide range of animals with and without signs of disease. Reports of disease in association with these fungi document isolated episodes or situations where the disease is secondary to some other condition or environmental or managemental stressor. In addition, many agents are saprophytes and/or common in the air, soil or water and most are presumed to be widespread in the Australian environment and fauna. Foggin (1987) considers that the few fungal infections observed in farmed crocodiles in Zimbabwe were probably secondary infections and of little importance. For these reasons, none of the fungi isolated from crocodilians meet the criteria for further consideration in this IRA.

Parasites

Crocodiles, in common with other reptiles, usually harbour a wide variety of protozoans and metazoan endo- and ecto-parasites. The parasitic burden is often very heavy but, even so, it can be difficult to determine the clinical significance of the infestation. There are no reports of epizootics associated with parasitism of wild reptile populations but many parasites can be responsible for illness and death in captive reptiles (Jacobson 1986). Many protozoan species are limited by host specificity or vector requirements. However, Telford (1971) and Willette-Frahm *et al* (1994) warn that parasite species normally benign in nature, can become pathogenic in non-adapted atypical host species; a situation which may arise in captivity.

Some parasitic infections are direct, eg. *Entamoeba invadens*, while others require one or more intermediate hosts for completion of the life cycle, with important implication for risk management. The life cycle of many reptilian parasites remains to be elucidated (Jacobson 1986).

There is considerable literature on parasites of crocodilians but usually from authors with a taxonomic interest. Usually the report has no indication of the host response to the parasite or pathological effects associated with the infection (Riley *et al* 1990).

Protozoa

Protozoans in the subphylum Mastigophora (Phylum Sarcomastigophora) in the genera *Enteromonas*, *Trimitus*, *Giardia*, *Trepomonas*, *Monocercomonoides* and *Tritrichomonas* are recorded as parasites of reptiles in general (Frank 1984). These parasites are considered to be harmless commensals of low or nil pathogenicity (Barnard and Upton 1994) and are not considered further in this review.

Protozoans in the genus *Spironucleus* (*Hexamita*, *Octomitus*) can cause lethargy, anorexia and weight loss in some reptiles (Barnard and Upton 1994) and are recorded in association with reptiles in general by Frank (1984). However, Barnard and Upton (1994) did not specifically include crocodilians in the host list for these parasites and they are not considered further.

The genera *Trypanosoma* and *Leishmania*, within the order Kinetoplastida are recorded in crocodiles. Ladds *et al* (1994) describe a granulomatous enteritis which appeared to be a primary cause of death and ill thrift in hatchlings in farmed *C. porosus* in Northern Australia and Papua New Guinea. The suspected causative agent resembled the amastigote of *Leishmania* sp. of mammals. This disease agent has been recorded in Australia and so is not considered further in the review.

Trypanosomes have been reported in crocodilians. *Trypanosoma* sp. in *Caiman crocodilus yacare* (Nunes and Oshiro 1990), *T grayi* in the Nile crocodile (Hoare 1929) and *T cecili* in *Caiman*

crocodilus crocodilus (Lainson 1977). These trypanosomes produce no clinical disease (Nunes and Oshiro 1990; Campbell 1996). Species infecting mammals have not been recorded in reptiles. Trypanosomes are not considered further in the IRA.

Entamoeba invadens (subphylum Sarcodina; phylum Sarcocystidophora) may potentially infect all reptile species (Barnard and Upton 1994), including crocodilians (Frank 1984; Ippen 1959). Crocodilians are considered to be resistant carriers (Cubas 1996) of this parasite which can be a significant pathogen for other reptiles (Ippen and Zwart 1996). Losses averaging 5% and epizootics with up to 100% mortality in other reptiles held in zoo collections have been attributed to infection with *E. invadens* (Frank 1984; Cubas 1996). Snakes and carnivorous lizards are highly susceptible; death in these species often occurs 2 to 10 weeks after exposure (Barnard and Upton 1994). *E. invadens* infection of crocodilians has not been reported in Australia and this parasite is included for further consideration.

Blood parasites collectively termed haemogregarines are within the phylum Apicomplexa; family Haemogregarinidae. *Haemogregarina* sp. (Jacobson 1989; Ladds *et al* 1995) and *Hepatozoon* sp. (Barnard and Upton 1994) have been reported in crocodilians and reptiles in general.

Haemogregarines have been recorded in a farmed *Crocodylus* species in Irian Jaya (Ladds *et al* 1995) and Nile crocodiles in Uganda [Hoare 1932 (cited in Frank 1984)]. Infection is usually asymptomatic in natural hosts (Campbell 1996) but heavy parasite burdens may result in anaemia and inanition (Barnard and Upton 1994). *Hepatozoon* spp. are not considered to be very host specific and can be transferred from one reptile species to another via mosquito vectors (Marcus 1981). In unnatural host species, hepatitis, pancreatitis and splenitis may result from infection (Wozniak *et al* 1994). Haemogregarines have not been reported in crocodilians in Australia and they are included for further consideration.

Coccidia (phylum Apicomplexa; family Eimeriidae) in genera *Eimeria* (Barnard and Upton 1994; Ladds *et al* 1995; Frank 1984; Jacobson 1989; Lainson 1968), *Goussia* (Obwolo and Zwart 1992; Gardiner *et al* 1986; Levine 1987) and *Isospora* (Lainson 1968) have been reported in crocodilians. In nature, most coccidia cause infections that are usually self-limiting and evidence of pathogenicity in their natural hosts is lacking (Telford 1971). However, under captive conditions coccidia may become pathogenic, causing illness or death in younger animals (Barnard and Upton 1994; Foggin 1992a). Coccidiosis is considered to be one of the major disease of captive crocodiles in Zimbabwe (Foggin 1987) and Irian Jaya (Ladds and Sims 1990). Coccidiosis is also considered to be one of the major disease affecting farmed crocodiles in Queensland and the Northern Territory (Buenviaje *et al* 1994). As coccidia cause disease in crocodiles in Australia they are not included for further consideration in the IRA.

Cryptosporidium spp. may be recovered from many reptile species (Barnard and Upton 1994) including Nile crocodiles (Siam *et al* 1994). Cryptosporidia have been isolated from reptiles during routine monitoring at several zoological parks in Australia (McFetridge and Burrell 1991; William Meikle, personal communication). These parasites lack host specificity and cross infection may occur between reptile species (Urquhart *et al* 1996) and potentially from crocodile to humans (Siam *et al* 1994). This parasite is not considered further as it affects a range of animal species in Australia and is considered to be enzootic.

The haemosporines, *Progammaria archosauriae* (Lainson 1995) and *Plasmodium* sp. (Sigler 1992), have been reported in crocodilians. They are considered to be of low or nil pathogenicity (Barnard and Upton 1994) and are not considered further in this IRA.

Representative genera of the phylum Ciliophora recorded in aquatic reptiles are *Geimania* and *Sicuophora* (Barnard and Upton 1994). Symptoms in infected hosts have not been reported (Barnard and Upton 1994) and these parasites are not included in this review.

A representative of *Blastocystis* was recovered from a single captive, apparently healthy, *C. porosus* in Singapore (Teow *et al* 1992). The relationship between *Blastocystis* species recovered from reptiles and *B hominis*, which is usually non-pathogenic but can cause diarrhoea in humans, is unknown. This parasite, of uncertain taxonomic status and unrecorded pathogenicity, is not considered further.

Metazoan endoparasites

Nematodes of the genera *Terranova*, *Brevimulticaecum*, *Multicaecum*, *Dujardinascaris* (*Gedoelstascaris*), *Ortleppascaris*, *Micropleura*, *Eustrongylides*, *Contraecum*, *Paratrichosoma*, *Goezia*, *Hartwichia*, *Trispioulascaris*, *Trichinella*, *Crocodylocapillaria* and *Capillaria* are reported from crocodylians (Machida *et al* 1992; Catto and Amato 1994a; Ladds *et al* 1995; Goldberg *et al* 1991; Ashford and Muller 1978; Sprent 1984; Huchzermeyer 1997; Moravec and Spratt 1998). *Dujardinascaris* spp., *Eustrongylus* spp. *Contraecum* spp., *Physaloptera* spp., *Paratrichosoma* spp., *Crocodylocapillaria* and *Goezia fluviatis* have been reported from crocodiles in Australia (Webb *et al* 1982; Moravec and Spratt 1998).

Gastrointestinal nematode infections are usually asymptomatic in crocodiles but may be occasionally associated with disease. Infection with *Dujardinascaris* may cause disease and can be associated with gastric ulceration (Ladds and Sims, 1990) and runting in hatchlings (Foggin 1992a). This ascarid is considered to be of economic importance for crocodile farming (Foggin 1987). Several species (*D. mawsonae*, *D. taylorae* and *Dujardinascaris* sp) have been reported from crocodiles in Australia (Machida *et al* 1992; Webb *et al* 1982) but several others have not (*D. philippinensis*, *D. woodlandi*, *D. gedoelsti* and *D. dujardini*). Nematodes belonging to the the genus *Dujardinascaris* are included for further consideration in this IRA.

The remaining nematode genera are not associated with disease and considered to be of lesser importance. These will not be considered further in the risk analysis.

Nematode larvae in meat of slaughtered Nile crocodiles from farms in Zimbabwe were tentatively identified as *Trichinella spiralis nelsoni* (Foggin and Widdowson 1996). Kapel *et al* (1998) questioned this identification and suggested that the parasitism may not have been caused by a *Trichinella* or that the nematode observed may represent a new taxon of *Trichinella*. *Trichinella spiralis nelsoni* can transmit to livestock and humans. The distribution of *T spiralis* is regarded as cosmopolitan apart from Australia and several other South American, Asian and African countries. *Trichinella* nematodes (other than *T pseudospiralis*) are included for further consideration in this review.

Capillaria spp. and *Crocodylocapillaria longiovata* may infect crocodylians; usually inhabiting the intestinal tract, but other organs may also be involved (Lane and Mader 1996; Ladds *et al* 1995). Nematodes of these genera have been reported from crocodiles in Australia (Buenviaje *et al* 1994; Moravec and Spratt 1998). Most infestations are subclinical. Intestinal capillarids are considered to be of low pathogenicity and are not considered further.

Larval migratory tracts of the capillarid nematode genus *Paratrichosoma* are reported in crocodiles in Zimbabwe, New Guinea, India and Thailand (Youngprapakorn, *et al* 1994; Foggin 1987; Ashford and Muller 1978; Jacobson 1989). *Paratrichosoma* is considered to be of economic significance in crocodiles farmed in Zimbabwe (Foggin 1987). This parasite is often encountered in wild Australian crocodiles (*C porosus*) (Buenviaje *et al* 1998; Webb and Manolis 1983) but because the species responsible is unknown, and the parasite has the potential to cause significant damage to crocodile skins it is included in this review.

The following genera of filarid worms have been noted in crocodilians; *Oswaldofilaria*, *Befilaria*, *Conofilaria*, *Piratuba*, *Piratuboides* and *Solafilaria* (Lane and Mader 1996). Jacobson (1986) notes that there are relatively few reports documenting clinical signs and gross lesions associated with infection. Heavy infestations have been recorded and these can be associated with thrombosis (Lane and Mader 1996). *Oswaldofilaria kanbaya*, has been identified in *C porosus* in Australia (Manzanell 1986). Apart from this, filarids have not been reported in crocodilians in Australia and are included in this review.

The trematodes *Protocaecum dorsale*, *Caimanicola marajoara*, *Proterodiplostomum* sp *Cystodiplostomum hollyi*, *Prolecithodiplostomum constrictum*, *Paradiplostomum abbreviatum*, *Pseudotelorchis* sp. and *Herpetodiplostomum caimancola* are described in crocodilians (Catto and Amato 1993a, b; Catto and Amato 1994a,b; Ladds *et al* 1995). These parasites were not considered to be the cause of significant disease in their crocodilian hosts and are not included in this review.

The blood fluke *Griphobilharzia amoena* has been identified in crocodiles in association with clinical illness in Irian Jaya (Ladds *et al* 1995) and Australia (Ladds 1996). Kidney flukes have been reported in crocodiles in Irian Jaya (Ladds *et al* 1995), Zimbabwe (Foggin 1992a) and Australia (Blair *et al* 1989). These flukes are not considered further in the risk analysis.

The acanthocephalan, *Polyacanthorhynchus rhopalorhynchus* (Catto and Amato 1994a) was identified in *Caiman crocodilus yacare*. This report gave no indication of any pathology associated with infection. By contrast, *Acanthostomum loossi* was recovered from farmed *C acutus* and *C rhombifer* in Cuba in association with poor health and growth rates (Perez-Benitez *et al* 1980). These authors suggested that the parasite was a significant cause of economic loss. As this is an isolated report the parasite is not considered in this review.

Ectoparasites

Pentastomes often occur in crocodilians in great numbers, both as adults and also as larvae and nymphs. Despite this, and their capacity to migrate through tissue, infections with this parasite in natural hosts may be asymptomatic with little accompanying inflammatory reaction. In other situations, there may be significant damage (Marcus 1981; Lane and Mader 1996). Migration of larvae through tissues can result in pulmonary haemorrhage (Boyce *et al* 1984) and other lung pathology (Rego 1987). Infection has been associated with meningitis, pneumonia and mortalities (Cherry and Ager 1982; Boyce *et al* 1984). Pentastomiasis is regarded as a major disease on crocodile farms in Australia (Buenviaje *et al* 1994).

The following pentastomid genera have been reported from crocodilians; *Alofia*, *Sebekia*, *Subtriquetra*, *Diesingia*, *Selfia* and *Leiperia* (Riley 1994; Riley and Huchzermeyer 1995a, b; Riley and Huchzermeyer 1996; Riley *et al* 1990; Youngprapakorn, *et al* 1994; Boyce *et al* 1984; Cosgrove *et al* 1984). Some of these genera have not been reported in Australia. Pentastome infections can be associated with significant disease and these parasites are included for further consideration in the IRA.

Leeches (Hirudinea) (Phylum Annelida) are common on crocodiles in Thailand (Youngprapakorn *et al* 1994), and American alligators (Jacobson 1989). Leeches were detected on 9.7% of animals examined in one study of crocodiles in the NT (Webb and Manolis 1983). The buffalo leech, *Hirudinaria manillensis*, was recovered from the lungs of an estuarine crocodile, *C. porosus*, which died at Zoo Negara, Malaysia, from causes unrelated to its presence (Jeffery *et al* 1990). The leech, *Placobdella multilineata* was found on *C. porosus* in a Chinese zoo (Yang and Davies 1985). This leech and *P. papillifera* are common parasites of *A. mississippiensis* (Cherry and Ager 1982). *P. papillifera* and *P. multilineata* are intermediate hosts for *Haemogregarina* spp. in *A. mississippiensis*

(Forrester and Sawyer 1974 Telford and Campbell 1981). There is no disease syndrome associated with leech infestations and these parasites are not included in this review.

2.1.3 Summary list of disease agents

Viruses

Poxvirus

Adenovirus-like agent (viral hepatitis and enteritis)

Bacteria

Mycoplasma

Chlamydia

Protozoa

Entamoeba invadens

Haemogregarines

Metazoan endoparasites

Paratrichosoma

Dujardinascarids

Filarids

Trichinella

Pentastomes

2.2 Exposure pathways

Exposure pathways trace the potential route by which disease agents harboured by imported animals are exposed to animals and humans in this country.

The number of animals to be imported in the future is expected to be small. This estimate is based on AQIS's records of applications for permits to import (one application since 1992) and the declared acquisition plans of the members of the reptile taxon advisory group (TAG) (ARAZPA 1998).

2.2.1 Theoretical exposure pathway

For an exotic pathogen to become established in Australia through the importation of crocodylians from other countries, the following sequence of events must occur:

- a) the pathogen is present in or on the animal⁵ and the animal remains infectious after importation
- b) the pathogen is transmitted to another susceptible host (or vector) or survives exposure to environmental stressors, eg. desiccation, ultraviolet light
- c) one or more exposed hosts become infected as a result of exposure
- d) the pathogen spreads from the index case(s) to a sufficient number of susceptible hosts to sustain infection in the population and become established.

⁵ Animal in this context includes eggs.

2.2.2 Significant exposure pathways for crocodilians in Australia

Potential exposure pathways can be identified for imported live crocodilians and their eggs. The nature of the animals and their intended use limits the number of pathways which are considered significant in this IRA.

Initially, all imported crocodilians are held in zoos or other premises for a period of post arrival quarantine (PAQ)(refer appendix 1) overseen by AQIS officers and zoo personnel. Crocodilians released from PAQ will be detained in quarantine or under quarantine surveillance in a AQIS approved premises. AQIS may approve transfer to another premises which meets requirements consistent with the pest risk of the animal (ie. there is little potential for animals to be released to the wild). Animals which die in these premises are usually incinerated or disposed of by deep burial after post mortem.

Imported exotic and native crocodilians are held in premises in both urban and country locations throughout Australia and are thus exposed to a wide range of environments and potentially to animals inhabiting these environments.

Two routes of exposure following release from PAQ are considered to be significant in relation to this risk analysis.

The first and currently the most probable pathway follows on from crocodilians held permanently in A Class zoos. In this situation direct exposure of resident zoo animals to imported crocodilians is unlikely as animals are usually housed as separate species. Other crocodilians or reptiles in the zoo may be indirectly exposed to imported animals via fomites, personnel or pests. A class zoos are located in urban areas so that direct exposure of imported crocodilians to populations of native reptiles, eg. lizards, is unlikely, but can occur. A range of non-reptile species, for example sparrows, rats, mice, flies and cockroaches, is highly likely to come in contact with crocodilians in captivity (especially those held in external enclosures) and may go beyond the confines of the zoo.

A number of indirect pathways are possible. Contamination of bedding, personnel or equipment can occur. Waste disposal is generally via the municipal sewerage system or may be direct into a natural waterway.

Crocodilians are also held on premises, eg. wildlife parks or farms, which may be less secure than A class zoos and may be located in country areas. This alternative pathway may become more significant if recommendations in this risk analysis are adopted. Proximity to natural habitats increases the potential for direct contact between imported animals and native fauna. Small reptiles, birds, other animals may enter these premises and come in direct contact with imported reptiles. In addition, water draining from these establishments may be discharged without treatment into the surrounding environment. In this situation aquatic animal species (including native reptiles) which are susceptible to infection or can serve as intermediate hosts may be exposed to pathogens.

Rehabilitation and captive breeding programs are carried out in a number of zoos and wildlife parks. When natives reptiles are not isolated from collection reptiles and released into the natural environment, another potential pathway may be created for the spread and establishment of exotic pathogens. This pathway may arise from either of the two major pathways considered above.

3 Risk assessment and risk management options

A qualitative approach has been used to assess the level of risk for each of the identified hazards. Insufficient information is available for a quantitative assessment. The methodology used is based on the guidelines in the *International Animal Health Code* of the Office International des Epizooties (OIE) as presented in the IRA Handbook. A summary of relevant information on each disease agent

associated with imported crocodylians and identified as a hazard is presented in Appendix 2. Risk assessments are based on this information.

3.1 Risk assessment

3.1.1 Release assessment (probability of agent entry)

Release assessment is an estimation of the probability that the importation of crocodylians will introduce (release) disease agents. This stage of the analysis considers the likelihood that the first stage of the theoretical exposure pathway (section 2.2.1) is completed, viz. the pathogen must be present in or on the animal or egg and the infection persists during transit.

Whether the pathogen is present in or on the animal or egg and the infection persists during transit depends on the probabilities that the animal selected for importation is infected and remains infected and undetected at the time of entry (ie. on leaving post arrival quarantine). An evaluation of the likelihood that animals in the source country will be infected is based on a knowledge of the distribution and prevalence of the disease caused by the agent and the host range of the pathogen. The likelihood that infected animals will be imported and released from quarantine depends on the level of official health controls and the availability and accuracy of tests available to detect animals harbouring the disease agent.

3.1.1.1 Probability that animals in the source population are infected

The likelihood that infected animals will be sourced for importation depends, amongst other things, on the life stage of animals sourced. A notable example is crocodylian eggs, which are unlikely to carry disease agents not vertically transmitted.

Data on outbreaks of disease are the only source of information available to describe the distribution of disease agents. In terms of determining individual country status this approach has a low sensitivity, therefore individual exporting countries are grouped into regions and crocodylian disease status is determined on a regional basis. If an infectious disease is reported within a country's borders, or if its presence is suspected, crocodylians in all countries in the whole region are considered to be infected.

For the purposes of this risk analysis countries are grouped into the following regions:

- Asia and the Pacific region
- North America
- South America
- Africa
- Europe

Surveillance and monitoring programmes on which prevalence estimates are based are almost non-existent for wild species. Some disease surveillance occurs on animals kept in zoos, wildlife parks or farms. Generally suspicious cases of disease are investigated and the cause may be determined. In some establishments, reptiles are periodically tested for the presence of particular disease agents. The Code recommends that in the absence of quantitative data, an assigned prevalence is given to the occurrence of diseases based on reports to the OIE or in the literature. However there is insufficient information available to assign prevalence estimates for any of the disease agents in this IRA. Consequently, for the purposes of this IRA, the prevalence of each disease is assumed to be high in regions in which the disease agent is reported (table 1).

Table 1 **Reported outbreaks of disease and host range of disease agents**

Disease agent	Countries in which disease outbreaks reported	Regions in which the disease is estimated to occur at high prevalence	Host range
Adenovirus-like agent	Zimbabwe	Africa	<i>C niloticus</i>
Poxvirus	Zimbabwe Australia, Zambia, South Africa, USA , Brazil, Germany.	All	Saltwater crocodile (<i>Crocodylus porosus</i>), freshwater crocodile (<i>C. johnstoni</i>), Nile crocodile (<i>C. niloticus</i>), Yacare caiman (<i>Caiman yacare</i>), spectacled caiman (<i>Caiman crocodilus</i>).
<i>Mycoplasma</i>	Zimbabwe, USA	Africa, North America	<i>C niloticus</i> American alligator (<i>A. mississippiensis</i>)
<i>Chlamydia</i>	South Africa, Zimbabwe	Africa	<i>C. niloticus</i>
<i>Entamoeba invadens</i>	Worldwide	All	Potentially all crocodylian species and other reptiles
Haemogregarines	Africa, India, Sri Lanka, Indonesia, North and South America.	Africa, Asia Pacific, North and South America.	Crocodiles, caimans, alligators and gharials.
<i>Dujardinascaris</i>	the Philipines, India, USA, Africa, Australia	Asia Pacific, North and South America, Africa	Potentially all crocodylian species
<i>Paratrichosoma</i>	New Guinea, India, Australia, Thailand	Asia Pacific, Africa	Nile crocodile, Muzzer crocodile (<i>C. palustris</i>), New Guinea crocodile (<i>C. novaeguineae</i>), <i>C. niloticus</i> , Siamese crocodile (<i>C. siamensis</i>), <i>C. johnstoni</i> and <i>C. porosus</i> .
Filarids	Worldwide	All ⁶	Potentially all crocodylian species
<i>Trichinella</i>	Eastern and southern Africa, southern USSR, Bulgaria, Switzerland	Africa, Asia Pacific ⁷ Europe	<i>C. niloticus</i> , wild pigs, canids and rodents
Pentastomes	Worldwide	All	Potentially all crocodylian species

3.1.1.2 Probability that infected animals will be imported⁸

Whether infected reptiles are imported depends on the agent's susceptibility to treatments (including vaccination) and the capacity to detect infected reptiles before they are released from quarantine in Australia. Detection of infection depends on the biological properties of the disease agent, including its predilection sites, the course of disease, the mode of transmission and the available diagnostic methods.

There is little information on the incubation period, the course of disease and predilection sites in crocodylians for most disease agents in this paper. Further, for some diseases, eg. viral hepatitis and

⁶ For the purposes of this IRA it is assumed that the distribution of filarids in crocodylians is worldwide. This is based on knowledge of the large number of genera and hosts reported.

⁷ Southern USSR is considered here to be part of Asia.

⁸ Imported, for the purposes of this IRA, means release from post arrival quarantine (PAQ).

enteritis, the causative agent is not known, and consequently there is no information on the biology of the presumed viral agent responsible.

A summary of information which is available and relevant to this section is given in Table 2.

In the absence of knowledge to the contrary, it is assumed that all disease agents considered in the IRA may be harboured without the host showing signs of disease. Where diagnostic methods for use in living animals prior to export are not available, the most definitive guide to the infection status of an animal destined for export is the absence or presence of disease in the group from which the animal is sourced.

Table 2 Detection of disease agents

Disease agent	Disease signs in crocodilians	Confirmation of infection	Tests to detect disease agent in asymptomatic animals
Adenovirus-like agent	Non-specific	Histopathology, electron microscopy	No
Poxvirus	Skin lesions	Histopathology, electron microscopy	No
<i>Mycoplasma</i>	Arthritis, mortalities	Isolation of mycoplasmas from infected joints	No
<i>Chlamydia</i>	Mortalities	Isolation of chlamydia from infected liver and spleen	No (serology is used to detect infection in birds and livestock)
<i>Entamoeba invadens</i>	No signs ⁹	Detection of cysts or trophozoites in faecal preparations	Yes
Haemogregarines	Anaemia, inanition	Blood smears	Yes
<i>Dujardinascaris</i>	Non specific	Detection of eggs by faecal flotation	Yes
<i>Paratrichosoma</i>	Typical tracks on belly skin	Detection of eggs in skin tracks	No
Filarids	Cardiovascular	Blood smears	Yes ¹⁰
<i>Trichinella</i>	No signs ¹¹	Detection of cysts in affected muscles	No (serology is used to detect infection in other species)
Pentastomes	Non specific	Eggs may be detected in faeces	Yes

3.1.1.3 Summary of release assessment

A summary of information from Tables 1 and 2 is presented in Table 3 for live crocodilians and Table 4 for eggs. The capacity of disease agents to transmit vertically is an important consideration in the release assessment for eggs. Table 4 draws on information in table 6 as well as that in Tables 1 and 2. Both Tables 3 and 4 give an estimate of the probability that crocodilians or eggs imported from a particular region of the world will be infected and that the infection will remain undetected.

The assignment of a probability that infected animals are imported from a particular region, is based on available information. It is acknowledged that this estimate may only reflect the level of reporting of crocodilians diseases rather than a true prevalence.

If the probability that infected animals are imported from the region is low then the probability of agent entry is estimated as low, regardless of likelihood of detection.

⁹ Clinical signs have not been reported in crocodilians; significant signs including mortalities are reported in snakes and other reptiles.

¹⁰ The appearance of microfilaria in blood may be intermittent.

¹¹ Clinical signs in horses and humans (Geering et al 1995).

If the probability that infected animals are imported from the region is high and the probability of detection is high then the probability of agent entry is estimated to be moderate. If the probability that infected animals are imported from the region is high and the probability of detection is low then the probability of agent entry is estimated to be high.

Because of the level of uncertainty associated with these estimates, the descriptors have been limited to three, viz. low, moderate and high, rather than using an expanded range (negligible, slight, etc.) which would imply a greater level of certainty than is currently valid.

Table 3 Estimated probability of disease agent entry through the importation of live crocodilians

Disease agent	Probability that infected animals are detected	Probability that infected animals are imported from the region					Probability of agent entry
		North America (NA)	South America (SA)	Asia (A)	Africa (Af)	Europe (E)	
Adenovirus-like agent	low	low	low	low	high	low	low (NA, SA, A, E) high (Af)
Poxvirus	low	high	high	high	high	high	high (all countries)
<i>Mycoplasma</i>	low	high	low	low	high	low	low (SA, A, E) high (NA, Af)
<i>Chlamydia</i>	low	low	low	low	high	low	low (NA, SA, A, E) high (Af)
<i>Entamoeba invadens</i>	high	high	high	high	high	high	moderate (all countries)
Haemogregarines	high	high	high	high	high	low	moderate (NA,SA,A,Af) low (E)
<i>Dujardinascaris</i>	high	high	high	high	high	low	moderate (NA, SA, A, Af) low (E)
<i>Paratrichosoma</i>	low	low	low	high	high	low	low (NA, SA, E), high (A,Af)
Filarids	low	high	high	high	high	high	high (all countries)
<i>Trichinella</i>	low	low	low	high	high	high	low (NA,SA) high (Af,A,E)
Pentastomes	high	high	high	high	high	high	moderate (all countries)

Table 4 Estimated probability of disease agent entry through the importation of crocodylian eggs

Disease agent	Probability that infected animals are detected	Probability of vertical transmission	Probability that infected eggs are imported from the region					Probability of agent entry (Release assessment)
			North America (NA)	South America (SA)	Asia (A)	Africa (Af)	Europe (E)	
Adenovirus-like agent	low	high	low	low	low	high	low	low (NA, SA, A, E) high (Af)
Poxvirus	low	low	low	low	low	low	low	low (all countries)
<i>Mycoplasma</i>	low	high	high	low	low	high	low	low (SA,A,E), high (NA,Af)
<i>Chlamydia</i>	low	low	low	low	low	low	low	low (all countries)
<i>Entamoeba invadens</i>	high	low	low	low	low	low	low	low (all countries)
Haemogregarines	high	low	low	low	low	low	low	low (all countries)
<i>Dujardinascaris</i>	high	low	low	low	low	low	low	low (all countries)
<i>Paratrichosoma</i>	low	low	low	low	low	low	low	low (all countries)
Filarids	low	low	low	low	low	low	low	low (all countries)
<i>Trichinella</i>	low	low	low	low	low	low	low	low (all countries)
Pentastomes	high	low	low	low	low	low	low	low (all countries)

3.1.2 Exposure assessment (probability of exposure of animals to the agent in the importing country)

Article 1.4.2.4. of the Code (1998) states that “The probability of exposure is the likelihood that the commodity is exposed to animals or humans in the importing country, and that agent transmission, infection, disease, and disease spread occur combined with the likelihood of these events being detected”.

The exposure assessment describes and estimates the probability of exposure of susceptible species in the importing country to potentially hazardous disease agents. Exposure depends on the pathways and intended location of animals, the numbers imported, characteristics of agent transmission and the number and location of susceptible species.

Section 2.2.2 of this paper describes the two most significant pathways for imported crocodylians. The pathways both involve a PAQ period and then diverge. The two pathways following PAQ have been considered, for the purposes of this risk assessment, in terms of direct and indirect exposure.

Both forms of exposure may occur in B class zoos and other registered premises. Direct exposure is much less likely in A class zoos provided different animal species are not held together. Factors operating at each stage of these pathways for both forms of exposure are shown in Figure 1 and discussed in the following sections.

3.1.2.1 Intended location of imported crocodilians

Potential exposure pathways leading to contact between the disease agent and susceptible animals or humans in Australia are limited due largely to the nature of the animals imported. The Animal and Plant Control Commission (formerly the Vertebrate Pest Committee) (APCC) lists crocodilians in category 2, as “animals limited to restricted collections” and as such to be held permanently in secure premises. Even so, the potential remains for crocodilians to come into direct contact with other animals which may result in the transfer of pathogens. The likelihood of transfer depends on the capacity of the agent to infect other hosts and/ or to survive exposure to environmental stressors. This is considered in the following sections.

3.1.2.2 Survival in the environment

Resistance to environmental stressors, such as desiccation, ultraviolet light exposure and heat, increase the ability of disease agents to survive away from the host. This in turn, increases the probability of indirect exposure of susceptible animals to the disease agent. Table 5 presents an estimate of the capacity of disease agents to survive free in the environment based on information specific to the disease agents considered in this IRA (refer Appendix 2). The type of transmission and structure of infective stages are assumed to be key indicators of resistance. Where this information is not available, an estimate of survival capacity is based on descriptions (refer footnotes) of comparable agents, usually those affecting birds.

Table 5 Estimated capacity of disease agents to survive on exposure to environmental stressors

Disease agent	Survival in the environment ¹²
Adenovirus-like agent	High ¹³
Poxvirus	High ¹⁴
<i>Mycoplasma</i>	Low ¹⁵
<i>Chlamydia</i>	Low ¹⁵
<i>Entamoeba invadens</i>	High
Haemogregarines	Low ¹⁵
<i>Dujardinascaris</i>	Low
<i>Paratrichosoma</i>	High ¹⁶
Filarids	Low
<i>Trichinella</i>	High
Pentastomes	Low

¹² Environment in this context means any site external to any of the susceptible hosts of the pathogen. Generally no information is available on this but an estimate has been made based on a knowledge of the biological properties of the crocodilian and other closely related pathogens.

¹³ Avian adenoviruses may be spread via fomites and aerosol (McFerran 1997).

¹⁴ Poxvirus virions are relatively stable in dry conditions at room temperature (Murphy *et al* 1995).

¹⁵ Avian mycoplasmas, chlamydia and blood protozoans are generally regarded as susceptible to a wide range of environmental stressors (Calnek *et al* 1997).

¹⁶ Capillarid eggs are generally resistant to environmental stressors.

3.1.2.3 Mode of transmission

Both the mode of transmission and the infectious dose affect the likelihood infection will result from direct or indirect exposure of susceptible species. Information on the infectious dose is not available and is not included in Table 6.

The life cycles of a number of disease agents, for example *Dujardinascaris* and pentastomes, rely on intermediate hosts or vectors. If the required intermediate hosts are not present in this country the probability of transmission is negligible. There is not enough information on the host ranges of specific pathogens to dismiss the possibility that such hosts may also be present in Australia and allow completion of life cycles.

The likelihood that vertical transmission occurs for many of the disease agents is unknown. It is presumed not to occur if this mode of transmission has not been noted for comparable, but well-studied, disease agents. For example, vertical transmission of poxviruses in crocodilians has not been reported and is presumed to be unlikely as egg transmission of other poxviruses does not occur. The mode of transmission for each of the disease agents is presented in Table 6.

Table 6 Mode of transmission of disease agents

Disease agent	Horizontal transmission	Estimated probability of vertical transmission
Adenovirus-like agent	Probably direct	High ¹⁷
Poxvirus	Direct, or via vector	Low
<i>Mycoplasma</i>	Direct horizontal	High ¹⁸
<i>Chlamydia</i>	Direct horizontal	Low
<i>Entamoeba invadens</i>	Direct horizontal	Low
Haemogregarines	Via vector	Low
<i>Dujardinascaris</i>	Direct or via intermediate host	Low
<i>Paratrichosoma</i>	Probably via intermediate host ¹⁹	Low
Filarids	Via vector	Low
<i>Trichinella</i>	Via intermediate host	Low
Pentastomes	Via intermediate host	Low

3.1.2.4 Host range and distribution of susceptible animals

An estimate of the distribution of susceptible animals is given for each disease agent in Table 7 based on information presented in Table 1. A report of disease in any crocodilian species has been extrapolated to apply to all crocodilians. Further, it is assumed that the host range of pathogens is restricted to crocodilians, unless reports indicate otherwise. The distribution of wild and captive representatives of this group of reptiles in Australia is generally along the coast and watercourses of northern Australia and in registered zoos. The host range of some pathogens is broader, eg. *Entamoeba*. In these cases, the distribution of potentially susceptible animals (including intermediate hosts and vectors) is difficult to determine. Distribution is assumed to extend to all reptiles in Australia, largely because of the lack of knowledge of the susceptibility of Australian reptiles to these pathogens.

¹⁷ Avian adenovirus infections are spread by vertical transmission (McFerran 1997)

¹⁸ Avian mycoplasmas transmit vertically (Kleven, 1997)

¹⁹ This is based on the observation that *Paratrichosoma* does not transmit horizontally in farm situations (Buenviaje *et al* 1998).

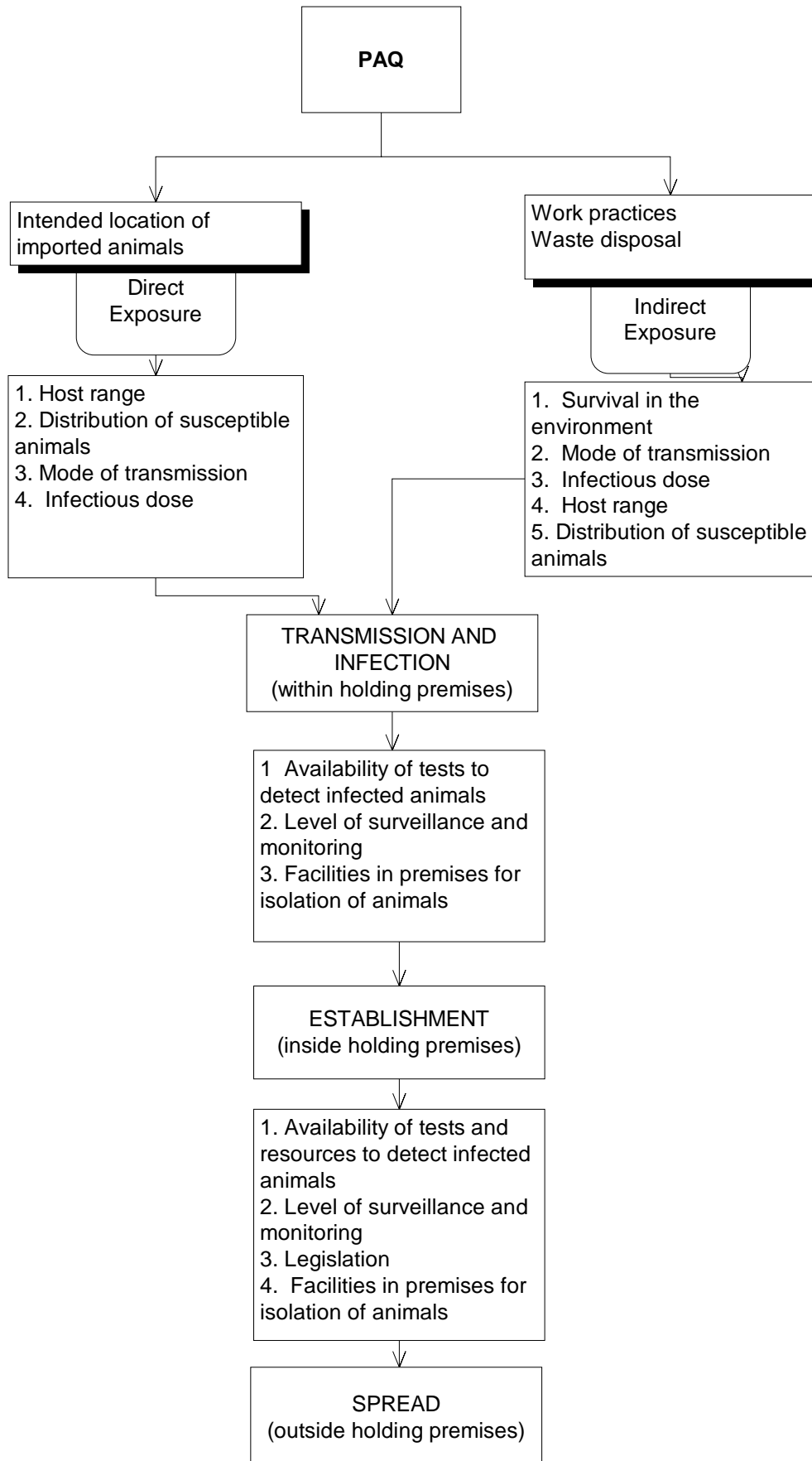


Fig. 1 Factors operating at stages of the exposure pathway following PAQ

3.1.2.5 Detection of disease

In the event that an exotic disease outbreak occurs due to the importation of crocodilians, ie infection of susceptible animals leads to spread of infection, the capacity to limit spread depends firstly on the detection of disease. This depends on the level of health surveillance in the zoos or other premises, the availability of trained personnel and diagnostic tests, the status of animal health legislation and the level of compliance. These factors influence the likelihood that an outbreak will be detected and that appropriate steps will be taken to control and eradicate the disease. Detection methods for disease agents are presented in Table 2.

On a larger scale, a technical response plan is described for disease situations in relation to all zoo species in the Australian Veterinary Emergency Plan (Ausvetplan) interim document. Strategies recommended in this response plan aim to prevent the spread of suspected exotic disease involving a zoo. Legislation both at the Commonwealth and State/Territory levels has been enacted for the purpose of controlling exotic diseases in these situations.

Table 7 Estimated distribution of susceptible animals and vectors

Disease agent	Assumed host range ²⁰ ,	Distribution of host species	Vectors or intermediate hosts	Distribution of vectors
Adenovirus-like agent	Crocodilians	Northern Australia and zoos	Unknown	Unknown
Poxvirus	Crocodilians	Northern Australia and zoos	Unknown	Unknown
<i>Mycoplasma</i>	Crocodilians	Northern Australia and zoos	Unknown	Unknown
<i>Chlamydia</i>	Crocodilians	Northern Australia and zoos	Unknown	Unknown
<i>Entamoeba invadens</i>	All reptiles	Throughout Australia	No	
Haemogregarines	Crocodilians	Northern Australia and zoos	Ticks, mites, flies, bugs and leeches	Throughout Australia
<i>Dujardinascaris</i>	Crocodilians	Northern Australia and zoos	Fish	Unknown
<i>Paratrichosoma</i>	Crocodilians	Northern Australia and zoos	No	
Filarids	Crocodilians	Northern Australia and zoos	Mosquitos	Throughout Australia
<i>Trichinella</i>	Crocodilians, wild canids, pigs and rodents	Throughout Australia	Wild canids, pigs and rodents	Throughout Australia
Pentastomes	Crocodilians	Northern Australia and zoos	Fish	Susceptible Australian species not known

3.1.2.6 Probability of transmission and spread of infection to susceptible animals

Transmission of disease agents from imported crocodilians to other animals in the holding premises constitutes spread. Spread of infection may go beyond the boundaries of the zoo or other premises and lead to establishment of disease in the wider Australian reptile population. Whether these events occur depends on a number of factors as shown in figure 1. The ability to detect infected

²⁰ The estimate of host range in this column is based on information in table 1.

animals and the managerial and legislative capacity to control spread are important determinants.

3.1.2.7 Summary of the probability of exposure of susceptible animals to pathogens

In this assessment of the probability of exposure to agents carried by imported crocodilians leading to establishment of disease two key factors are considered. Firstly, information on the host range and distribution (including vectors and intermediate hosts) is used to estimate the opportunity for contact between imported crocodilians and susceptible species. The other factor is the ease of transmission. The following estimate ratings for ease of transmission are assigned. Direct transmission with a high level of resistance to environmental stressors is rated as high. Direct transmission of more labile agents or indirect transmission with a wide range of vectors or intermediate hosts is rated moderate. Indirect transmission with a narrow range of vectors or intermediate hosts is rated low. These two estimates are combined as follows to yield ratings for the probability of exposure leading to establishment of infection.

Contact opportunity	Ease of transmission	Probability of exposure
High	High	High
	Moderate	High
	Low	Moderate
Moderate	High	High
	Moderate	Moderate
	Low	Moderate

Table 8 presents estimates of contact opportunity and ease of transmission for each disease agent based on information in Tables 2, 5, 6 and 7. These estimates are used to determine a rating for the probability of exposure and establishment. For the purposes of this assessment it is assumed that no risk management measures are used. Detection of disease agents is not included here as this factor has already been used to estimate probability of agent entry (release assessment).

Table 8 Estimated probability of exposure

Disease agent	Estimated contact opportunity	Estimated ease of transmission	Probability of exposure ²¹
Adenovirus-like agent	moderate	high	high
Poxvirus	high	high	high
<i>Mycoplasma</i>	moderate	moderate	moderate
<i>Chlamydia</i>	moderate	moderate	moderate
<i>Entamoeba invadens</i>	high	high	high
Haemogregarines	moderate	moderate ²²	moderate
<i>Dujardinascaris</i>	high	moderate	high
<i>Paratrichosoma</i>	moderate	moderate	moderate
Filarids	moderate	moderate ²¹	moderate
<i>Trichinella</i>	high	low ²³	moderate
Pentastomes	high	moderate	high

3.1.3 Consequence assessment

3.1.3.1 Direct consequences

²¹ In situations where there is an almost complete absence of relevant information it is assumed that the probability of exposure is high.

²² Haemogregarines and filarids employ a wide range of vectors (refer appendix 2)

²³ The probability of exposure of susceptible hosts to *Trichinella* is considered to be low due to assumed disposal practices of dead crocodiles.

Direct consequences may be measured in terms of production losses caused by disease and public health consequences. Table 9 lists whether or not losses are likely to occur if disease agents become established. Production losses likely to be suffered by commercial farms and zoos are those arising through death or ill-thrift of infected animals. For zoos, these losses may be measured in economic terms but also as losses in genetic diversity. This is especially significant where endangered species are held or bred. For some disease agents losses may occur in reptiles apart from crocodilians, for example *E invadens*.

Public health losses occur when a disease agent infects humans. The only example considered here is *T spiralis nelsoni*.

Crocodile farming is a relatively new industry but, since 1982, has developed into a significant livestock industry in many countries (Luxmore 1992). The two species of crocodiles native to Australia (*C. porosus* and *C. johnstoni*) are farmed for their skins and flesh in the Northern Territory, Western Australia and Queensland. The Northern Territory has eight crocodile farms, Queensland has five and Western Australia has two farms. The product of principal interest is the skin for the exotic leather trade, accounting for around 80% of the value of production (Brown et al 1997).

Zoos import crocodilians to maintain collections for purposes of exhibition and tourism and to increase genetic diversity of the species held. Zoos and fauna parks may also participate in co-operative international breeding programs for endangered species and be involved in research in this area. Crocodilians are held in a number of zoos and fauna parks in Australia. Section 1.1 provides the location of the exotic species held in these establishments.

Table 9 Direct consequences of infection

Disease agent	Production losses probably caused by disease	Probable public health consequences
Adenovirus-like agent	Yes	No
Poxvirus	Yes	No
<i>Mycoplasma</i>	Yes	No
<i>Chlamydia</i>	Yes	No
<i>Entamoeba invadens</i>	Yes	No
Haemogregarines	Yes	No
<i>Dujardinascaris</i>	Yes	No
<i>Paratrichosoma</i>	Yes	No
Filarids	Yes	No
<i>Trichinella</i>	No	Yes
Pentastomes	Yes	No

3.1.3.2 Indirect consequences

Indirect consequences may be measured in terms of surveillance and control costs, compensation costs, potential trade losses and adverse effects on the environment. Table 10 lists an estimate of which of these losses may occur for each disease agent. It is argued here that, with the exception of *T spiralis nelsoni*, public resources would not be allocated for surveillance and control in the event that disease outbreaks occurred, if caused by the disease agents considered in this paper. Similarly, trade losses would generally not result from the establishment of the non-OIE listed disease agents in reptile populations in Australia. The exceptions here are poxviruses and *Paratrichosoma* which have the potential to impact on trade due to the skin lesions produced.

Native crocodilian species, *Crocodylus porosus* and *C. johnstoni*, are distributed along the coastal regions of northern and north-eastern Australia. The impact of disease agents on native reptiles and

other Australian fauna is unknown. Acknowledging this uncertainty, it is assumed that potentially harmful consequences for the environment would follow establishment of all listed agents.

Table 10 Indirect consequences of infection

Disease agent	Probable surveillance and control costs	Probable potential trade losses	Probable adverse effects on the environment
Adenovirus-like agent	No	No	Yes
Poxvirus	No	Yes	Yes
<i>Mycoplasma</i>	No	No	Yes
<i>Chlamydia</i>	No	No	Yes
<i>Entamoeba invadens</i>	No	No	Yes
Haemogregarines	No	No	Yes
<i>Dujardinascaris</i>	No	No	Yes
<i>Paratrichosoma</i>	No	Yes	Yes
Filarids	No	No	Yes
<i>Trichinella</i>	Yes	Yes	Yes
Pentastomes	No	No	Yes

3.1.3.3 Summary consequence assessment

Each of the disease agents included in the risk analysis has been reported in association with significant harm, either in crocodylians or other species. Also, the effect of these pathogens on Australian reptiles is largely untested and is assumed to be potentially significant. The risk of establishment of most agents is estimated to be low in zoos mainly because of the high probability that infected animals will be detected and spread prevented. The converse applies where infection has been transmitted to wild animals. Failure to detect infected animals means that the spread and establishment of disease in wild populations will be highly likely. In all cases the estimated consequences of establishment are considered to be significant, either measured in terms of losses of wild or farmed crocodylians or in ecological terms.

3.1.4 Risk estimation (integrated risk assessment)

3.1.4.1 Qualitative risk levels

An estimate of the risk for each disease agent was obtained by combining the risk ratings for the release assessment, exposure assessment and consequence assessment. These risk estimates for live crocodylian imports are in Table 11 and those for eggs in Table 12. The final output expresses the estimated probability that a disease agent establishes in susceptible animals in this country and results in significant harm in human, economic or environmental terms.

Ratings are assigned as follows. The probability of harmful consequences is estimated to be high in all cases. If the probability of agent entry is low then the integrated risk is considered to be low irrespective of the rating assigned in the exposure or consequence assessment. In this risk analysis, risk management measures are considered unnecessary for disease agents with a low rating. If the probability of entry is moderate and the probability of exposure is either moderate or high, the overall risk is rated as moderate or high respectively. If the probability of entry is high and the probability of exposure is moderate or high the overall risk is rated as high.

3.1.4.2 Description of the level of uncertainty of estimates

Compared with domestic animal species, little information is available on disease agents affecting crocodylians; especially that required for an assessment of quarantine risk. This risk analysis is undertaken acknowledging that data used are incomplete and often uncertain and that assumptions

and recommendations have been based on the limited data which are currently available. Such judgements are provisional, and subject to change based on new information. Significant examples of this level of uncertainty are the estimations of probability that crocodilians in particular regions are infected (table 3). The estimates for Africa and North America may reflect a greater level of disease surveillance and reporting in these regions rather than a higher disease prevalence.

Generally, for all disease agents, the quarantine controls applied to the movement of crocodiles with regard to each agent within the country or internationally are expected to be minimal with the exception of Australia and New Zealand. The relatively unrestricted movement of animals between zoological collections in different countries imposes a high level of uncertainty on estimations of disease agent distribution on a regional basis and consequently estimations of probability of disease agent entry. Unrestricted movement of animals into zoos, would also be expected to demonstrate the capacity of disease agents to enter and spread to other crocodilians and/or reptiles.

Table 11 Risk estimates for crocodilian disease agents for live crocodilians

Disease agent	Release assessment					Exposure assessment	Consequence assessment	Risk estimation (integrated risk assessment)				
	North America	South America	Asia	Africa	Europe			North America	South America	Asia	Africa	Europe
Adenovirus-like agent	low	low	low	high	low	high	high	low	low	low	high	low
Poxvirus	high	high	high	high	high	high	high	high	high	high	high	high
<i>Mycoplasma</i>	high	low	low	high	low	moderate	high	high	low	low	high	low
<i>Chlamydia</i>	low	low	low	high	low	moderate	high	low	low	low	high	low
<i>Entamoeba invadens</i>	moderate	moderate	moderate	moderate	moderate	high	high	high	high	high	high	high
Haemogregarines	moderate	moderate	moderate	moderate	low	moderate	high	moderate	moderate	moderate	moderate	low
<i>Dujardinascaris</i>	moderate	moderate	moderate	moderate	low	high	high	high	high	high	high	low
<i>Paratrichosoma</i>	low	low	high	high	low	moderate	high	low	low	high	high	low
Filarids	high	high	high	high	high	moderate	high	high	high	high	high	high
<i>Trichinella</i>	low	low	high	high	high	moderate	high	low	low	high	high	high
Pentastomes	moderate	moderate	moderate	moderate	moderate	high	high	high	high	high	high	high

Table 12

Risk estimates for crocodylian disease agents in eggs

Disease agent	Release assessment					Exposure assessment	Consequence assessment	Risk estimation (integrated risk assessment)				
	North America	South America	Asia	Africa	Europe			North America	South America	Asia	Africa	Europe
Adenovirus-like agent	low	low	low	high	low	high	high	low	low	low	high	low
Poxvirus	low	low	low	low	low	high	high	low	low	low	low	low
<i>Mycoplasma</i>	high	low	low	high	low	moderate	high	high	low	low	high	low
<i>Chlamydia</i>	low	low	low	low	low	moderate	high	low	low	low	low	low
<i>Entamoeba invadens</i>	low	low	low	low	low	high	high	low	low	low	low	low
Haemogregarines	low	low	low	low	low	moderate	high	low	low	low	low	low
<i>Dujardinascaris</i>	low	low	low	low	low	high	high	low	low	low	low	low
<i>Paratrichosoma</i>	low	low	low	low	low	moderate	high	low	low	low	low	low
Filarids	low	low	low	low	low	moderate	high	low	low	low	low	low
<i>Trichinella</i>	low	low	low	low	low	moderate	high	low	low	low	low	low
Pentastomes	low	low	low	low	low	high	high	low	low	low	low	low

3.2 Risk management

3.2.1 Risk evaluation

Risk reduction measures are considered warranted for disease agents which have a risk estimate of moderate to high (refer Tables 11 and 12). Consequently, risk reduction measures for disease agents are warranted for crocodilians and eggs imported from the following regions.

Adenovirus-like agent	live crocodilians and eggs from Africa
Poxvirus	live crocodilians from all regions
Mycoplasma	live crocodilians and eggs from North America and Africa
Chlamydia	live crocodilians from Africa
<i>Entamoeba invadens</i>	live crocodilians from all regions
Haemogregarines	live crocodilians from all regions except Europe
<i>Dujardinascaris</i>	live crocodilians from all regions except Europe
<i>Paratrichosoma</i>	live crocodilians from Asia and Africa
Filarids	live crocodilians from all regions
<i>Trichinella</i> sp	live crocodilians from Asia Pacific, Africa and Europe
Pentastomes	live crocodilians from all regions

The OIE Code does not recommend international sanitary standards for the movement of live crocodilians and their eggs.

3.2.2 Option evaluation

The following options are identified as measures capable of reducing the risk associated with the importation of live crocodilians.

- choice of the origin of the animal
- pre-export quarantine (PEQ)
- post-arrival quarantine (PAQ)
- diagnostic testing
- vaccination
- treatment
- restrict the destination (avoidance of exposure to susceptible animals, vector or intermediate host)
- importation of eggs

Choice of the origin of the animal

The risk of introducing particular disease agents would be reduced by sourcing animals from regions considered free of diseases of concern (Table 1).

The risk associated with the importation of animals from regions which are not considered to be disease free can be reduced by sourcing animals from a Government registered or licensed farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. It is presumed that the health of animals in these groups will be closely monitored and the introduction of new animals, eg wild-captured animals, will occur infrequently and will be regulated. A period of 12 months in these premises is presumed to allow sufficient time for observation and health monitoring. This should reduce the likelihood that animals affected by the pathogens under consideration but not showing signs of disease may be included in consignments for importation. It is assumed that if a pathogen is present in one or more animals in the

group, disease will manifest itself in at least some of the members of the closed group and will be detected during the normal course of health surveillance. It is recommended that this measure is also applied to animals used for the production of eggs.

Pre-export quarantine

PEQ isolates animals to be exported from other susceptible or infected animals reducing the risk of disease transmission. PEQ also provides an opportunity for more rigorous observation, disease diagnosis and treatment. These observations and interventions generally reduce the likelihood that significant disease agents remain undetected. In addition, transient infections may be resolved during PEQ. In both situations, the risk of exporting animals while they are infected, is lessened.

Post-arrival quarantine

The benefits of PAQ are equivalent to those of PEQ. PAQ isolates imported animals from other susceptible animals reducing the risk of disease transmission. PAQ also provides an opportunity for observation, disease diagnosis and treatment. These observations and interventions generally reduce the likelihood that significant disease agents remain undetected before animals are released from PAQ. In addition, transient infections may be resolved during PAQ. In both situations, the risk of releasing infected animals, is lessened.

Diagnostic testing

Testing may be used to detect asymptomatic infected animals. The capacity of testing to reduce the risk depends on the availability and use of tests with high sensitivity. Animals yielding a positive result to testing may be treated or removed from PEQ or PAQ. Testing may also, depending on the test sensitivity and specificity, lead to disqualification or treatment of cohorts in the whole consignment.

Vaccination

Vaccination against specific disease agents reduces the risk that the animals sourced from infected regions become infected and/or show signs of disease. Reliable, commercial vaccines are not available for crocodylian diseases. This measure is therefore not available.

Treatment

Treatments to eliminate or reduce the pathogen load of disease agents reduce the risk that the animals sourced from infected regions remain infected and/or show signs of disease. Treatments for many of the crocodylian pathogens have been reported. Studies of the efficacy and safety of different drugs reported for use in crocodylians have not been published so proposed treatments are not rigidly prescriptive.

Restrict the destination (avoidance of exposure to susceptible species, vectors or intermediate hosts)

Holding animals in regions where there are no susceptible hosts reduces the risk of spread of disease. Similarly, maintaining imported animals in regions free from intermediate hosts or vectors will reduce the risk of infection, spread and establishment of disease agents with indirect life cycles. Long term restriction of the destination of animals to one geographical location is considered to be of uncertain value given the lack of knowledge of potential vectors and their distribution. This measure may be too difficult to regulate as crocodylians are generally long-lived.

Importation of eggs

Most of the disease agents considered in this risk analysis are not vertically transmitted. Consequently the risk posed by these disease agents through the importation of eggs is negligible.

3.2.3 Risk management recommendations

Of the range of measures considered above those which are relevant to each disease agent are discussed and recommendations proposed to minimise risk of entry.

Adenovirus-like agent

As this agent has only been reported from the African region, sourcing crocodylians from other regions may reduce the risk of introducing this agent.

This paper recommends that animals from Africa are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. Infection with adenovirus-like agent should not have been diagnosed in crocodylians held in the establishment during this period.

It is further recommended that animals and hatching eggs from Africa are held in PAQ for at least 6 months. This recommendation is based on a reported incubation period of up to 18 weeks and takes into account the current incomplete state of knowledge of the basic biology of this disease agent and the absence of tests to detect infected animals.

This paper recommends that the same conditions apply to animals producing eggs as apply to live crocodylians. This is because adenovirus-like agent is probably vertically transmitted so the risk of entry may not be reduced by importing eggs.

Poxvirus

Poxviruses have been reported worldwide. This paper recommends that animals from all regions are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. Infection with poxvirus must not have been diagnosed in crocodylians held in the establishment during this period.

It is also recommended that animals are held in PAQ for at least 8 weeks. This is based on a reported maximum incubation period of 4 weeks (Foggin *et al* 1992b) and takes into account the current incomplete state of knowledge of this virus group in crocodylians and that no tests are available to detect infected asymptomatic animals.

It is recommended that restrictions specifically aimed at reducing entry of this virus in eggs are not necessary as poxviruses are not known to be vertically transmitted.

Mycoplasma

Restrictions specifically aimed at reducing the risk of entry of crocodylian mycoplasmas are not needed for crocodylians and eggs sourced from regions other than North America and Africa.

It is recommended that crocodylians from Africa and North America are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. Infection with mycoplasmas should not have been diagnosed in crocodylians held in the establishment during this period.

Crocodylian mycoplasmas are probably vertically transmitted. It is, therefore, recommended that eggs may only be imported from North America and Africa crocodylians if animals are held under conditions described above.

Animals and hatching eggs from Africa and North America must be held in PAQ for at least 30 days. This requirement is based on a reported incubation period of up to 10 days (Mohan *et al* 1995) and takes into account the current incomplete state of knowledge of the biology of this disease agent and the absence of tests to detect infected animals.

Chlamydia

This agent has only been reported from the African region. Crocodylians and eggs from other regions will present a low risk of introducing this agent.

It is recommended that live crocodylians from Africa are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. Infection with *Chlamydia* must not have been diagnosed in crocodylians held in the establishment during this period.

The risk of entry of this disease agent is reduced by importing eggs as the disease is probably not vertically transmitted. It is recommended that eggs may be sourced from crocodylians in Africa with negligible risk of introducing this disease agent.

Entamoeba invadens

It is recommended that the faeces of crocodylians held in PEQ are tested on at least two occasions with an interval of at least 14 days. This testing will reveal cysts or trophozoites in infected animals. All animals in the imported consignment should be treated with metronidazole or dimetridazole or other antiprotozoal medication effective against *Entamoeba invadens* (refer Appendix 2 for dose rates) and retested. Only animals testing negative should be released from PEQ for export.

The risk of entry of this disease agent is reduced by importing eggs as the disease is not vertically transmitted.

Haemogregarines

It is recommended that blood samples taken from crocodylians held in PEQ are tested on at least three occasions with an interval of at least 14 days between each testing. This testing will reveal gametocytes within the cytoplasm of erythrocytes in infected animals. As no treatment is available infected animals in PEQ should not be exported. If infected animals are detected in PAQ they and the other animals in the consignment should be retested and if still positive re-exported or destroyed.

The risk of entry of haemogregarines is reduced by importing eggs as the disease is not vertically transmitted.

Dujardinascaris

It is recommended that the faeces of crocodilians held in PEQ are tested on at least two occasions with an interval of at least 14 days. Microscopic examination of samples subjected to the faecal flotation technique will reveal typical thick-shelled ascarid eggs in infected animals. These animals and others in the consignment should be treated with mebendazole, fenbendazole, thiabendazole or other antihelminthic medication effective against *Dujardinascaris* (refer Appendix 2 for dose rates) with a repeat dose in two weeks. Animals should be tested again 14 days post treatment and treated again if test results are positive. Only animals testing negative should be released from PEQ for export.

The risk of entry of infected animals is reduced by importing eggs as the disease is not vertically transmitted.

Paratrichosoma

This agent has only been reported from the Asia Pacific and African regions. Crocodilians and eggs from other regions will pose negligible risk of introducing this agent. It is recommended that quarantine measures are not necessary for animals imported from these other regions.

It is recommended that live crocodilians from the Asian Pacific and African regions are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12 months. Infection with *Paratrichosoma* must not have been diagnosed in crocodilians held in the establishment during this period.

The risk of entry of this disease agent is reduced by importing eggs as the disease is not vertically transmitted. It is recommended that eggs may be sourced from crocodilians in Asia Pacific and Africa with negligible risk of introducing this disease agent.

Filarids

It is recommended that blood samples from crocodilians held in PEQ are tested on at least three occasions with an interval between each sampling of at least 14 days. This testing will reveal microfilaria in the blood of infected animals. Testing on at least three occasions is recommended as the entry of microfilaria into the bloodstream is intermittent. As no treatment is available infected animals in PEQ should not be exported. If infected animals are detected in PAQ all animals in the consignment should be retested on at least three occasions with an interval between each sampling of at least 14 days. If an animal remains positive it should be re-exported or destroyed.

The risk of entry of infected animals is reduced by importing eggs as the disease is not vertically transmitted.

Trichinella

There are no OIE conditions specifically relating to the importation of crocodilians with regard to trichinosis.

Trichinella species other than *T pseudospiralis*, have been reported in all regions.

It is recommended that live crocodilians from all regions are sourced from a Government registered or licenced farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles on which they have been held continuously for at least 12

months. Infection with *T spiralis* should not have been diagnosed in crocodylians held in the establishment during this period.

The risk of entry of this disease agent is reduced by importing eggs as the disease is not vertically transmitted. Eggs may be sourced from crocodylians in all regions with negligible risk of introducing this disease agent.

Pentastomes

It is recommended that the faeces of crocodylians held in PEQ are tested on at least two occasions with at least a 14 day interval. Eggs, with larvae and hooks, will be found in infected animals. As no treatment is available infected animals in PEQ should not be exported. If infected animals are detected in PAQ they should be retested and, if still positive, re-exported or destroyed.

The risk of entry of infected animals is reduced by importing eggs as the disease is probably not vertically transmitted.

General recommendations

The quarantine conditions in the next section provide specific measures to reduce the risk of establishment of each disease agent considered in this IRA. In addition a number of general quarantine measures are included.

Crocodylians should be examined in PEQ and PAQ by experienced wildlife veterinarians to ensure they are clinically healthy. Also animals should be given prophylactic treatment with a broad spectrum anthelmintic and an approved ecto-parasiticide (suitable for use on reptiles). These recommendations aim to enhance the animal's chances of survival during transit and reduce the non-specific parasite load. Moreover, these measures may reduce the likelihood of introducing pathogens, as yet unidentified, which may cause harm to Australian animals.

General quarantine measures are also provided for egg collection and transport. These include a requirement that egg donors in all regions must have been held continuously for at least 12 months prior to egg collection in a Government registered or licensed farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles.

The quarantine conditions for live crocodylians require both PEQ and PAQ. Animals in PEQ are isolated from others not of the same health status for 60 days prior to the scheduled date of export. During PEQ blood and faecal samples can be collected and tested and treatments administered as necessary prior to export. More intensive observation of animals and clinical examinations may be carried out during this time.

The required period of PAQ for live crocodylians, eggs and hatchlings (6 months for animals from Africa and 90 days for animals from all other regions) is based on the known incubation periods of disease agents of concern and recommendations in the literature.. During PAQ the health of animals can be checked. The general quarantine measures are based on the 1997 interim quarantine requirements for the importation of reptiles (Appendix 1) and the recommendations of Jacobson (1993) and Miller (1996) for the quarantine of reptiles prior to captive release and entry into zoo collections. These authors recommend among other things,

- avoiding mixing different reptile species,
- separation of new animals from collection animals for a 90 day quarantine period,

- quarantine on an “all in; all out” basis in premises well separated from the main collection,
- provision of designated keepers for quarantine animals or facilities for handlers to wash and change before and after contact with collection animals,
- physical examination of animals,
- complete blood examination whenever possible,
- skin and faecal examinations and anti-parasitic treatment as required, and
- post mortem examination of any animals that die during quarantine.

4 QUARANTINE CONDITIONS

4.1 QUARANTINE CONDITIONS FOR THE IMPORTATION OF CROCODILIAN EGGS FROM ALL COUNTRIES

1. GENERAL

- 1.1 These conditions apply to the importation of hatching eggs of all members of the order Crocodylia, ie. crocodiles, alligators, gharials and caimans.
- 1.2 Each consignment of eggs must be accompanied by a copy of a valid "Permit to Import". The Permit is obtainable from the Chief Quarantine Officer (Animals) [CQO(A)] of the State of Australia to which the importation is to be made. A processing fee will be charged for the permit.
- 1.3 Permission to import crocodilian eggs must also be obtained from Environment Australia under the *Wildlife Protection (Regulation of Exports and Imports) Act 1982*. Further information may be obtained from:
- | | | |
|-----------------------|----------|---|
| The Director | Phone: | 02 6274 2291 |
| Wildlife Protection | Fax: | 02 6274 1921 |
| Environment Australia | Email: | wps@ea.gov.au |
| GPO Box 787 | Website: | http://www.biodiversity.environment.gov.au/plant/wildlife/intr.htm |
| CANBERRA ACT 2601 | | |
- 1.4 The animals must be accompanied by an Animal Health Certificate signed by an Official Veterinarian. An Official Veterinarian is a veterinarian authorised by the Veterinary Administration of the country of export to perform animal health and/or public health inspections of commodities and, when appropriate, perform certification in conformity with the provisions of chapter 1.3.2 of the Code. The Certificate is to be in English and in a language understood by the certifying Official Veterinarian and stamped on each page with an Official stamp. There must be a separate certificate for each consignment.
- 1.5 The Animal Health Certificate should be in the format of the OIE Animal Health Code Model Certificate No.9 (*Animal Health Certificate for Day-Old Chicks, Turkey Poults, other Newly-Hatched Avian Species and Eggs*) replacing all references to 'birds' with 'crocodilians'. It must provide details of the certifying authority, identification of each animal, premises of origin of the animals, consignor and consignee, destination and means of transport.
- 1.6 Eggs intended for importation may only be obtained from animals held continuously for at least 12 months prior to egg collection in a Government registered or licensed farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles.
- 1.7 Costs associated with the selection, testing, transport, quarantine (including any extension to the quarantine period for whatever reason) and any Australian Government veterinary supervision of the animals during each quarantine period and during transport to Australia will not be met by the Australian Government.
- 1.8 Live crocodilians and crocodilian eggs are required to remain in quarantine or under quarantine surveillance pursuant to *Quarantine (Animals) Regulations* 35 and 36. Under

section 46A of the *Quarantine Act* 1908 a quarantine officer may approve premises for the purposes of quarantine. The quarantine premises may be managed under a compliance agreement (Section 66B of the *Quarantine Act* 1908). Non-compliance with the criteria or any breach of quarantine may result in registration of the premises being withdrawn or suspended and legal action instigated.

- 1.9 These requirements may be varied or reviewed at any time at the discretion of the Australian Director of Quarantine (herein called the Director).

2. CERTIFICATION

The Animal Health Certificate must attest that:

- 2.1 Eggs were obtained from animals held continuously for at least 12 months prior to egg collection in a Government registered or licensed farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles (specify the identification number and premise of origin of each egg-layer).
- 2.2 There has been no clinical or other evidence of infection with adenovirus-like agent (viral hepatitis and enteritis) or *Mycoplasma* sp in the source population during the 12 months immediately prior to the collection of eggs for export to Australia.
- 2.3 The source animals were free of clinical signs of disease for 60 days prior to the laying and collection of eggs for export to Australia.
- 2.4 The eggs in this consignment were
 - (i) clean when collected
 - (ii) indelibly marked so that batches of eggs from individual egg-layers may be clearly identified
 - (iii) packed in clean, inert material in new cartons or containers, approved for transport to Australia
 - (iv) stored in containers to prevent leakage in the event of breakage during transport
 - (v) only mixed with batches of other crocodylian eggs collected for export to Australia
 - (vi) stored since the end of the collection period until export in an approved secure place.
- 2.5 The vehicles for the transport of the eggs to the port of export were cleaned, disinfected and disinfested (wooden materials) prior to loading. The egg packing material, eg. vermiculite, must be new or sterilised prior to use. The containers used for transporting the eggs were new or were cleaned and disinfected prior to loading. Containers constructed of timber were treated against insect infestation or fumigated prior to loading as required by AQIS.

Note Items 2.1 to 2.5 inclusive should be certified by the Official Veterinarian.

3. TRANSPORT

- 3.1 The eggs must be consigned directly to Australia either by air or sea by a route approved by AQIS. They may not be accompanied by other eggs or animals without the approval of

AQIS. Transit or transshipment on route will need prior approval from AQIS and relevant authorities in the country or countries of transit or transshipment.

- 3.2 The design of the containers, the preparation for transport and the disinfection and disinsection (if wooden) of the interior of the containers must be in accordance with the recommendations of the OIE Code unless otherwise agreed by AQIS.
- 3.3 In the event of a consignment arriving in Australia in a leaking container, or with inadequate certification, the consignment may not be permitted entry into Australia, may be retained in quarantine, returned to the country of origin or destroyed without recompense.

4. ENTRY AND POST-ARRIVAL QUARANTINE REQUIREMENTS

- 4.1 Imported eggs will be taken directly to a quarantine premise for post arrival quarantine (PAQ); that is a zoo, scientific establishment or other premises which has been previously approved by the Director. The animals which are hatched from these eggs will remain in post-arrival quarantine (PAQ) for 6 months if from Africa or 90 days from all other regions.
- 4.2 After completion of PAQ, each hatchling from eggs imported under these conditions will be detained in quarantine or under quarantine surveillance in an approved premises. AQIS may approve transfer to another premises which meets requirements consistent with the pest risk of the animal.
- 4.3 During PAQ and while the imported animals and eggs remain under quarantine surveillance, they, and any in-contact animals or eggs, may be subjected to any testing or treatment prescribed by the Director, at the importer's expense.
- 4.4 If any eggs or animals fail a test or shows signs of disease, that animal and any or all other animals in the PAQ premises may, at the Director's discretion, be detained in quarantine and be subjected to any testing, treatment, observation or destruction ordered by AQIS at the importer's expense. Animals or eggs may be re-exported at the importers expense or destroyed without recompense.
- 4.5 All services provided by the AQIS will be at the importers expense. If animals die or are destroyed during transit or during any period of control, compensation will not be paid by AQIS.

4.2 QUARANTINE CONDITIONS FOR THE IMPORTATION OF LIVE CAPTIVE CROCODILIANS FROM ALL COUNTRIES

1 GENERAL

- 1.1 These conditions apply to the importation of all members of the order Crocodylia, ie. crocodiles, alligators, gharials and caimans.
- 1.2 The animals must be accompanied by a copy of a valid "Permit to Import". The Permit is obtainable from the Chief Quarantine Officer (Animals) [CQO(A)] of the State of Australia to which the importation is to be made. A processing fee will be charged for the permit.
- 1.3 Permission to import crocodilians must also be obtained from Environment Australia under the *Wildlife Protection (Regulation of Exports and Imports) Act 1982*. Further information may be obtained from:
- | | | |
|-----------------------|----------|---|
| The Director | Phone: | 02 6274 2291 |
| Wildlife Protection | Fax: | 02 6274 1921 |
| Environment Australia | Email: | wps@ea.gov.au |
| GPO Box 787 | Website: | http://www.biodiversity.environment.gov.au/plant/wildlife/intr.htm |
| CANBERRA ACT 2601 | | |
- 1.4 The animals must be accompanied by an Animal Health Certificate signed by an Official Veterinarian. An Official Veterinarian is a veterinarian authorised by the Veterinary Administration of the country of export to perform animal health and/or public health inspections of commodities and, when appropriate, perform certification in conformity with the provisions of Chapter 1.3.2 of the Code. The Certificate is to be in English and in a language understood by the certifying Official Veterinarian and stamped on each page with an Official stamp. There must be a separate certificate for each consignment.
- 1.5 The Animal Health Certificate should be in the format of the OIE Animal Health Code Model Certificate No. 8 (*Animal Health Certificate for Birds*), replacing all reference to 'birds' with 'crocodilians'. It must provide details of the certifying authority, identification of each animal, premises of origin of the animals, consignor and consignee, destination and means of transport.
- 1.6 Animals may only be imported if they have been held continuously for at least 12 months on a Government registered or licensed farm, wildlife park, zoo, scientific establishment or other Government approved establishment for holding reptiles.
- 1.7 Costs associated with the selection, testing, transport, quarantine (including any extension to the quarantine period for whatever reason) and any government veterinary supervision of the animals during each quarantine period and during transport to Australia will not be met by AQIS.
- 1.8 Live crocodilians and crocodilian eggs are required to remain in quarantine or under quarantine surveillance pursuant to *Quarantine (Animals) Regulations 35 and 36*. Under section 46A of the *Quarantine Act 1908* a quarantine officer may approve premises for the purposes of quarantine. The quarantine premises may be managed under a compliance agreement (Section 66B of the *Quarantine Act 1908*). Non-compliance with the criteria or

any breach of quarantine may result in registration of the premises being withdrawn or suspended and legal action instigated.

- 1.9 These requirements may be varied or reviewed at any time at the discretion of the Australian Director of Quarantine (herein called the Director).

2. CERTIFICATION

The Animal Health Certificate must attest that:

- 2.1 There has been no clinical or other evidence of infection with adenovirus-like agent (viral hepatitis and enteritis), crocodilian poxvirus, *Mycoplasma* sp., *Chlamydia* sp., *Trichinella* sp. or *Paratrichosoma* sp. in the source population during the 12 months immediately prior to export.
- 2.2 The animals were held immediately prior to export in pre-export quarantine (PEQ) for a minimum period of 60 days and during this time were isolated from all other animals not of equivalent or better health status. During PEQ animals were held in premises which have been approved by an Official Veterinarian for holding animals for export to Australia.
- 2.3 During PEQ each animal remained healthy and free from clinical signs of infectious disease.
- 2.4 During PEQ blood samples were taken from each animal on at least three occasions no less than 14 days apart and examined according to the methodology indicated in brackets with negative results for the parasite in each case.
 - (i) haemogregarines (microscopic examination of Giemsa stained blood smears)
 - (ii) filarids (microscopic examination of thick blood smears, refer Appendix 2)
- 2.5 During PEQ faecal samples were taken from each animal on at least two occasions no less than 14 days apart and examined according to the methodology indicated in brackets with negative results for the parasite in each case.
 - (i) *E. invadens* (faecal flotation, refer Appendix 2). Animals infected with *E. invadens*. may be treated with Metronidazole at 125 mg/kg repeated twice at 72 and 96 hrs (or other treatment of equivalent or better efficacy) and re-tested.
 - (ii) *Dujardinascaris* sp. (faecal flotation, refer Appendix 2). Animals infected with *Dujardinascaris* sp. may be treated with Mebendazole at 25 mg/kg by mouth on 2 occasions 2 weeks apart (or other treatment of equivalent or better efficacy) and re-tested.
 - (iii) Pentastomes (faecal examination, refer Appendix 2).
- 2.6 Each animal was examined by, or under the direct supervision of, an Official Veterinarian within 24 hours prior to leaving the PEQ premises for the port of export and was free from clinical evidence of infectious disease and external parasites and appeared fit to travel.
- 2.7 The vehicles for the transport of the animals to the port of export were cleaned and disinfected prior to loading. The containers used for transporting the animals were new or were cleaned and disinfected prior to loading and met the requirements in Section 4.4 of the OIE Code and Container requirement 42 in the International Air Transport Association (IATA) Live Animal

Regulations. Containers constructed of timber were treated against insect infestation or fumigated prior to loading as required by AQIS.

- 2.8 The compartment of the aircraft or vessel to be occupied by the animals and all removable equipment and containers were cleaned and disinfected prior to loading.
- 2.9 At the time of loading each animal appeared healthy and fit to travel and any chemical or physical restraint were in accordance with appropriate animal welfare guidelines.

Note Items 2.1 to 2.9 inclusive should be certified by the Official Veterinarian.

3. TRANSPORT

- 3.1 The animals must be consigned directly to Australia either by air or sea by a route approved by AQIS. They may not be accompanied by other animals without the approval of AQIS. Transit or transshipment on route will need prior approval from AQIS and relevant authorities in the country or countries of transit or transshipment.
- 3.2 The design of the containers, the recommended species requirements, the preparation for transport and the disinfection of the interior of the aircraft or vessel and containers must be in accordance with the recommendations of the OIE Code (Section 4.4) and IATA Live Animal Regulations (Container requirement 42) and animal welfare considerations unless otherwise agreed by AQIS.
- 3.3 The use of hay or straw as bedding during transport by air is not permitted; the use of stretchers is recommended. Alternatively, treated wood shavings, sterilised peat and soft board may be used.

4. ENTRY AND POST ARRIVAL QUARANTINE

- 4.1 The imported animals will be taken directly to a quarantine premises, ie. a zoo, scientific or other premises which has been previously approved by the Director, for post-arrival quarantine (PAQ). The animals will remain in PAQ for 6 months if from Africa and 90 days from all other regions.
- 4.2 As soon as possible after arrival, when acclimated and within 5 days post-arrival, animals will be clinically examined by a government veterinarian. Thereafter, all animals must be carefully observed for evidence of behavioral problems and signs of disease. Any abnormalities must be reported immediately to AQIS.
- 4.3 After completion of PAQ, each animal imported under these conditions will be detained in quarantine or under quarantine surveillance in an approved premises. AQIS may approve transfer to another premises which meets requirements consistent with the pest risk of the animal.
- 4.4 During PAQ and while the imported animals remain under quarantine surveillance, they, and any in-contact animals, may be subjected to any testing or treatment prescribed by the Director, at the importer's expense.
- 4.5 If any animal fails a test or shows signs of disease, that animal and any or all other animals in the PAQ premises may, at the Director's discretion, be detained in quarantine and be subjected to any

testing, treatment, observation or destruction ordered by AQIS at the importer's expense. Animals may be re-exported at the importers expense or destroyed without recompense.

- 4.6 All services provided by the AQIS will be at the importer's expense. If animals die or are destroyed during transit or during any period of control, compensation will not be paid by AQIS.

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6 Appendices

Appendix 1 Interim Quarantine Requirements for the Importation of Reptiles

18 February 1997

DOCUMENTATION

Each consignment of reptiles must be accompanied by a copy of a valid AQIS Permit to Import, an International Animal Health Certificate and an Import Permit from Environment Australia Biodiversity Group (formerly the Australian Nature Conservation Agency).

The Animal Health Certificate must give details of

- the identification of each animal including species, sex, age, and identifying marks, registration number or microchip implant number;
- name and address of exporter and registered, licensed or approved premises of origin;
- name and address of consignee;
- nature and identification of means of transport.

The Animal Health Certificate must be in English and signed by an Official Veterinarian who must certify that:

- the premises of origin is a government registered or licensed zoo or wildlife park or a government approved facility for the holding of reptiles;
- each animal has been continuously resident in the premises of origin for at least 12 months prior to certification;
- as far as can be determined, no case of aeromonas septicaemia, inclusion body disease of boids, paramyxovirus infection, salmonellosis, septicaemic cutaneous ulcerative disease (SCUD), tuberculosis, ulcerative shell disease (USD) or ulcerative stomatitis has been diagnosed at the premises of origin, in any member of the species of reptile to which this certificate applies, during the 12 months prior to certification;
- the animals were isolated from other animals not of the same health and residency status for 30 days prior to the scheduled date of export, and they and all in-contact animals were free from clinical signs of infectious or contagious disease during that period;
- either *the premises of origin is in an area free from Eastern and Western equine encephalomyelitis; or *the animals have been protected from exposure to insects for 120 days prior to export; (*delete one)
- within 96 hours prior to export, each reptile was treated with an effective external parasiticide suitable for use on reptiles;
- he/ she or another Official Veterinarian, or a veterinarian recognised by the Official Veterinarian as having expertise in the diagnosis of disease in reptiles, has inspected each animal within 72 hours prior to export and found it to be healthy and fit to travel;
- after due enquiry he /she is satisfied that each animal will be shipped in a container that meets the container requirements specified in the International Air Transport Association (IATA) Live Animals Regulations [General Container Requirements for Reptiles, Amphibians and Shellfish, and Container Requirements 41 - 47 as appropriate].

Note: An Official Veterinarian is a civil service veterinarian or a specially appointed veterinarian authorised to certify on behalf of the Veterinary Administration.

TRANSPORT

The animal/s must be consigned directly to Australia by an approved route. During transport to the port of export, during shipment, and during transport from the port of importation to the post-arrival quarantine facility, the animal/s must have no contact with animals not of the same consignment.

QUARANTINE

Each animal must undergo at least 30 days post-arrival quarantine isolation in an A class zoo or another approved quarantine facility and be inspected and found free from signs of infectious disease before release from quarantine isolation.

During post-arrival quarantine isolation the animal/s may be subjected to such tests and/or treatments as are specified by the Chief Quarantine Officer (Animals) in the State of import at the importer's expense. If any animal fails any test or shows evidence of an exotic disease during post-arrival quarantine, it may be detained in quarantine isolation, exported at the importer's expense or destroyed.

Each imported animal must remain in a registered A or B class zoo after release from post-arrival quarantine isolation unless otherwise agreed by the Director of Animal and Plant Quarantine (Australia).

REVIEW

These conditions may be reviewed at any time at the discretion of the Director of Animal and Plant Quarantine (Australia).

Appendix 2 Information on disease agents

ADENOVIRUS –LIKE AGENT

Agent

Viral particles are not enveloped and measure from 75-80 nm in diameter with hexagonal outlines and an electron dense core (Jacobson et al 1984). It is unclear whether this disease is caused by one or two adenovirus-like agents (Foggin 1987). Strain variations have not been reported.

Distribution

This disease has been reported in farmed crocodiles in Zimbabwe (Jacobson et al 1984; Foggin 1987) and South Africa (Foggin 1992b). This agent has not been recorded or suspected in Australia.

Host range

This disease has been recorded in Nile crocodiles (*C. niloticus*) (Jacobsen et al 1984).

Zoonotic Potential

Disease or infection have not been reported in humans.

Signs

The disease has been reported most frequently in “runt” hatchlings less than 5 months old on farms in Zimbabwe (Foggin 1992a). Symptoms are non-specific with lethargy and anorexia (Foggin 1992b). Affected crocodiles are usually found moribund or dead. It has been suggested that viral damage to the intestine predisposes crocodiles to secondary bacterial and parasitic infections (Foggin 1985). Chronic inflammation of the liver due to the virus could cause runting (Foggin 1992a). Hatchlings and juveniles up to 5 month old are usually affected (Foggin 1985; Jacobsen et al 1984) and within this age-group, slower-growing individuals are affected (Foggin 1992a).

Pathology

Major organs affected are liver or intestines (Foggin 1992b). The pancreas is frequently involved in both hepatic and intestinal forms of the disease (Foggin 1992b). The liver is usually markedly swollen and pale. In the intestinal syndrome the intestinal wall is swollen and congested (Foggin 1987; 1992b). Multifocal to diffuse areas of hepatic necrosis and necrotizing enteritis may occur; both in association with basophilic nuclear inclusions in affected cells (Jacobsen et al 1984). On electron microscopic examination, inclusions are revealed as crystalline arrays of viral particles. Portal fibrosis and bile duct hyperplasia are often associated with previous (Huchzermeyer et al 1994) or chronic adenoviral infection (Foggin 1992a)

Diagnosis

The disease is diagnosed by the typical intra-nuclear inclusions in the liver and intestines (Huchzermeyer et al 1994).

Transmission

The early development of disease in hatchlings indicates that vertical transmission may occur (Foggin 1992b). The disease can be transmitted by oral dosing with a preparation comprising liver from naturally infected crocodiles (Foggin 1992b). The incubation period for experimental infection was from 2 to 18 weeks (Foggin et al 1989).

Course of Infection

Not reported.

Treatment/Prevention

It is suggested that the disease may be prevented by only introducing new stock from farms with no history of disease (Foggin 1992b).

Pathogen survival

Unknown.

POXVIRUS

Aetiology

Elliptical virions, having dumb-bell shaped nucleoids typical of poxvirus were identified in transmission electron microscopy studies of infected tissue (Buenviaje *et al* 1992; Gerdes 1991; Horner 1988; Jacobson *et al* 1979). However, the viral particles described in crocodilians are smaller than other described poxviruses (Jacobson *et al* 1979).

Spectacled caiman poxvirus and Nile crocodile poxvirus are differentiated and listed by the International Committee on Taxonomy of Viruses as unassigned pox viruses (Murphy *et al* 1995). Characteristics of the virions have led to the suggested inclusion in both of the genera Orthopoxvirus and Parapoxvirus (Gerdes 1991); both are within the subfamily Chordopoxvirinae (Murphy *et al* 1995).

Variations in the clinical signs of disease and the dimensions of virions associated with disease have been reported from cases of poxvirus infection (Penrith *et al* 1991). This may indicate infection with different viruses or strains of viruses are responsible for the disease episodes reported.

Virion dimensions	Host	Country	Reference
220x110 nm	<i>Crocodylus porosus</i> <i>C. johnstoni</i>	Australia	Buenviaje <i>et al</i> 1992
200 x 100nm	<i>Caiman crocodilus</i>	Florida, USA	Jacobson <i>et al</i> 1979
285 x 195 x 135 nm	crocodile	South Africa	Huchzermeyer <i>et al</i> 1991
236-252 x 185-228nm	crocodile	South Africa	Gerdes 1991
233 x 195nm	caiman	South Africa	Gerdes 1991
not stated	<i>Crocodylus niloticus</i>	Zimbabwe	Foggin 1987
160x200x230nm	<i>C. niloticus</i>	Zambia	Pandey <i>et al</i> 1990
	<i>Caiman crocodilus</i>	Brazil	Cubas 1996
100 x 200 nm	<i>C. niloticus</i>	South Africa	Horner 1988

Distribution

Poxvirus infection has been reported in crocodilians in Australia (Buenviaje *et al* 1992), Zambia (Pandey *et al* 1990), South Africa (Horner 1988; Huchzermeyer *et al* 1991), Florida, USA (Jacobson *et al* 1979), Zimbabwe (Horner 1988), Brazil (Cubas 1996), Germany (Vetesi *et al* 1981), Malawi and Kenya (Foggin 1992b).

Infection with poxvirus has been reported in saltwater (*C. porosus*) and freshwater crocodiles (*C. johnstoni*) on a number of crocodile farms in Queensland and Northern territory (Buenviaje *et al* 1992; Buenviaje *et al* 1998). Australian reports of the disease differ from those overseas in that usually neither the clinical history nor gross appearance of lesions was suggestive of infection with the virus. In early reports infection was detected on light microscopy and confirmed on electron microcopy of tissues as incidental findings (Buenviaje *et al* 1992). Poxvirus infection may have been associated with high hatchling mortality (Hutton and Webb 1990).

Host species

Poxvirus infections have been reported in the saltwater crocodile (*C. porosus*), freshwater crocodile (*C. johnstoni*), (Buenviaje *et al* 1992), the Nile crocodile (*C. niloticus*) (Horner 1988; Pandey *et al* 1990), caiman (*C. crocodilus*) (Jacobson *et al* 1979; Vetesi *et al* 1981; Cubas 1996).

Zoonotic potential

Disease or infection have not been reported in humans.

Signs

Cubas (1996) reported high morbidity and low mortality due to a pox virus dermatitis. Caimans (*Caiman crocodilus yacare*) from 5 to 9 months were affected with superficial grey-white lesions. The disease is common only in hatchling crocodiles less than 1 year old (Foggin 1992b).

In the single outbreak reported in Zambia, yellowish or occasionally brownish nodules which varied in size from 2-3mm up to 6 mm in diameter were observed on the skin of Nile crocodiles (Pandey *et al* 1990). High mortalities (27.3%) in crocodiles ranging from 1 to 2 year of age occurred during this outbreak.

Lesions in Nile crocodiles in South Africa were described as brownish and wart-like, varying in size from unraised spots 2 to 3 mm diameter to raised, uneven nodules up to 8 mm in diameter appearing throughout the body but especially on the head (Horner 1988). Over 40 % of 9 month old animals were affected. No mortalities could be attributed to pox virus infection during the episode described by Huchzermeyer *et al*(1991).

The outbreak in captive spectacled caimans in the US, commenced with a single pox usually on an eyelid. Later lesions were distributed over the body as scattered grey-white, slightly raised, circular lesions 1-3 mm in diameter (Jacobson *et al* 1979; Vetesi *et al* 1981). Foggin (1992b) observed that lesions healed without permanent damage to the skin.

Pathology

Eosinophilic intracytoplasmic inclusion bodies (Borrel and Bollinger bodies) were present in affected hypertrophied epithelial cells in captive caimans (Jacobson *et al* 1979), farmed Nile crocodiles (Horner 1988) and a saltwater and freshwater crocodile (Buenviaje *et al* 1992). Epidermal lesions were reported to consist of large focal areas of acanthosis accompanied by hyperkeratosis and necrosis (Pandey *et al* 1990; Jacobson *et al* 1979).

Examination of pox lesions by transmission electron microscopy revealed the viral particles to be morphologically similar to poxvirus (Horner 1988; Jacobson *et al* 1979; Buenviaje *et al* 1992).

Diagnosis

Pox virus infection may be diagnosed by the detection of pox-like lesions on clinical examination, and by the characteristic histopathological changes in infected tissues on light or electron microscopic examination.

Transmission

Disease spread occurred horizontally amongst a group of Nile crocodiles involving animals of all sizes, on an affected farm in South Africa (Horner 1988). Huchzermeyer *et al* (1991) suggested that asymptomatic wild-caught crocodiles are carriers of the virus when introduced onto farms as breeding stock.

Course of Infection

Lesions in Nile crocodiles may regress after 3 to 4 weeks (Horner 1988) or persist for up to 5 months (Huchzermeyer *et al* 1991). Lesions in affected spectacled caimans may persist for months, even until death (Vetesi *et al* 1981). The incubation period on experimental infection extends from 9 days to 4 weeks (Foggin 1992b).

Treatment/ Prevention

An autogenous vaccine has been produced from lesion material in affected Nile crocodiles. Lesions healed more quickly in vaccinated crocodiles than in unvaccinated controls (Horner 1988). The disease may be prevented by not importing wild-caught stock or crocodiles from affected farms (Foggin 1992b).

Pathogen survival

Not reported.

MYCOPLASMAS

Agent

Fifteen rapidly growing *Mycoplasma* isolates were cultured from sick and dead crocodiles in Zimbabwe (Mohan *et al* 1995). The crocodile isolates described by Mohan *et al*(1995) shared the main biochemical characteristics of *Mycoplasma capricolum*, but they differed serologically from this species. They also differed serologically from the avian mycoplasma strains tested. Goats, but not crocodiles, were resistant to experimental infection with crocodile

strains (Mohan *et al* 1995). Grenard (1997) reported a recent outbreak of mycoplasma infection in captive alligators and suggested that the isolate represents a new strain.

Distribution

Mycoplasma infection of crocodiles has been reported in Zimbabwe (Mohan *et al* 1995) and the USA (Grenard 1997). Mycoplasma infection has not been recorded or suspected in Australia

Host range

Mycoplasma infection has been recorded in *C niloticus* (Mohan *et al* 1995) and captive alligators in Florida, USA (Grenard 1997).

Zoonotic potential

Not reported.

Signs

Polyarthritis was described in farmed crocodiles on 5 farms among the rearing stock aged 1-3 years with 10% morbidity and low mortality (Mohan *et al* 1995). The sick animals displayed swollen limb joints with progressive lameness and paresis. Grenard (1997) recorded high mortality in captive alligators due to *Mycoplasma* infection.

Pathology

The synovial structures in subacute cases contained mycoplasmas and excess turbid mucus which, at a later stage of the disease, became yellowish and sterile. Evidence of pneumonia was observed on post mortem (Mohan *et al* 1995).

Cellular changes in the joint capsule included oedema, necrosis of the superficial layers of membrane, lymphocytic infiltration and fibrosis. The lungs showed extensive areas of consolidation and oedema and the alveoli were filled with a mixture of polymorphs, mononuclears and erythrocytes (Mohan *et al* 1995).

Diagnosis

Mycoplasma spp. can be cultured from the joints of affected crocodiles (Mohan *et al* 1995).

Transmission

Not reported.

Treatment/Prevention

Sick animals in Zimbabwe recovered after a single intra muscular injection of long-acting tetracycline (10 mg/kg) followed by oxytetracycline mixed in the feed at 550 mg/kg for 10 days. However, animals may recover without treatment within 6-8 weeks (Mohan *et al* 1995). Mohan *et al*(1997) report on the use of a vaccine to protect crocodiles from disease.

Course of Infection

On experimental infection, lameness with swollen joints developed 7-10 days following intraperitoneal infection (Mohan *et al* 1995). Experimentally infected animals recovered within 6-8 weeks (Mohan *et al* 1995).

Pathogen survival

Not reported.

CHLAMYDIA

Agent

A species of *Chlamydia* was isolated from the livers of dead hatchlings on a farm in South Africa. However Koch's postulates for this agent have probably not been satisfied. The one reported isolate shared antigenic characteristics with the ovine strain of *C psittaci* and is regarded as a crocodile specific strain of this species (Huchzermeyer *et al* 1994).

Distribution

Chlamydial infection in crocodiles has been reported in Zimbabwe and South Africa (Huchzermeyer *et al* 1994). Chlamydial infection has not been recorded in Australian crocodilians.

Host range

Chlamydial infection has been reported in *Crocodylus niloticus* (Huchzermeyer *et al* 1994). Whether this chlamydia infects avian and mammalian hosts is unknown.

Zoonotic potential

Chlamydia infection may be a potential zoonosis, but there is no evidence that workers on infected crocodile farms have been affected (Foggin 1992a).

Signs

The disease affects all sizes of hatchlings and is characterised by sudden death (Huchzermeyer *et al* 1994). A retrospective study of cases of hepatitis in Zimbabwe suggests that chlamydial hepatitis is an important cause of losses in young crocodiles (Huchzermeyer *et al* 1994).

Pathology

Prominent pathological findings were acute hepatitis with intracellular chlamydial colonies and generalised oedema (Huchzermeyer *et al* 1994). Affected animals had pale, mottled, enlarged livers and slightly enlarged spleens. All animals had mild ascites with severe hydropericardium (Huchzermeyer *et al* 1994). Severe lymphoplasmacytic hepatitis was noted with congestion, mild bile duct proliferation, and oedema in the lungs. Foci of necrosis in the abdominal fat body and splenic necrosis were also observed (Huchzermeyer *et al* 1994).

Diagnosis

Chlamydial colonies can be detected in liver impression smears stained with Giemsa and a chlamydia presumed to be *C psittaci* can be isolated from livers of affected animals (Huchzermeyer *et al* 1994).

Transmission

Huchzermeyer *et al*(1994) suggest that the recorded outbreak of chlamydial hepatitis may have resulted from water contaminated by the African clawed toad, a possible reservoir of infection (Howerth 1984). Adult crocodiles may also act as reservoir hosts for the infection of stressed younger crocodiles (Huchzermeyer *et al* 1994). An initial disease episode in Zimbabwe spread via infected hatchlings to at least 4 other properties (Foggin 1992b).

Course of Infection

Acute mortality (Huchzermeyer *et al*1994). Chlamydial species may remain latent for prolonged periods in reptiles (Jacobson *et al* 1989).

Treatment/Prevention

Treatment of surviving animals with oxytetracycline prevented further mortalities (Huchzermeyer *et al* 1994).

Pathogen survival

Not reported

ENTAMOEBA INVADENS

Agent

This parasite is almost indistinguishable from *E. histolytica* which causes disease in humans. *E. invadens* differs from the human species in requiring culture at lower temperatures (<31 C) (Frank 1984).

Variation have been recorded in the length, width and nuclear diameter of trophozoites, in addition to differing sites of recovery in the intestine or liver ((Frank 1984). Telford (1971) suggests that strain differences occur in parasites found in different geographic locations.

Distribution

E. invadens is considered to have a world-wide distribution among reptiles in captivity (Frank 1984). *E. invadens* has been reported in captive reptiles in an Australian zoo (William Meikle, personal communication) but has not been reported in Australian free ranging species.

Host range

Entamoeba sp., probably *E. invadens*, has been reported in *C. porosus* (Ippen 1959) and crocodiles can serve as reservoirs for this parasite (Lane and Mader 1996; Cubas 1996). When strains from one geographic area are permitted to infect hosts from another area, disease with invasion of tissues can occur (Telford 1971). *E. invadens* may be harboured as a commensal in a range of reptile species (Barnard and Upton 1994). Crocodiles and turtles serve as reservoir hosts.

Zoonotic potential

E. invadens is not known to be pathogenic for humans (Marcus 1981) but is considered to share features with *E. histolytica* of humans (Frank 1984).

Signs

In snakes, some tortoise species and to a lesser degree, in lizards, this disease causes high morbidity and mortality (Marcus 1981) and is considered to be the most serious protozoan disease of snakes (Keymer 1981). Some species of snakes may also be carriers of this parasite. Reptiles in the families *Boidae*, *Pythonidae*, *Crotalidae*, *Elapidae*, *Hydrophiidae*, *Viperidae* and *Varanidae* are reported to develop clinical dysentery (Lane and Mader 1996). A description of clinical disease in the crocodile is limited to a single report (Ippen 1959). In this report it is not clear whether the signs described apply to infection in the crocodile or the other reptiles considered in the paper. Crocodiles are generally regarded as resistant to disease (Lane and Mader 1996).

Pathology

Pathology in crocodilians has not been reported.

Diagnosis

Detection of amoebiasis depends on the demonstration of either the cysts or trophozoites in tissue sections, fresh faecal preparations or in wet mounts of an intestinal scraping. A direct smear of fresh faeces will reveal trophozoites and cysts. Cysts may also be recovered by faecal flotation. Accurate identification of cysts or trophozoites is important as other non-pathogenic amoebas can occur in reptiles (Lane and Mader 1996). Culture of the protozoan is possible from fresh faeces or colonic wash material (Frank 1984).

Transmission

The life cycle of *E. invadens* is direct. Infected animals shed trophozoites and cysts in the faeces (Frank 1984). Cysts in the faeces of an infected reptile may be transferred mechanically by cockroaches, ants and flies (Frank 1984) to susceptible hosts. There is no evidence that arthropods act as true intermediate hosts (Keymer 1981).

Reptilian amoebiasis cannot be transmitted from reptiles to birds or mammals (Ippen and Zwart 1996).

Course of Infection

The direct life cycle can lead to rapid spread within captive collections. In a new host cysts pass into the stomach and start to excyst in the posterior midgut/colon area. The released amoebulae grow to fully developed trophozoites in the

gut contents and, after repeated cell division, either penetrate the mucosa or encyst. In reptile collections latent carriers continuously shed cysts and function as reservoirs. Crocodylians are considered to be amongst the most important carriers (Frank 1984).

Treatment/Prevention

Metronidazole and dimetridazole are effective treatments (Jacobson 1986; Barnard and Upton 1994). Metronidazole can be given at a dose of 125mg/kg repeated twice at 72 and 96 hrs (Lane and Mader 1996). Spread of the disease may be minimized by preventing faecal contamination of feed, including cockroach control (Foggin 1992b).

Pathogen survival

E. invadens cysts can survive outside a host for 7 weeks at 4⁰C (Frank 1984) and several days at 37⁰C (Barnard and Upton 1994).

HAEMOGREGARINES

Agent

Hepatozoon spp. and *Haemogregarina* spp. are not easily distinguished by their gametocytes within host tissues and are usually placed under the general term haemogregarines when detected in blood smears. Blood borne parasites of genera *Hepatozoon* have been recorded in crocodylians (Keymer 1981). Many parasites classed as *Haemogregarina* may belong to the genus *Hepatozoon* as the parasites can only be separated on the basis of their endogenous schizogony or type of development in a particular vector which is often not known.

Distribution

Haemogregarines have been recorded in crocodylians in Africa, India, Sri Lanka, Sumatra, North and South America (Telford *et al* 1984). *Crocodylus* sp. in Irian Jaya (Ladds *et al* 1995) and Nile crocodiles in Uganda (Hoare 1932, cited in Frank 1984). *Hepatozoon* are not considered to be very host specific and can be transferred from one reptile species to another via mosquito vectors (Marcus 1981). Haemogregarines have not been recorded in crocodylians in Australia.

Host range

Crocodylus sp., *Caiman* sp. are hosts for *Hepatozoon* sp. (Keymer 1981). *Crocodylus* sp., *Caiman* sp., *Caiman* sp. and *Gavialis* sp. are hosts for *Haemogregarina* species (Keymer 1981). Telford *et al* (1984) writes that haemogregarines are known from crocodiles, alligators and gavials. Haemogregarines are a relatively non-host-specific group of protozoa that can be transmitted across reptile species (Wozniak *et al* 1994).

Zoonotic potential

Not known.

Signs

These protozoans rarely cause clinical disease but heavy infections may be associated with anaemia and inanition (Barnard and Upton 1994; Campbell, 1996). Moreover haemogregarines cause clinically significant disease in unnatural host species (Wozniak *et al* 1994).

Pathology

Heavy parasitaemias are commonly seen in reptiles, but little investigation of the associated pathology has been undertaken (Telford *et al* 1984). In unnatural host species, hepatitis, pancreatitis and splenitis may result from infection (Wozniak *et al* 1994).

Diagnosis

Parasitaemia may be intense and can be life-long in infected reptiles (Telford 1984; Campbell, 1996). Microscopic examination of thin Giemsa stained blood smears during parasitaemia reveals sausage- or banana-shaped, non-

pigmented gametocytes within the cytoplasm of erythrocytes on blood smear. Infected cells may have the nucleus pushed to one side and the cells may be irregular in shape and size (Lane and Mader 1996). Extra cellular *Hepatozoon* spp. may also be detected in smears (Campbell 1996). A PCR has been developed for detecting reptilian haemogregarines (Wozniak *et al* 1994).

Transmission

Hepatozoons are transmitted to crocodiles by invertebrate intermediate hosts (Lane and Mader 1996). Ticks, mites, flies, bugs and leeches may serve as vectors (Campbell, 1996). Transmission may be mechanical only or show sporogonic development within the vector (Telford 1984).

Course of Infection

Generally, haemogregarine infections of reptiles occur when infective sporozoites enter the host from the vector by inoculation or ingestion. Sporozoites enter the bloodstream and are carried to various organs, most commonly liver, spleen and lungs. Hepatozoon sporozoites enter capillary endothelial cells of the liver developing into schizonts. Haemogregarine sporozoites enter erythrocytes which may be sequestered in some viscera in which schizogony occurs. The merozoites formed invade new erythrocytes; this schizogony continues so that parasitaemia can become intense (Telford 1984). Schizonts occur in the liver, spleen and other organs and gametocytes in the blood (Keymer 1981).

Treatment

Not known.

Pathogen survival

Not known.

DUJARDINASCARIS

Agent

The nematode ascarid genus, *Dujardinascaris*, (syn. *Gedoelstascaris*) contains eleven species, all parasites of crocodilians (Goldberg *et al* 1991).

Distribution

Dujardinascaris spp infection extends to the whole geographic range of their hosts (Sprent 1984) and are reported in crocodilians in Australia, Philippines, India, Florida, Africa and South America (Cherry and Ager 1982; Sprent 1990; Machida *et al* 1992).

Host range

This ascarid occurs throughout almost the whole range of host groups (Sprent 1984). *D. taylorae* and *D. mawsonae* are reported in Australian crocodiles. *D. waltoni* are reported in alligators in Florida (Cherry and Ager 1982), *D. malapteruri*, *D. dujardini*, *D. madagascariensis*, *D. gedoelsti*, *D. puylaerti* and *D. graberi* are reported in African crocodiles (Sprent 1977). *D. longispicula*, *D. paulista* and *D. chabaudi* are reported in South American caimans (Sprent 1990).

Dujardinascaris spp. were recovered from 96% of samples in one study of Northern Territory crocodiles (Webb *et al* 1982).

Larval stages of *Dujardinascaris* spp. also occur in fish (Sprent 1984).

Zoonotic potential

Not reported

Signs

The clinical signs are usually non specific. Infected animals may be anorectic and slowly lose weight. Anaemia, regurgitation, bloat, signs of obstruction and wasting may also be observed in association with infection.

Pathology

Gastric ulceration may be observed in animals due to *Dujarnascaris* infection (Ladds and Sims 1990). Foggin (1992b) noted large numbers of the parasite in the stomach of runt hatchlings, excluding ingesta.

Diagnosis

Typical thick-shelled ascarid eggs may be identified in stomach washings or in faeces using the faecal flotation technique (Jacobson 1986).

Transmission

Infestation may be direct through ingestion of a third stage larva or via an intermediate host resulting from feeding freshly caught fish harbouring the parasite (Foggin 1987). There is a tendency for host specificity, in that each genus and species has a preferred host group.

Course of Infection

Infection can occur in young animals from 5 months of age (Sprent 1977; Goldberg *et al* 1991).

Eggs from adult worms in the intestinal tract of the definitive host pass out and undergo larval development to an infective stage in an intermediate host. Transmission of *Dujarnascaris* occurs when the infected intermediate host, usually fish, is eaten by a crocodilian and the third stage larvae emerge (Goldberg *et al* 1991). The fourth stage larvae and adults are probably closely associated with the stomach mucosa.

Treatment/Prevention

Mebendazole²⁴, fenbendazole²⁵ or thiabendazole²⁶ have been used to treat crocodilians infected with this ascarid (Foggin 1987; Jacobson 1986). Parasitism may be avoided by not feeding fresh, unfrozen fish (Foggin 1992b).

Pathogen survival

Eggs of *Dujardinasca* species are thin shelled (Frank 1981).

PARATRICHOSOMA

Agent

Nematode worms in the genus *Paratrichosoma* spp. lay eggs under the skin. Emerging larvae create serpentine tracks under the skin reducing its value.

Distribution

Papua New Guinea, India, Australia, South Africa and Thailand (Ashford and Muller 1978; Jacobson 1989; Buenviaje *et al* 1998; Youngprapakorn *et al* 1994).

Host range

New Guinean crocodile (*C. novaeguineae*), the Mugger crocodile, the Nile crocodile (*C. niloticus*), *C. johnstoni* and *C. porosus* (Buenviaje *et al* 1998; Jacobson 1989; Foggin 1992b).

Zoonotic potential

Not reported

Signs

²⁴ 20-25 mg/kg PO repeat in 2 weeks (Funk 1988)

²⁵ 50-100 mg/kg PO repeat in 2 weeks (Funk 1988)

²⁶ 50-100 mg/kg PO repeat in 2 weeks (Funk 1988)

Serpentine tunnels in the belly skin of infected crocodiles (Jacobson 1989).

Pathology

Not reported

Diagnosis

Clinical examination of belly skin and the detection of characteristic serpentine tunnels.

Transmission

Infection is apparently restricted to crocodiles in the wild or recently introduced into captivity. The parasite does not transmit horizontally in the farm pen situation (Foggin 1992b; Buenviaje *et al* 1998).

Course of Infection

Not reported

Treatment/Prevention

Treatment not reported. Infection may be prevented by raising animals in farm pens, thereby avoiding exposure to intermediate hosts.

Pathogen survival

Not reported

FILARID WORMS

Agent

The following genera of nematode, filarid worms have been reported in crocodilians; *Oswaldofilaria*, *Befilaria*, *Conofilaria*, *Piratuba*, *Piratuboides* and *Solafilaria* (Lane and Mader 1996; Telford *et al* 1984).

Distribution

A number of genera have been reported from a range of crocodilians. One report describes a filarid infection of caimans in South America (Telford *et al* 1984). Manzanell (1986). Manzell (1986) describes a new species of *Oswaldofilaria*, *O kanbaya* from *C porosus* in Australia. *Oswaldofilaria medemi* was obtained from the thoracic wall of an adult smooth-fronted colombian caiman, *Paleosuchus trigonatus* (Marinkelle 1981) *Oswaldofilaria bacillaris* is described from South American caimans (Prod'hon and Bain 1972).

Host range

Filarid worms have been described in a wide range of reptiles, including alligators and caimans (Telford *et al* 1984; Manzanell 1986). Filarids are apparently not very host specific (Frank 1981) and infection of aberrant hosts may occur (Lane and Mader 1996).

Zoonotic potential

Not reported

Signs

Jacobson (1986) notes that there are relatively few reports documenting clinical signs and gross lesions associated with infection. Heavy infestations have been recorded and these can be associated with thrombosis, aneurisms and vessel blockage (Lane and Mader 1996).

Diagnosis

Infection may be diagnosed in living animals by finding microfilaria in the blood. Jacobson (1986) notes that there are relatively few reports documenting clinical signs and gross lesions associated with infection. Heavy infestations have been recorded and these can be associated with thrombosis (Lane and Mader 1996).

Pathology

Accumulations of filaria can produce thromboarteritis verminosa. Accumulations of dead calcified worms may be found in distal parts of aneurysms and may block arteries (Frank 1981).

Transmission

Completion of the filarid life-cycle usually requires mosquitos as intermediate hosts. Frank (1981) considers the specificity for arthropod intermediate hosts to be low suggesting that several species may serve as the intermediate host for a filarid in nature. Prod'hon and Bain (1972) describe larval development of the South American caiman filarid (*Oswaldofilaria bacillaris*) in *Anopheles stephensi*.

Course of infection

Adult worms are usually found in the posterior vena cava and renal portal vein and produce microfilaria. Adults may also be found free in the body cavity or beneath the skin. Microfilariae in host blood are ingested by vector arthropods feeding on the reptile. Larvae enter the vector's haemocoel, undergo development and moulting to become infective (third stage). These larvae are transmitted to new crocodilian hosts via a blood meal (Frank 1981).

Treatment

Not known.

Pathogen survival

Not reported

TRICHINELLA SPIRALIS NELSONI

Agent

Tspiralis .nelsoni (*T nelsoni*) is a small filiform nematode which circulates among wild carnivores and pigs in eastern and southern Africa. Some authors recognise *T.nelsoni* as a species others regard *T.nelsoni* as a strain of *T. spiralis*. This species (strain) has also been found in the southern USSR, Bulgaria and Switzerland. There are several geographic *Trichinella* variants which differ in some of their physiological and morphological properties, including their infectivity for pigs and rodents.

Distribution

Tspiralis nelsoni has been reported in crocodiles in Zimbabwe (Huchzermeyer 1997) and wild carnivore and pigs in eastern and southern Africa, southern USSR, Bulgaria and Switzerland (Acha and Szyfres 1987).

A different species, *T. pseudospiralis* has been identified in wild animals in Australia (Geering *et al* 1995).

Kapel *et al* (1998) have questioned the identification of the *Trichinella* species reported in Nile crocodiles. There are no other reports of *Trichinella* spp. infecting poikilothermic hosts and they suggest that the infection observed was more likely to have been a genus other than *Trichinella* or a new taxon of *Trichinella*. Experimental infection of caimans with *Trichinella* species including *T nelsoni* was unsuccessful (Kapel *et al* 1998).

Host species

Nile crocodile (*C. niloticus*) (Foggin and Widdowson 1996), wild carnivores and pigs (Acha and Szyfres 1987).

Zoonotic potential

Trichinosis is included in the WHO's 'top ten' parasites (Bryant 1993).

Signs

Clinical signs of infection in all host species are rare (Geering *et al* 1995).

Pathology

Cysts are well encapsulated and spindle-shaped with an enclosed larva. Old lesions are calcified. There is minimal inflammatory response around cysts in animals (Geering *et al* 1995).

Diagnosis

An ELISA is available for the detection of antibodies in the serum of infected pigs. It is sensitive for the diagnosis of trichinellosis in animals which have been infected by the parasite for 4 weeks or longer (Geering *et al* 1995).

Transmission

Encysted larvae are very resistant to physical and chemical treatments. Larvae have remained viable in meat after 4 months of decomposition. Larvae are also resistant to desiccation, smoking and salting.

Course of Infection

When the host ingests meat containing the encapsulated infective larvae, the larva frees itself in the stomach and lodges in the villi and glandular crypts of the small intestine. Here it continues development until reaching adult stage in 2 to 3 days. Females deposit larvae that have hatched from eggs in utero. Larvae appear 4 to 7 days after ingestion of infected meat and may continue to be released for several weeks. Larvae cross the intestinal wall, enter the lymphatic vessels and travel via the thoracic duct to the superior vena cava and heart to be distributed to organs and tissues by the arterial circulation. Larvae remain for a limited time, re-enter the circulatory system and leave capillaries to lodge in striated muscle, where they encapsulate. Larvae lodging in other tissues die after a short period. The host's tissues surround the larvae forming a lemon shaped capsule. The parasite infests a new host via ingestion of this infected muscle. Encapsulated larvae may survive for years; from 5 to 10 years in human tissue (Acha and Szyfres, 1987).

Treatment

Not reported

Pathogen survival

Trichinella cysts are destroyed by storing infected meat at -15°C or less for 20 days or by heating to a core temperature of 60°C (Geering *et al* 1995). Encapsulated larvae can survive for long periods in carcasses.

PENTASTOMES

Agent

Adult pentastomes are large obligate endoparasites which are currently classified in a separate phylum, Pentastomida (Johnson-Delaney, 1996) Pentastomids in genera *Alofia*, *Sebekia*, *Subtriquetra*, *Diesingia*, *Selfia* and *Leiperia* have been reported from crocodylians (Riley, 1994; Riley *et al* 1995; Riley and Huchzermeyer, 1995a; Riley and Huchzermeyer 1995b; Riley and Huchzermeyer 1996; Riley *et al* 1990; Youngprapakorn, *et al* 1994; Boyce *et al* 1984; Cosgrove *et al* 1984).

Distribution

Pentastomid species are reported worldwide (Lane and Mader, 1996).

Host range

The adults of most pentastomes are parasites of reptiles with a wide host range (Frank 1981). Immature forms occur in reptiles, other vertebrates, especially fish or rarely in invertebrates (Cosgrove *et al* 1984; Junker *et al* 1998). Pentastomes were identified in Rainbow fish (*Nematocentrus rubostriatus*) and perchlets (*Ambassis* sp.) in the Northern Territory (Barrow 1988).

Zoonotic potential

Humans may become infected, serving as incidental intermediate hosts if viable eggs are ingested with food or water or if raw or undercooked meat is consumed (Johnson-Delaney, 1996).

Signs

Migration, encystment, muscular activity and armature of hooks have the potential to cause lesions in the host. Infections may be asymptomatic or accompanied by significant damage. Adult worms occasionally pierce the lung and body wall and protrude from the skin (Lane and Mader, 1996). The presence of adults is sometimes associated with anaemia, anoxia or hypoproteinaemia. Migration of larvae through tissues can result in tissue damage; the severity is dependent on the number and stage of invading larvae, the immune status of the host and the presence of concurrent disease (Lane and Mader, 1996). Infection has been associated with meningitis, pneumonia and mortalities (Cherry and Ager 1982; Boyce et al 1984). Young hatchling crocodiles in captivity seem the most susceptible, with anorexia, weight loss and respiratory distress reported (Lane and Mader, 1996).

Pentastomiasis is regarded as a major disease on crocodile farms in Australia (Buenviaje *et al* 1994).

Pathology

Mechanical trauma from the four moveable hooks may cause haemorrhage in the lungs which may become secondarily infected (Cosgrove *et al* 1984). Alveolar wall thickening may occur interfering with oxygenation. Eosinophilic granulomas or abscesses may form around dead larvae; healing is by fibrosis and calcification. (Cosgrove *et al* 1984).

Diagnosis

Eggs, containing larvae with hooks, may be found in the faeces (Lane and Mader, 1996). Radiological examination may reveal adult worms or calcified cyst remains (Cosgrove *et al* 1984).

Transmission

Larvae and nymphs are found in a wide variety of species of intermediate hosts and are not considered to be highly host specific (Cosgrove *et al* 1984).

Most infections in crocodiles in captivity are self limiting as infection is usually dependent on access to fish which serve as intermediate hosts (Lane and Mader 1996; Junker *et al* 1998). *Gambusia affinis* (mosquito fish) is intermediate host to at least two species of pentastomids infecting alligators (Boyce *et al* 1984; Boyce *et al* 1987). *Gambusia* species have become widely established in water bodies in Australia (Arthington *et al* 1986).

Course of infection

The adult usually resides in the lungs of the definitive host and eggs are coughed up, swallowed and passed in faeces. Eggs develop to an infective stage and are ingested by an intermediate host in which larval development occurs. Infective nymphs in the intermediate host are ingested by the definitive reptile host. The larval stage of the parasite perforates the intestinal wall and migrates through the body to the lungs (Cosgrove *et al* 1984; Lane and Mader, 1996). Auto-infection of reptiles may also occur if faecal passage of eggs is delayed so that intra-intestinal hatching occurs (Cosgrove *et al* 1984). Fish are intermediate hosts for *Sebekia oxycephala* infections of alligators (Cosgrove *et al* 1984).

Treatment/Prevention

Effective medical treatment is not available. Surgical removal of adults or nymphs is possible. The disease may be prevented by not feeding fresh fish.

Pathogen survival

Freezing mosquito fish (*Gambusia officinalis*) at -10⁰ C for 72 hrs killed larval *S. oxycephala* (Lane and Mader, 1996).

