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Research article

Translocation of european captive pink pigeons *Nesoenas mayeri* to Mauritius: disease risk analysis for production of a pre-export quarantine protocol

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Abstract

The Mauritian pink pigeon *Nesoenas mayeri* is at risk of extinction; only 470 birds remain in the free-living population. Being a recovered bottleneck species, this population suffers from genetic loss and inbreeding depression. Genetic variation in the European Association of Zoos and Aquaria (EAZA) Ex situ Programme (EEP) population is absent from free-living birds. EEP birds will be translocated to the captive breeding colony in Mauritius for genetic rescue. A disease risk analysis for this translocation was performed, following a four-step process outlined in International Union for Conservation of Nature guidelines: 1) problem description, 2) hazard identification, 3) risk assessment and 4) risk management. Potential pathogens were assessed and categorised as low, medium or high risk. Twenty-one pathogens were medium or high risk to the pink pigeon population, requiring risk mitigation strategies. Some pathogens were considered a risk to the poultry industry or endemic wild birds. A pre-export quarantine protocol was created which includes mitigation strategies to reduce these pathogens' risk rating to low. Recommendations include 30 days quarantine; general clinical examination and visual inspection for ectoparasites and pigeon pox lesions; radiography; blood sampling, cloacal swabbing, pharyngeal swabbing, faecal sampling and crop swabbing for various pathogens; and prophylactic endoparasite and ectoparasite treatment. Due to potential impacts on poultry and endemic wild birds, additional testing for avian influenza, Newcastle disease (avian paramyxovirus-1) and *Mycoplasma* spp. should be considered. This protocol can guide future EEP pink pigeon translocations to Mauritius.

Introduction

The Mauritian pink pigeon *Nesoenas mayeri* was driven to near-extinction in the 1970s, with only 20 birds remaining due to habitat destruction, predation by introduced mammals, parasitic diseases from invasive bird species and seasonal food shortages (Swinnerton 2001; Swinnerton et al. 2005a). Due to in-situ and ex-situ recovery programmes established in 1977 by the Mauritian Wildlife Foundation (MWF), the Durrell Wildlife Conservation Trust (DWCT–formerly Jersey Wildlife Preservation Trust), the International Council for Bird Preservation (now BirdLife International) and the Government of Mauritius, the free-living population now numbers 470 birds and has been downlisted to Vulnerable on the International Union for Conservation of Nature (IUCN) Red List (BirdLife International 2021). As part of this programme, breeding facilities were established on Mauritius and five founder birds (two pairs and a juvenile) were transported to DWCT headquarters in Jersey to establish a European captive population (Hartley 1977). This captive population has been managed as a European Association of Zoos and Aquaria (EAZA) Ex situ Programme (EEP) since 1992. The pink pigeon is classified as Critically Depleted on the IUCN Green Status assessment, with a species recovery score of 17%, and without historical conservation actions would almost certainly be extinct today (Tatayah 2021).

As a recovered bottleneck species (Jones et al. 1992), the pink pigeon population on Mauritius suffers from reduced genetic variation and inbreeding depression (Swinnerton et al. 2004). Due to the negative effects on fitness of inbreeding depression

and a reduction in adaptive potential, there is concern that the pink pigeon could be entering an extinction vortex. A population viability analysis has shown that the pink pigeon could become extinct in 100 years without intervention, with population models suggesting genetic rescue gives the best chance for long-term survival (Ryan 2020). Genetic rescue involves the introduction of genetically distinct individuals from one population into another, to reduce genetic load caused by the accumulation of harmful variants, and provide genetic variation (Hedrick and Fredrickson 2010; Ralls et al. 2018). It has been used successfully to increase fitness and genetic diversity in New Zealand robin *Petroica australis* (Heber et al. 2013) and Florida panther *Puma concolor coryi* (Johnson et al. 2010). Genetic variation in the captive pink pigeon population is absent from the free-living population (Jackson et al. in prep. 2020). Thus, translocation of individuals from the EEP captive population to the captive breeding colony on Mauritius is part of the EEP Long-term Management Plan (Francescon and Schad 2014). The captive breeding colony's progeny will be released to increase genetic diversity and fitness, reduce genetic load and create a more sustainable free-living population.

Conservation translocations and reintroductions are being increasingly employed (Bobadilla Suarez et al. 2017; Brown et al. 2017; Goodman et al. 2012; Keller and Hartup 2013). Although they can reinforce declining populations and improve genetic diversity, they also present disease risks to the translocated population and recipient ecosystem, which may result in significant mortality or reduced fitness, offsetting potential advantages (Aiello et al. 2014). When translocated individuals originate from an ex-situ population, they may carry pathogens novel to the destination population and they risk being introduced as symptomless carriers (Hartley and Sainsbury 2017). These animals may pose a higher risk if they have been housed in mixed species exhibits, have had contact with free ranging wildlife or have not been managed in a biosecure manner.

A disease risk analysis (DRA) can be qualitative, quantitative or semi-quantitative and is used to direct and perform translocations more effectively (Deem 2012). DRA is a structured, evidence-based process utilised prior to translocations to maximise translocated animals' health and minimise the risk of introducing potential pathogens to the destination population (IUCN/SSC 2013). IUCN Guidelines (IUCN/SSC 2013; Jakob-Hoff et al. 2014) highlight the importance of considering transmissible diseases in translocations, and provide frameworks for assessing risks.

Given inbreeding depression's negative effects on fitness (Ortego et al. 2007; Smallbone et al. 2016; Trinkel et al. 2011), the Mauritian pink pigeon population likely has an elevated susceptibility to disease, and the introduction of novel vectors and pathogens to Mauritius could pose a serious threat to its survival. This was seen with *Trichomonas gallinae*, believed to be introduced by invasive Columbiformes (Bunbury et al. 2007a). Additionally, during previous reintroductions, clinical disease became apparent as the population size increased (Peirce et al. 1997; Swinnerton 2001; Wheler et al. 1996). Therefore, risk mitigation strategies are hugely important for this translocation.

A four-step process recommended by the IUCN (Jakob-Hoff et al. 2014) was followed, incorporating a qualitative risk analysis and production of a pre-export quarantine protocol.

Materials and methods

Step 1: Problem description

MWF manages the free-living and captive breeding populations of pink pigeons in Mauritius. In January 2020, there were 470 free-living and 12 captive birds in Mauritius, as well as 97 captive birds across 13 European collections (Species360 2020a). The initial cohort of birds for translocation to Mauritius will have been bred and housed at Jersey Zoo, with the most genetically suitable birds selected for export. To increase fecundity and improve rearing success, most EEP captive pink pigeon neonates are raised by foster parents, often Barbary doves *Streptopelia risoria*. Around two months of age, birds are removed from foster parents and kept either isolated or in mixed species exhibits. The use of foster parents is popular in captive breeding and avian reintroduction programmes (Jones et al. 1983; Vigo-Trauco et al. 2021) and offers a more natural approach than hand-rearing, allowing normal species imprinting and socialisation (Jones and Merton 2012). After selection for export, birds will be housed in quarantine facilities, in line with mitigation strategies guided by this DRA. Birds will then be translocated to Mauritius, and on arrival will enter aviaries geographically distant from the breeding colonies. After a further quarantine period and post-import disease screening as deemed necessary, these birds will be moved to the captive breeding aviaries to breed with resident birds. Selection of individuals, management of these birds in quarantine facilities prior to export and the procedures post-arrival in Mauritius are discussed further in Whitford et al. (in prep. 2022).

This DRA focuses on the export of the first cohort of birds from Jersey Zoo. Prior to performing the risk analysis, the aim, desired outcome, limitations and acceptable level of risk were discussed with stakeholders (Table S1).

Step 2: Hazard (disease) identification

To identify potential pathogens associated with the translocation, a literature search on diseases found in captive and wild Columbiformes in Europe using the search engines BIOSIS, CAB Abstracts, and MEDLINE was performed. Results from a PhD thesis reviewing diseases in wild Columbiformes in the United Kingdom were considered (Pennycott 2016), as well as diseases detected by the Animal Plant and Health Agency during avian species disease surveillance in Great Britain (APHA 2014–2016, 2018a–c, 2019). Potential pathogens identified in a retrospective mortality review and health screen of the EEP captive pink pigeon population were also assessed (Shopland et al. 2020a, b).

Step 3: Risk assessment

The priority for this risk assessment was to consider the potential impact translocation could have on the recipient pink pigeon population (both free-living birds and those in the captive breeding facilities). In a full DRA, it is also important to consider any significant economic (poultry) or conservation (endemic wild birds) risks. Each potential pathogen was assessed based on its likelihood of occurrence and possible disease severity for pink pigeons. When assessing likelihood of occurrence, factors such as geographical range, requirement for a vector, seasonality and identification of the pathogen in the mortality review or health screen were considered. When assessing disease severity, consideration was given to how the disease could affect both free-living birds and adults and squabs within the captive breeding facilities. Combining these two factors allowed allocation of an overall risk rating to the pink pigeon population of low, medium or high (Table 1). In addition, risk to poultry and endemic wild birds was assessed, considering, where possible, whether the pathogen was already present in Mauritius, and therefore whether the translocation would modify disease risk. Based on all these factors, an overall assessment was made for each pathogen. Diseases rated moderate or high risk for pink pigeons, or that were deemed a significant risk to poultry or endemic wild birds, were subject to risk mitigation strategies.

Step 4: Risk management

Several potential pathogens that could be associated with a significant risk to the pink pigeon population, poultry and endemic

Table 1. Risk assessment matrix used to assess pathogens identified as a risk to the pink pigeon population.

Pathogen severity	Likelihood of occurrence -->			
	Rare	Unlikely	Possible	Likely
	Extremely low likelihood, sporadic reports in Columbiformes	Reported infrequently but known pathogen of Columbiformes	Reported commonly worldwide in Columbiformes	Reported very commonly worldwide and locally in captive/wild Columbiformes
Negligible Subclinical, no treatment	Low	Low	Low	Low
Minor Low level discomfort, basic first aid	Low	Low	Low	Medium
Moderate Can cause illness, often treatable	Low	Low	Medium	Medium
Major Severe illness, intensive treatment, death	Low	Medium	Medium	High

wild birds were identified. Based on the agreed acceptable level of risk determined with stakeholders, mitigation strategies were recommended to reduce each pathogen's risk to low. Management strategies were assigned based on: disease screening practicality (sample type, volume required, handling requirements, number of tests needed); test availability and reliability (specificity, sensitivity); available prophylactic treatments; and practicality of husbandry and environmental management practices to exclude the disease.

Results

Pink pigeon population

Seventy-six assessed pathogens were classified as high risk (n=4), medium risk (n=17) and low risk (n=55) to the pink pigeon population (Table 2). Seventeen pathogens were detected in the retrospective mortality review (n=14) or health screen (n=3) (Shopland et al. 2020a, b), seven of which have a medium or high risk (*T. gallinae*, *Eimeria* spp., *Chlamydia psittaci, Mycobacterium avium, Yersinia pseudotuberculosis, Ixodes* spp. and *Ornithostrongylus* spp.).

Endemic wild birds and poultry

The risk assessment also identified several pathogens that might be associated with risks to poultry or endemic wild birds. Those not already highlighted for mitigation strategies due to risks to the pink pigeon population include: avian influenza (AI), Newcastle disease (avian paramyxovirus-1: APMV-1), *Mycoplasma* spp., *Dermanyssus gallinae, Ornithonyssus sylviarum, Knemidokoptes mutans* and *K. laevis*. Due to lack of data for many other parasites regarding presence in Mauritius, ability to act as vectors and potential disease severity in different species, prophylactic treatment for endoparasites and ectoparasites is recommended.

Full considerations for all high and medium risk pathogens, alongside proposed management strategies and actions to take if mitigation strategies are not effective, are provided in Table S2. The suggested mitigation strategies would reduce all pathogens to low risk. General risk mitigation strategies for all pathogens include a 30-day quarantine period, increased biosecurity and appropriate housing with minimal disturbance to reduce potential for pathogen transmission and prevent unnecessary stress. The recommended pre-export protocol, which combines these risk mitigation strategies, is given in Table 3.

Viral pathogens

Eight viral pathogens required risk mitigation strategies: pigeon adenovirus (PiAV), pigeon herpesvirus (PiHV), pigeon circovirus (PiCV), rotavirus (RV), pigeon paramyxovirus-1 (PPMV-1), pigeon pox virus (PPV), AI and APMV-1.

Adenoviruses, PiCV and PiHV are widespread in Europe and have been found in cases of young pigeon disease syndrome (YPDS), with PiCV believed to play a major role (Duchatel and Szeleszczuk 2011; Herdt and Pasmans 2009; Stenzel et al. 2012). Lesions suggesting PiCV infection have been seen in young feral pigeons *Columba livia* in the UK (Pennycott 2016). Pigeon herpesvirus caused mortality of captive neonatal pink pigeons in the US believed to have been infected by asymptomatic foster parents (Snyder et al. 1985). Adenoviruses are associated with two disease states in Columbiformes: classic adenovirus causing vomiting, diarrhoea and weight loss in birds <1 year old and necrotising hepatitis, found in birds of all ages, with mortality rates ranging from 30–100% (Crespo et al. 2018; Herdt and Pasmans 2009; Pennycott 2008). Adenoviral hepatitis and mortality is recorded in wild feral and wood pigeons *Columba palumbus* in the UK (APHA 2018b). Different PiAV variants have been found in clinically healthy and sick domestic pigeons *Columba livia,* which makes testing challenging (Ballmann and Harrach 2016; Teske et al. 2017; Wan et al. 2018). Spillover infection with circoviruses has been reported from passerines to pigeons (Sarker et al. 2019), whilst PiHV has caused death in raptors (Phalen et al. 2017) and has been detected in other non-columbids (Woźniakowski et al. 2013). Fowl adenovirus can cause significant economic losses in poultry and has been detected in wild pigeons, demonstrating interspecies transmission (Niczyporuk et al. 2020). Although these viruses have never been detected in the EEP captive population, recommended risk mitigation strategies include PiCV, PiHV and pan-adenovirus polymerase chain reaction (PCR) testing due to the potential consequences of infection for naïve squabs in captive breeding

Table 2. Assessed risk of 76 potential pathogens to the resident pink pigeon population (free-living birds and those in captive breeding facilities). Categorisation based on 'likelihood of occurrence' and 'pathogen severity'.

Assessed pathogen risk	Pathogen type	Pathogen
High	Bacteria	*Chlamydia psittaci, Salmonella* spp.
	Endoparasites	*Trichomonas gallinae, Eimeria* spp.
Medium	Viruses	Pigeon adenovirus, pigeon circovirus, pigeon herpesvirus, rotavirus, pigeon paramyxovirus-1, pigeon pox virus
	Bacteria	*Mycobacteria avium, Yersinia pseudotuberculosis*
	Haemoparasites	*Leucocytozoon* spp., *Haemoproteus* spp., *Plasmodium* spp.
	Endoparasites	Protozoa: *Hexamita columbae*
		Nematodes: *Ornithostrongylus* spp., *Ascaridia columbae, Capillaria* spp.
	Ectoparasites	Lice: *Pseudolynchia canariensis*
		Ticks: *Ixodes* spp.
Low	Viruses	Avian influenza, avian paramyxovirus-1, avian paramyxovirus-7, Usutu virus, West Nile virus, pigeon parvovirus
	Bacteria	*Campylobacter* spp., *Escherichia coli, Streptococcus* spp., *Staphylococcus* spp., *Pasteurella* spp., *Mycoplasma* spp., *Pseudomonas* spp., *Haemophilus* spp., *Clostridium perfringens, Citrobacter* spp., *Klebsiella* spp., *Erysipelas* spp., *Enterobacter* spp.
	Endoparasites	Protozoa: *Toxoplasmosis* spp., *Sarcocystis* spp., *Giardia* spp., *Cryptosporidium* spp., *Enterocytozoon* spp., *Encephalitozoon* spp.
		Nematodes: *Dispharynx* spp., *Tetrameres* spp., *Syngamus trachea, Heterakis gallinarum*
		Cestodes: *Raillietina* spp., *Hymenolepis* spp., *Davainea proglottina*
		Trematodes: *Paratanaisia* spp., *Echinostoma* spp., *Echinoparyphium, Hypoderaeum conoideum, Brachylaemus commutatus*
	Ectoparasites	Lice: *Columbicola columbae, Campanulotes bidentatus*
		Mites: *Dermanyssus gallinae, Megninia columbae, Falculifer rostratus, Ornithonyssus sylviarum, Knemidokoptes mutans, Citodites nudus, Knemidokoptes laevis, Hypodectus propus, Myialges* spp., *Laminosioptes cisticola*
		Ticks: *Argas reflexus*
		Fleas: *Ceratophyllus columbae*
		Flies: *Culicoides* spp.
	Fungal	*Aspergillus* spp., *Candida albicans, Cryptococcus* spp.

facilities and impacts on endemic birds and the poultry industry.

PPV is found worldwide with variable mortality. Increasing infection frequency, and appearance in previously unaffected species, suggests this is an emerging viral disease (Lawson et al. 2012; Pello and Olsen 2013). Characteristic lesions take either a cutaneous form with hypertrophic epithelial proliferation predominantly around the beak and eyelids or a diphtheritic form with pseudomembranous lesions in the oral cavity (Herdt and Pasmans 2009). Pox-viral infections can cause significant economic losses in domestic poultry (Tripathy and Reed 2013) and are believed to be a factor in bird population declines in Hawaii, the Galapagos Islands and New Zealand (Atkinson and LaPointe 2009; Ha et al. 2011; Parker et al. 2011). In general, avian poxviruses are believed to be order-specific, although this may be influenced by ecological niche, habitat and geography (Gyuranecz et al. 2013). Advances in molecular sequencing have shown that many avian pox virus strains can infect multiple host species (Williams et al. 2021). PPV has been found in wood and feral pigeons in the UK (APHA 2014, 2016, 2019; Pennycott 2016) but not in the EEP population. It is a concern due to the possibility of disease in multiple species, however avian pox has been seen frequently in Mauritius, with historical reports of pink pigeons suffering from lesions on Ile aux Aigrettes (Swinnerton 2001) and mortality reported in fodies *Foudia rubra* (Cristinacce et al. 2009). Therefore, visual inspection for evidence of cutaneous or oral diphtheritic lesions and ectoparasite treatment to control vectors are considered adequate mitigation strategies for this translocation.

PPMV-1 is a variant of APMV-1 causing neurotropic disease in Columbiformes (Alexander 2011; Gogoi et al. 2015). Morbidity is usually high, whilst mortality varies with strain (Herdt and Pasmans 2009). It is endemic in Europe (Alexander 2011; Pennycott 2016). Outbreaks and mortality have occurred in UK wood pigeons, feral pigeons and collared doves *Streptopelia decaocto* (APHA 2014, 2018a, 2018c). Although PPMV-1 has not been detected in the EEP population, screening using a haemagglutination-inhibition test (HAIT) is recommended due to the frequency with which PPMV-1 is recorded in the UK, its potential to cause high mortality and the absence of known disease in Mauritius. Due to Newcastle disease's potential impact on the poultry industry, an oropharyngeal and cloacal swab for APMV-1 is also recommended.

Racing pigeons in the UK are routinely vaccinated against PPMV-1 (Cousquer and Parsons 2007) and there are vaccines for PPV. Vaccinating against these diseases was considered, but

Table 3. Recommended pre-export protocol for translocation of EEP captive pink pigeons to Mauritius.

Timescale	Procedure	Details, test requirements and treatments
30-days pre-export	Start of quarantine	Quarantine in rodent-, wild bird- and insect-proof enclosure Biosecurity measures to reduce inadvertent spread of disease from staff, other animals in the collection and environment e.g. disinfectant foot dips, personal protective equipment (boots, overalls, gloves), restricted access to facilities
	General anaesthesia	Sevoflurane or isoflurane in oxygen by mask, intubation with 2–2.5 mm endotracheal tube
	Clinical examination	Check ID: rings, microchips Physical examination to assess general health Body condition score and weight Visual examination for signs of infectious disease including: -Evidence of ectoparasites -Evidence of characteristic pox lesions -Oral examination for lesions suggesting diphtheritic form of pigeon pox virus or *Trichomonas gallinae* infection
	Venepuncture	Using the medial metatarsal or ulnar veins collect 0.5 ml blood (into a lithium heparin tube) and three blood smears for: -Direct microscopy of a Giemsa-stained blood smear for haemoparasites (*Haemoproteus* spp., *Leucocytozoon* spp. and *Plasmodium* spp.). Blood smear to be stained and stored -Haematology and biochemistry Pigeon paramyxovirus-1 (HAIT; 100 µl in plain tube) Evaluation of blood results to assess for signs of infectious disease: -Leucocytosis (with monocytosis) may suggest *Chlamydia psittaci* or *Mycobacterium avium* -Anaemia may suggest haemoprotozoa
	Radiography	Positioning: right lateral and ventrodorsal whole body views Screening to assess general health Screening for signs suggesting specific pathogens: hepatomegaly and splenomegaly (*C. psittaci*), skeletal abnormalities (*M. avium*)
	Crop swab	Culture for *T. gallinae* (InPouch®TV, Biomed Diagnostics, OR 97503, US). Incubated for 5 days at 37°C, microscopic examination daily
	Pharyngeal swab	Pigeon herpesvirus, avian influenza, avian paramyxovirus-1, *Mycoplasma* spp. (all PCR)
	Cloacal swab	Pigeon circovirus, adenovirus, avian influenza, avian paramyxovirus-1 (all PCR)
25–29 days pre-export	Pooled (five-day) faecal	*C. psittaci* (PCR) Culture for *Salmonella* spp. Direct microscopy and faecal flotation for endoparasites
Administration to coincide with testing or routine handling where possible	Prophylactic treatments (suggested products that have been used by the authors in pink pigeons; dosage)	Carnidazole (Harkers Spartrix, Petlife International Ltd, Bury St Edmund, UK; 25 mg/kg once orally) Ivermectin (Panomec, Boehringer Ingelheim, Bracknell, UK; 0.2–0.4 mg/kg once by intramuscular injection) Toltrazuril (Baycox, Bayer Reading, UK; 25 mg/kg orally SID for 2 days) Fipronil (Frontline 0.25%, Boehringer Ingelheim; one pump under each wing, approximately 2.5mg fipronil per bird) Doxycycline (Ornicure 150 mg/g, Oropharma nv, Deinze, Belgium; 1 sachet per 850 ml of drinking water daily for 30 days minimum—as per manufacturer's instructions)
Export	Further diagnostics, treatments or withdrawal from export to be decided based on results (see Table S2)	

booster vaccinations would be required to maintain immunity, vaccination may interfere with export screening tests and there was mortality from haemorrhage following PPMV-1 vaccine noted in the mortality review (Shopland et al. 2020a). Additionally, the level and duration of immunity that would be acquired from vaccination is unknown. In a study in racing pigeons, only 25.2% of juveniles and 74.9% of adults showed detectable antibodies after APMV-1 vaccination, which were overall at a relatively low level, indicating a potentially poor ability to mount an antibody response compared to other species (Teske et al. 2013). However, circulating titres cannot be reliably used to estimate protection against challenge. It was concluded that risk mitigation strategies other than vaccination would be adequate. However, if in future translocations the risk of these diseases changes, vaccination should be reconsidered.

RV is associated with recent outbreaks and mortality in Columbiformes in Europe and Australia and was found during a retrospective analysis of post-mortem examinations in the US (Blakey et al. 2019; Hunnam et al. 2019; McCowan et al. 2018; Rubbenstroth et al. 2018). Infection in Europe is associated with vomiting, diarrhoea and hepatic necrosis (Rubbenstroth et al. 2018). RV was recently identified in a racing pigeon with liver necrosis submitted to a UK laboratory (APHA 2018c). This disease is of concern due to its high mortality rate and frequent reports in Columbiformes in recent years, suggesting it may be an emerging disease. This pathogen was classed as 'major' for pathogen

severity but 'unlikely' to occur due to outbreaks being reported in only a few locations. Therefore, suggested risk mitigation strategies revolve around disease surveillance, including increased vigilance for clinical disease signs, and awareness of any reported cases local to the source population.

Highly pathogenic avian influenza (HPAI) is noteworthy due to its devastating effects on some endemic wild bird species and the poultry industry. Pigeons are susceptible to infection with HPAI, but do not tend to be clinically affected. They are believed to be ineffective disease propagators, shedding levels below the threshold required to infect other species (Abolnik 2014). Therefore, a suitable quarantine period and proper biosecurity should mitigate risks. However, as HPAI virus is constantly evolving and adapting to new hosts, oropharyngeal and cloacal swabs for PCR testing pre-export are recommended. Vaccination against AI could be considered for future translocations if the risk level changes.

Bacterial pathogens

As birds will be translocated to a captive facility, environmental bacterial load may accumulate more quickly than in a free-living environment. The most significant bacterial pathogens identified were: *C. psittaci, Salmonella* spp., *M. avium* and *Y. pseudotuberculosis.*

Chlamydia psittaci is a zoonotic disease found in Columbiformes worldwide (Kaleta and Taday 2003; Magnino et al. 2009). In pigeons it is often a chronic disease with subclinical carriers, but epizootic infections can occur (Stenzel et al. 2014). It has been reported as the cause of death of feral pigeons and collared doves in the UK (Beckmann et al. 2014; Pennycott 2016) and found as a comorbidity in a European captive pink pigeon post-mortem examination (Shopland et al. 2020a). The EEP population screened negative for this disease via PCR and serological testing (Shopland et al. 2020b). This pathogen's human health risks must be considered, as contact with birds is believed to be the primary risk factor and infection has been seen in numerous bird species, including poultry (Balsamo et al. 2017). Multiple testing methods exist for *C. psittaci*; molecular methods are preferred over serology for active infection detection (Balsamo et al. 2017). Serology is less useful for detecting current infections due to anti-chlamydial antibodies' long-term persistence post-exposure and false negatives that can occur in acute infection or in birds treated with certain antibiotics (World Organisation for Animal Health 2018). Given its global distribution, *C. psittaci* is likely already present in Mauritius. Risk mitigation strategies include PCR testing on a pooled faecal sample as well as physical examination, haematology, biochemistry and radiography to look for clinical disease indications. Given the human health risks, discussing requirements with the importing country's regulatory authorities is important prior to translocation. For this translocation, prophylactic treatment with a 30-day course of doxycycline is required.

Salmonellosis, most often caused by *S. typhimurium*, is an important Columbiformes bacterial disease (Harlin and Wade 2009; Herdt and Pasmans 2009). Many infected birds remain long-term carriers, excreting bacteria intermittently in their faeces. Mortality has occurred in feral pigeons and wood pigeons in the UK (Pennycott 2016; Pennycott et al. 2006). This disease has not been detected in the EEP population but is likely to be present in Mauritius. *S. typhimurium* has also caused severe disease and mass mortality in passerine species (Brunthaler et al. 2021; Mather et al. 2016). *S. pullorum* and *S. gallinarum* are responsible for pullorum disease and fowl typhoid respectively, which can have serious economic impacts on the poultry industry (Daoust and Prescott 2008). Although not commonly reported, both serotypes have been isolated from Columbiformes (EFSA AHAW Panel et al. 2017; Harlin and Wade 2009). Risk mitigation strategies include faecal culture for *Salmonella* spp. As both *C. psittaci* and *Salmonella* spp. can be shed intermittently, testing a pooled faecal sample may reduce the risk of false negative results.

Mycobacterium avium and *Y. pseudotuberculosis* are commonly reported in Europe and can persist for a long time in environmental and animal reservoirs (Carpenter 2013; Dhama et al. 2011; Gandolf and Weaver 2018). Clinical cases of *Y. pseudotuberculosis* occur most commonly in winter (Allchurch 2003; Shopland et al. 2020a). Clinical signs depend on disease chronicity and vary from lethargy, anorexia and weight loss, to sudden death (Crespo et al. 2018). There is no seasonal pattern to *M. avium*, and although it can present similar clinical signs to *Y. pseudotuberculosis*, pink pigeons with this disease are often in poor body condition (Shopland et al. 2020a). Both pathogens have occurred in wood pigeons in the UK (APHA 2014, 2019), with *M. avium* also causing mortality in collared doves and feral pigeons (Pennycott 2016). Both have caused significant mortality in EEP captive pink pigeons (Shopland et al. 2020a) and are likely present in Mauritius. *Y. pseudotuberculosis* is probably of less concern due to its preference for low temperatures. Bacterial transmission probability to the free-living pink pigeon population is low. The highest risk is to the individual being translocated and to birds in the captive breeding facility from faecal contamination of the environment by infected shedders. *M. avium* diagnosis in live birds remains challenging, particularly in the disease's early stages. The gold standard for diagnosis is culture, however this often requires a prolonged time for growth and has a low sensitivity and specificity in faecal samples due to inconsistent organism shedding and environmental contamination (Riggs 2012; Lennox 2016). PCR is preferred for ante-mortem diagnosis as it can detect low organism numbers and provides rapid results. However, ante-mortem testing via faecal screening is complicated by unreliable organism shedding and organ biopsy would be too invasive considering the risk levels (Riggs 2012). Columbiformes with chlamydiosis and mycobacteriosis can present with significant haematological and radiographic changes, most noticeably leucocytosis (with monocytosis) (Crespo et al. 2018; Kriz et al. 2015), and hepatosplenomegaly (*C. psittaci*) (Pennycott 2008). Therefore radiography and haematology should be performed as part of risk mitigation strategies for these pathogens. To reduce *Y. pseudotuberculosis* risk good pest control is essential, as rodents and wild birds can act as disease reservoirs (Allchurch 2003). The retrospective mortality review suggested that birds should be transferred during warmer seasons where possible (Shopland et al. 2020a). For both pathogens, preventing environmental build-up is a key management strategy. Therefore, maintaining optimum environmental hygiene is essential throughout translocation.

Many bacteria assessed as low risk are considered opportunistic pathogens, such as *Mycoplasma columbinum, M. columborale and M. columbinasale*, causing clinical disease only when the host is already immunocompromised (Cousquer and Parsons 2007). Although not known causes of disease in Columbiformes, *M. gallisepticum* and *M. synoviae* can cause respiratory disease in poultry, with *M. gallisepticum* also responsible for large-scale conjunctivitis epidemics in passerines in America. They can infect a wide range of avian hosts including wild Columbiformes (Sawicka et al. 2020). It is unknown whether these pathogens already exist in Mauritius, therefore a pharyngeal swab for *Mycoplasma* spp. testing should be considered.

Haemoparasites

Leucocytozoon spp., *Plasmodium* spp., and H*aemoproteus* spp. all received a medium risk rating (Table 2). *Leucocytozoon marchouxi* is associated with reduced pink pigeon survival (Bunbury et al. 2007b; Peirce et al. 1997), although mortality may depend

on the presence of other stressors (Swinnerton et al. 2005b). *Leucocytozoon* spp. are present in Europe, with several different lineages identified in feral pigeons in Italy and in Columbiformes in the UK (Dunn et al. 2017; Scaglione et al. 2015). As the free-living Mauritian population has already been exposed to *L. marchouxi*, these birds may have some immunity and therefore the greater risk is to young birds in the captive breeding facilities. *Haemoproteus* spp. have been reported in Columbiformes in Europe (Dunn et al. 2017; Pennycott 2016; Scaglione et al. 2015). Infected birds are often asymptomatic, however, clinical disease can be seen in young, debilitated individuals (Harlin and Wade 2009), with morbidity and mortality occurring worldwide (Hussein and Abdelrahim 2016; Joshi et al. 2017; Nebel et al. 2020). *Plasmodium* spp. have been reported in Columbiformes, with mortality in young racing pigeons (Da Silva et al. 2021). Chronic haemoparasite infections have a negative effect on fitness, reproductive success and immunity (Asghar et al. 2015; Tomas et al. 2007). *Plasmodium* spp. and *Haemoproteus* spp. cause mortality in many wild and captive bird species (Bueno et al. 2010; Derraik et al. 2008; LaPointe et al. 2012; Ortiz-Catedral et al. 2019). *Plasmodium* spp. may be lethal for highly susceptible hosts that have evolved in its absence, as in Hawaii where *Plasmodium* spp. was implicated in the significant decline in native forest birds (Atkinson and LaPointe 2009; Atkinson and Samuel 2010). Neither *Plasmodium* spp. nor *Haemoproteus* spp. have been detected in pink pigeons in Mauritius, despite the abundance of potential vectors (mosquitoes and hippoboscid flies respectively) (Bunbury et al. 2007b). However, both have historically been detected in other Mauritian birds, including *Haemoproteus* spp. in feral pigeons (Peirce et al. 1977). Although these haemoparasites have not been detected in the EEP population, they still pose a risk. Mitigation strategies include blood smear microscopy and anti-parasite treatment to control possible vectors.

Endoparasites

Several endoparasites can cause disease in Columbiformes (Harlin and Wade 2009; Herdt and Pasmans 2009). Many are found at low levels in clinically healthy birds with disease occurring when individuals are immunocompromised or when environmental conditions allow parasitic load to increase. The highest-ranking endoparasite hazards are *T. gallinae* and *Eimeria* spp., followed by *H. columbae, Ornithostrongylus spp., A. columbae* and *Capillaria* spp.

Protozoa

T. gallinae is responsible for high mortality in the Mauritian pink pigeon and is believed to be a threat to the species' recovery (Bunbury et al. 2007a, 2008a; Swinnerton et al. 2005a). *T. gallinae* is highly prevalent across Europe, with potentially pathogenic lineages causing morbidity and mortality in Columbiformes (Marx et al. 2017). Migrating birds may spread *T. gallinae*, as has been shown with finch trichomonosis (Lawson et al. 2011). In wild Columbiformes in the UK, *T. gallinae* is reported extensively and has caused mortality (APHA 2018c; Lennon et al. 2013; Pennycott 2016; Stockdale et al. 2015). Several birds in the source population were carrying *T. gallinae*. Genotyping in one bird confirmed a type C strain, which is believed to be apathogenic (Shopland et al. 2020b). However, due to the highly infectious nature of *T. gallinae* and its extensive presence in UK wild Columbiformes and the source population, this pathogen is rated high risk. Risk mitigation strategies include culturing for *T. gallinae* using the *Trichomonas vaginalis* pouch method (Shopland et al. 2020b), followed by prophylactic treatment of all birds with carnidazole (Harkers Spartrix, Petlife International Ltd, Bury St Edmund, UK; 25 mg/kg once orally). Birds with a positive *T. gallinae* culture should be retested after treatment. Optimal hygiene around feeders and drinkers is important, as indirect disease spread can occur by sharing food and water sources (Bunbury et al. 2008a).

Eimeria spp. can cause coccidiosis and were identified as high risk. Coccidial parasites are found worldwide, with sixteen named *Eimeria* species reported in Columbiformes (Ball et al. 2012). *Eimeria mauritiensis* has been reported in pink pigeons in Mauritius (Ball et al. 2012). Coccidial oocysts have been found incidentally during post-mortem examination of wild Columbiformes and captive pink pigeons in the UK, as well as during the EEP health screen (Pennycott 2016; Shopland et al. 2020a, b). Although pathogenicity is debated, stressful periods may lead to clinical disease resulting in increased oocyst shedding. Young birds are particularly susceptible (Krautwald-Junghanns et al. 2009; Yabsley 2008). As coccidian parasites have a direct life cycle, rapid intracellular multiplication and shedding may increase due to translocation stress. Risk mitigation strategies include optimal hygiene to reduce environmental contamination and re-infection risk, faecal screening (Shopland et al. 2020b) and prophylactic treatment with toltrazuril (Baycox, Bayer Reading, UK; 25 mg/kg orally SID for 2 days).

Hexamita columbae was assigned a medium risk. It is present in the UK and has caused disease in feral pigeons (Pennycott 2016). It is host-specific, causing disease mainly in young Columbiformes (Harlin and Wade 2009). In racing pigeons, it has been responsible for young bird mortality in the Netherlands (Zwart and Hooimeijer 1985) and has been found in YPDS cases in Northern Ireland (Scullion and Scullion 2007). A carrier state exists, with protozoa harboured in caecal tonsils. Risk can be reduced by direct microscopy of a fresh faecal sample or cloacal swabbing for protozoa. Prophylactic treatment with carnidazole (for *T. gallinae* risk mitigation) may have some effect on *H. columbae* infection (Carpenter et al. 2016).

Nematodes

Three nematodes were considered medium risk: *Ornithostrongylus* spp., *A. columbae* and *Capillaria* spp. These are found worldwide and in low numbers are clinically insignificant. There are many reports of avian species, including Columbiformes, testing positive on routine screening (Carrera-Játiva et al. 2018; Ilić et al. 2018; Rahmawati 2018). Severe infestations generally cause weakness, weight loss and diarrhoea. Helminthiasis has caused morbidity and mortality in wild Columbiformes (Pennycott 2016). During the EEP health screen strongyle ova were found, with no overt clinical disease signs. However, transportation stressors could cause increased shedding and parasite build-up. Recommended risk mitigation strategies include faecal screening for nematodes as well as prophylactic treatment with ivermectin (Panomec, Boehringer Ingelheim, Bracknell, UK; 0.2–0.4 mg/kg once by intramuscular injection). Although fenbendazole may be effective against nematodes, its use in this situation is not advised. Firstly, it can damage growing primary feathers and high doses can be toxic for Columbiformes (Howard et al. 2002). Secondly, in order to administer the full treatment course, birds would need to be handled daily for three days, which would increase the risk of stress and trauma.

Trematodes

All trematodes were considered low risk, but worthy of note are *Paratanaisia* spp. which have been recorded on post-mortem examinations of free-living pink pigeons in Mauritius (Bunbury et al. 2008b), with additional cases identified that are not recorded in the literature (Stidworthy personal communication). *Paratanaisia* spp. have been detected during captive Columbiformes post-mortem examination in the UK and can cause a range of clinical signs from subclinical to fatal, often culminating in renal failure (Unwin et al. 2013). There is no ante-mortem testing available

for *Paratanaisia* spp., but as this trematode is already present in Mauritius and requires an intermediate host, it remains in the low risk category.

Ectoparasites

Ectoparasites' pathological significance is often unclear, but high burdens may indicate a lack of preening due to illness or debilitation. Some ectoparasites may play a role as disease vectors (Harlin and Wade 2009; Sol et al. 2000) or may cause disease through irritation, restlessness and anaemia (Clayton et al. 2008; Taylor et al. 2016). Most ectoparasites assessed were considered low risk to pink pigeons, except ticks *Ixodes* spp. and the pigeon louse fly *Pseudolynchia canariensis.*

The tick *Ixodes frontalis* has been implicated in avian tick reaction syndrome (TRS). Clinical signs include depression, haemorrhage and oedema of the head and neck, and mortality (Monks et al. 2006). *I. frontalis* is found commonly on Columbiformes in the UK (Cull et al. 2018) and associated TRS has caused morbidity and mortality (APHA 2015; Chitty 2003; Monks et al. 2006). Ticks have caused morbidity and mortality in the captive pink pigeon population (Shopland et al. 2020a). Although no ticks were seen during EEP population screening, this may be due to the short duration for which they are attached to their host. Ticks are being more frequently recorded as a cause of morbidity in pink pigeons with five cases seen from 2000–2007 and ten from 2012–2018, suggesting this may be an emerging disease. Of these 15 cases, 8 were identified as *Ixodes* spp., of which six were *I. frontalis* (Flint personal communication, DWCT data on file). The pigeon louse fly is found throughout Europe (Sol et al. 2000; Taylor et al. 2016). It has been implicated in transmitting *Haemoproteus* spp. (Sol et al. 2000) and may be able to transmit some viruses (Harlin and Wade 2009). Several ectoparasites have the potential to negatively affect the poultry industry, including *Dermanyssus gallinae, Ornithonyssus sylviarum, Knemidokoptes mutans* and *K. laevis. D. gallinae*, one of the most damaging parasites of laying hens worldwide, may also act as a vector for several diseases, such as APMV-1 (Flochlay et al. 2017). These parasites' presence in wild birds in Mauritius is unknown, but they are likely already present in the poultry sector.

Although many parasites may be harmless at low levels and encourage the development of immunity, translocated birds will enter the Mauritian captive breeding facility where they may have contact with immunologically naïve squabs and experience stress during translocation which may increase pathogen shedding. Therefore, prophylactic endoparasite and ectoparasite treatment is recommended, including administering fipronil (Frontline 0.25%, Boehringer Ingelheim; one pump under each wing, approximately 2.5 mg fipronil per bird) and ivermectin (Panomec, 0.2–0.4 mg/kg once by intramuscular injection).

Fungal pathogens

Fungal pathogens were considered low risk as they are often secondary pathogens and ubiquitous in the environment. General risk mitigation strategies such as ensuring good hygiene and minimising unnecessary stressors will reduce the likelihood of occurrence.

Other considerations for the pre-export protocol

Additional information from the retrospective mortality review and health screen also influenced the pre-export protocol. Firstly, as impact trauma was a leading cause of mortality in pink pigeons (Shopland et al. 2020a), disturbance during quarantine should be minimised. Secondly, mortality during handling and conscious blood sampling has occurred, and during the EEP health screen comprehensive disease testing was not possible in all birds that were screened conscious. Therefore, pre-export screening should ideally take place under general anaesthesia, with tests aligned where possible to minimise stress and handling and allow more controlled haemostasis. Thirdly, as birds will be housed indoors in a less natural environment during quarantine, this period should not be extended unnecessarily. Ideally, birds will be housed in individual biosecure units to reduce the likelihood of group exclusion if an infectious disease is identified. Finally, birds must be in good general health and fit to travel. Clinical examination, radiographs, biochemistry, haematology and routine faecal screening should be performed to assess general health and screen for non-infectious diseases.

Recommended actions to take if pathogens are identified in a bird have been outlined (Table S2). The decision to treat an individual, rather than remove it from export, will depend on available time and logistics. There are limitations to the proposed pre-export protocol, due to lack of pharmacokinetic and pharmacodynamic data in this species for the proposed prophylactic treatments, and the absence of information regarding some of the proposed tests' sensitivity and specificity in pink pigeons. These limitations may be challenging to resolve due to the limited opportunities to carry out research on small populations. This can cause a degree of uncertainty for veterinarians involved in certain species translocations.

Post-export health monitoring

Based on the IUCN's guidelines, the extent to which imported birds are experiencing disease, adverse welfare conditions and mortality should be assessed (IUCN/SSC 2013; Jakob-Hoff et al. 2014). It is important that post-export monitoring continues to take place, for which an emphasis should be placed on screening translocated individuals and monitoring the population for infectious diseases. Captive-bred EEP pink pigeons will have different immunological challenges and pathogen exposure to those living in Mauritius, meaning translocated birds risk encountering novel pathogen strains, such as pox virus, *T. gallinae* and *L. marchouxi*. MWF conducts biannual health screening on pink pigeons, and this should continue. Any dead pink pigeons should receive full post-mortem examinations, with sample collection for further diagnostic testing (histopathology, microbiology and parasitology). Any evidence of wildlife or domestic animal disease in release areas that could be related to pathogens associated with imported pink pigeons should be fully investigated.

Conclusion

This export protocol has been designed to be evidence-based, practical, consider translocated birds' welfare and minimise the risk of introducing pathogens to the Mauritian pink pigeon population, endemic wild birds and the poultry industry. It is unknown whether many of the pathogens assessed are already present in Mauritius, which makes full evaluation of the degree of risk posed by this translocation difficult. In future, comprehensive health screening of the free-living Mauritian pink pigeon population would further understanding of disease susceptibility and exposure, add more evidence to the DRA and allow creation of effective preventative medicine protocols to safeguard translocated animals. This protocol can be used as a guide for future pink pigeon translocations, as well as for other endangered Columbiformes, and should be updated regularly to account for emerging infectious diseases and changes in the disease status of local wild bird or EEP pink pigeon populations. Mauritian authorities' requirements should be checked prior to each export and the protocol updated accordingly.

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Refereces

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