HEALTH SCREENING OF THE EUROPEAN ENDANGERED SPECIES PROGRAM CAPTIVE POPULATION OF THE PINK PIGEON (*NESOENAS MAYERI)*

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Abstract: The population of the Mauritian pink pigeon (*Nesoenas mayeri*) fell to fewer than 20 individuals in the 1970s. Following intensive conservation efforts, the free-living population is now estimated to be 470 individuals. However, due to the population bottleneck the species remains at risk of extinction due to genetic loss and inbreeding depression. A European captive population was established in 1977 and a European Endangered Species Program (EEP) was formalized in 1992. As birds in the EEP captive population possess unique alleles not observed in the surviving free-living birds, the EEP management plan recommends transferring EEP birds to Mauritius to improve genetic diversity. Health screening of the current EEP population to identify circulating pathogens was performed. Forty-two birds from three collections in the UK and one in Jersey were screened for a wide range of pathogens, present clinically or subclinically, including important viruses, bacteria, protozoa and helminths. Eleven birds tested positive for at least one pathogen: *Trichomonas* spp. (5), *Yersinia kristensenii* (2), *Yersinia aleksiciae* (1), coccidial oocysts (3) and strongyle ova (3). None of the positive birds showed overt signs of clinical disease, although two birds with *Trichomonas* spp. had suboptimal body condition. Genotyping of one *T. gallinae* sample revealed a type C strain (low pathogenicity). The results from this screening will contribute towards a disease risk assessment, to create a pre-export protocol for translocation of captive EEP birds to Mauritius.

Key words: Columbiformes, disease risk assessment, health screen, *Nesoenas mayeri*, pink pigeon, *Trichomonas gallinae*

INTRODUCTION

The Mauritian pink pigeon (*Nesoenas mayeri*) is a recovered bottleneck species.[44] The population fell to less than 20 individuals in the 1970s,[73,74] leading to the establishment in 1977 of a recovery program by the Mauritian Wildlife Foundation (MWF) and the Durrell Wildlife Conservation Trust (DWCT – formerly Jersey Wildlife Preservation Trust). Alongside *in situ* conservation efforts, captive breeding populations were established on Mauritius and at DWCT headquarters in Jersey.[36] This European captive population has been managed as a European Endangered Species Program (EEP) since 1992. By January 2020 the free-living population had recovered to 470 individuals (Tatayah, pers comm.). One major concern for ongoing recovery is reduced genetic variation, inbreeding depression and loss of adaptive potential.[75] Research shows genetic variation in the captive EEP pink pigeon population that is absent from the free-living population (Jackson *et al.*, unpubl. data.) and therefore translocation of EEP pink pigeons to the captive breeding colony in Mauritius is planned.[29] Their progeny will be released, with the aim of increasing genetic diversity and creating a more sustainable free-living population.

Species reintroductions and conservation translocations have been utilised worldwide with increasing frequency in recent years.[11,12,31,46] The success of any conservation translocation may depend on measures implemented to control the spread of disease associated with movement of hosts. When originating from an *ex situ* population, there is a possibility that translocated individuals could carry infectious agents novel to the final destination, and be introduced as symptomless carriers.[37] The International Union for Conservation of Nature Guidelines for Reintroductions and Translocations highlight the importance of considering

transmissible diseases, and the benefits of performing a disease risk assessment (DRA) to maximize the health of translocated animals and minimize the risk of introducing novel pathogens to the destination population.[41]

Columbiformes are susceptible to a variety of infectious diseases.[22,35,61] Trichomonosis, caused by *Trichomonas gallinae* and leucocytozoonosis, caused by *Leucocytozoon marchouxi* are two parasitic diseases of concern that have been observed in the Mauritian captive and free-living pink pigeon populations.[14,59] Trichomonosis has been responsible for high squab mortality and is believed to be a threat to the species' recovery.[16,73,74] Previous studies on *L. marchouxi* infection in pink pigeons show prevalence of 18.3-30%,[14,76] with reports of reduction in survival of infected birds.[14] *Plasmodium* spp. and *Hemoproteus* spp. have also caused mortality in many species of wild and captive birds,[13,24,48,56] with *Plasmodium* spp. being implicated in the significant decline of native Hawaiian forest birds.[6,7] These hemoparasites are reported in Columbiformes[25,67] and have been detected in Mauritian birds, but not specifically in pink pigeons.[58] Infected Columbiformes are often asymptomatic, however, clinical disease can be seen in young, debilitated individuals.[35]

Several viruses can infect Columbiformes. Pox viruses are not fatal to all avian species but may reduce population viability. Avian pox has been detected in Mauritius causing mortality in fodies (*Foudia rubra*),[23] and 10% of pink pigeons assessed on Il aux Aigrettes were found to be suffering from pox virus.[73] Avian pox is believed to be a factor in the decline of bird populations in Hawaii, the Galapagos Islands and New Zealand.[6,33,57] Increasing frequency and appearance in previously unaffected species worldwide, suggests this is an emerging viral disease.[50,60] Many viruses cause immunosuppression and an increased susceptibility to pathogens, particularly in young birds.[35,71] Pigeon circovirus, adenovirus and herpesvirus are

widespread, can have significant immunosuppressive effects and have been found concomitantly with young pigeon disease syndrome (YPDS).[26,27,35,71] Mortality rate is suggested to be around 20% and pigeon circovirus is believed to play an important role in this multifactorial disease.[26] Different variants of pigeon adenovirus exist and have been found in clinically healthy domestic pigeons (*Columba livia*).[77] There are several serotypes of paramyxoviruses, with *avian paramyxovirus*-1 (APMV-1) and *avian paramyxovirus*-7 reported to infect pigeons.[1] *Pigeon paramyxovirus*-1 (PPMV-1) is a variant of APMV-1. Morbidity is usually high, whilst mortality varies with strain.[39]

Several bacterial diseases can affect Columbiformes. Salmonellosis, most often caused by *Salmonella typhimurium*, is one of the most important.[35,39] Many infected birds remain long-term carriers, excreting bacteria intermittently in the faeces. *Chlamydia psittaci*, is a zoonotic disease found worldwide in a variety of avian species.[35,45,70] In pigeons it is often a chronic disease but can cause epizootic infections.[70] *Mycobacterium avium* and *Yersinia pseudotuberculosis* have a worldwide distribution, are commonly reported as pathogens in Europe, and can persist a long time in environmental and animal reservoirs.[18,30] Both have caused significant mortality in European captive pink pigeons.[68]

A large number of endoparasites and ectoparasites can cause disease in Columbiformes.[35,39] Many are found at low levels in clinically healthy birds with disease occurring when individuals are immunocompromised or environmental conditions allow parasitic load to increase.

This health screen was performed to identify potential pathogens currently present in the EEP pink pigeon population. Information obtained will contribute directly to the DRA for the

reintroduction, and the subsequent creation of the pre-export testing protocol for the translocation of EEP birds to Mauritius.

MATERIALS AND METHODS

The study was approved by the Animal Welfare and Ethical Review Board (AWERB) of the University of Bristol Veterinary School.

To formulate a list of potential pathogens a literature search on reported diseases in captive and wild Columbiformes was performed with the use of BIOSIS, CAB ABSTRACTS, and MEDLINE databases. This focused mainly on diseases in the UK and Channel Islands (from where the first cohort of birds will be transferred) but also considered emerging infectious diseases reported globally.[64] Infectious diseases identified in a retrospective mortality review of the European captive pink pigeon population were also taken into consideration.[68] Based on these results a list of diseases to be screened was formulated (Table 1). Some pathogens were not tested due to one or more of the following reasons: low risk of the pathogen causing clinical disease; lack of availability of commercial tests; lack of information relating to the current strain; limitations of handling and sampling small birds.

The study population consisted of all EEP pink pigeons (69 birds) during July 2017 – April 2018. Exclusions from this population included unfledged birds or any other reason for withdrawal by the housing collection. A total of 42/69 birds (61%) were screened across four collections in the UK and Jersey during this 10-month period. Most screening (69%) took place during the winter months (November – February).

Thirty-three birds were sampled conscious, coinciding with routine handling for health checks, nail clipping or moving aviaries. Sampling was performed on nine birds whilst anesthetized for routine health checks. General anesthesia was induced with inhalation of 5% isoflurane (Isoflo, Zoetis, Leatherhead, Surrey, KT22 7LP, UK) vaporized in 1.5L/min oxygen and maintained using 1-3% isoflurane vaporized in 1.5 L/min oxygen, either via a mask or after intubation with a 2.5mm stepped endotracheal tube.

37/42 birds were weighed. 31/42 birds were given a body condition score (BCS) using a subjective scale of 1-9, with 1 being emaciated, 9 being obese, and 5 being optimal. All birds received a clinical examination, including a visual inspection for evidence of ectoparasites and cutaneous lesions characteristic for pigeon pox virus. Oral examination was performed to look for lesions suggestive of the diphtheritic form of pox or *T. gallinae* infection. Nail clipping was performed if required.

Venepuncture was performed using the medial metatarsal or ulnar veins with a maximum of 0.5ml per 100g body weight taken using a 1ml syringe attached to a 25-27G needle. Digital pressure was applied after sampling to encourage hemostasis. Serum samples (200µl) were submitted for PPMV-1 and APMV-1 hemagglutination-inhibition tests (HIAT: Animal and Plant Health Agency, Weybridge, Surrey, KT15 3NB, UK). A minimum of 50µl whole blood was placed into 1ml of 96% ethanol for hemoparasite detection. For the polymerase chain reaction (PCR) based assays, DNA was isolated from blood samples following a salt extraction protocol[62] and diluted to 20 ng/µl. The PCR viability of the DNA samples was first confirmed using a PCR based sexing protocol for birds.[32] Only samples successfully amplifying a pink pigeon DNA fragment with this method were then used in the pathogen

screening. Samples were screened for presence/absence of hemoparasite infection using the nested PCR method previously described.[38] In order to further control for false negatives, samples were screened twice for both *Hemoproteus-Plasmodium* infection and *Leucocytozoon* infection. Light microscope examination (x400 and x1000) of Diff-quik (Rapi-Diff II Stain Kit, AtomScientific, Hyde, Cheshire, SK14 4GX, UK) stained blood smears was also performed to screen for hemoparasites. 50-100µl whole blood was used to saturate a paper disk for *C. psittaci* serology using the Immunocomb: Avian *C. psittaci* Ab. Test Kit (Biogal Galed Labs Asc Ltd., Kibbutz Galed, 1924000, Israel) according to the manufacturer's protocol. When only a small amount of blood could be collected, tests were prioritised as follows: hemoparasite PCR and microscopy, *C. psittaci*, PPMV-1, APMV-1. Residual whole blood (up to 100µl) was stored in 0.5-1ml of RNAlater for future genetic testing.

PCR testing was performed for *C. psittaci* (combined conjunctival, choanal & cloacal swab), *M. avium* (combined pharyngeal & cloacal swab), pigeon circovirus (cloacal swab) and pigeon herpesvirus (pharyngeal swab). Swabs were sent to Animal Genetics (St Austell, Cornwall, PL25 3LB, UK). A selective culture medium for the detection of *Trichomonas vaginalis* (TV pouch: InPouch® *Trichomonas vaginalis* culture kit, BioMed Diagnotics, Inc., White City, Oregon, 97503, USA), was used to screen for trichomonads, as previously used to culture *T. gallinae* from Columbiformes.[20,34] A crop swab was placed in the top chamber of the pouch and viewed immediately under a light microscope (x100 and x400). The double-chambered pouch was then mixed to allow the sample to disperse into the lower chamber and incubated at 37°C. The pouch was viewed every 24 hours for 5-days, for the presence of motile trichomonads. For positive TV pouches, a stabilate for the isolate was obtained,

revived and cultured as previously described.[4] Genotyping was performed using the method described by Chi et al 2013.[20]

A 3-day pooled fecal sample was collected for endoparasite screening. Fecal flotation was performed using zinc sulphate solution and samples were examined using light microscopy (x100 and x400) either at BZG or DWCT by an experienced technician. A cloacal swab or 3-day pooled fecal sample was cultured for *Yersinia* spp. and *Salmonella* spp. (Greendale Veterinary Diagnostics Limited, Woking, GU21 2EP, UK). To test for *Salmonella* spp., XLD agar and selentine broth were inoculated with feces and incubated at 37°C overnight. XLD plates were examined for non-lactose fermenting (NLF) colonies. The selentine broth was subcultured onto an XLD agar plate, incubated and examined as above. To test for *Yersinia* spp., Yersinia Selective Agar was inoculated with feces. This was incubated aerobically at 30°C for 48 hours, checking for growth each day. Any suspect colonies were subcultured onto blood agar and MacConkeys agar plates for further investigation. For positive samples, the species was identified using matrix-assisted laser desorption/ionization - time of flight mass spectroscopy (MALDI-ToF). Variation in sampling method (cloacal swab or pooled fecal sample) was due to addition of this test part way through the study period.

A summary of testing requirements for each disease can be seen in Table 1.

RESULTS

Of the 42 birds sampled there were 18 females and 24 males, confirmed by molecular sexing. Age ranged from 1 month 18 days to 12 years 7 months. Twenty-three birds were juveniles (31 days – ≤ 1-year-old) and 19 birds were adults > 1-year-old. The median age was 9

months and 27 days. All birds screened were negative for pigeon circovirus, pigeon herpesvirus, PPMV-1, APMV-1, *C. psittaci, M. avium, Salmonella* spp., *Leucocytozoon* spp., *Plasmodium* spp. and *Hemoproteus* spp.. 26.2% (7 males, 4 females) tested positive for at least one of the following: *T. gallinae* (5), coccidial oocysts (3), strongyle ova (3) and *Yersinia* spp. (*Yersinia kristensenii* (2) and *Yersinia aleksiciae* (1)). All *Yersinia* spp. cultured were obtained from cloacal swabbing. One bird had co-infection with *T. gallinae* and *Y. aleksiciae*. Two birds had co-infection with coccidial oocysts and strongyle ova. None of these birds showed overt signs of clinical disease. Not all birds were screened for every disease due to challenges with obtaining enough blood volume, the addition of extra testing after the health screening had commenced and tests being run in batches (Table 1).

Weight ranged from 200g – 392g. BCS ranged from 3-5/9. Most birds had an optimal BCS (5/9). 3/31 were BCS 4-5/9, which was deemed acceptable. 4/31 birds had a suboptimal BCS (3/9). Three were under 5 months old and one over 12 years old. Nineteen birds had overgrown nails (45.2%). Two birds had deformed keels (laterally deviated), four had foot abnormalities (pododermatitis, missing nails, toe injuries), seven had poor plumage (broken primaries and tail feathers) and three had evidence of skin trauma (scalping injury, carpal abrasions). No birds had visible pigeon pox lesions or ectoparasites. In six birds receiving conscious blood sampling, not enough blood was obtained to perform all tests. No adverse events occurred during the health screen.

Five birds, from two collections, were positive for *T. gallinae*: one at initial swabbing, three at 24 hours and one after 96 hours of incubation. Age ranged from 1 month 18 days to 12 years 7 months. One recently fledged female and one older male were in suboptimal body condition. No other pathogens were identified in these two birds. A budding yeast

overgrowth occurred in 5/42 TV pouches after 24 hours of incubation. Two positive pouches had a high bacterial load and therefore re-inoculation into a fresh TV pouch was performed at day five to increase chances of protozoal survival. Positive TV pouches were submitted for genotyping. Despite re-inoculation, bacterial contamination was still present, rendering them inadequate for genotyping. Of the remaining three samples, two died after stabilate formation. One sample was successfully genotyped as a type C strain.

DISCUSSION

Performing a DRA in order to inform pre-export disease screening is a valuable way to minimize disease risks involved in translocating animals from *ex situ* populations. Previous reintroductions illustrate the detrimental effects that anthropogenic movement of animals can have, such as the accidental introduction of *Batrachochytrium dendrobatidis* into the native Mallorcan ecosystem by reintroduction of captive bred midwife toads (*Alytes muletensis*).[81] A literature search is a commonly utilized tool for creating a DRA,[21,46,53,65] but historically DRAs have not always incorporated prospective data from health screening the source population. This may be due to debate between those who prefer minimal intervention and handling of captive wild animals, and those who would rather take a more proactive approach to screening populations involved in translocations. A balance needs to be reached between the potential stress and injury caused by the screening process, and the risk of subclinical disease going undiagnosed. In this health screen, sampling coincided with routine health examinations, nail trimming or aviary moves, and therefore, not all birds in the EEP population were screened.

Performing a health screen of the source population was a high priority for several reasons. Firstly, EEP pink pigeons are commonly raised by foster parents, usually Barbary doves (*Streptopelia risoria*), and adults are kept in outdoor aviaries, often in mixed species exhibits.[72] This results in frequent interactions with other avian species, including wild birds, and more potential encounters with exotic pathogens. Cross-species transmission of parasites from free-ranging birds to those in captive zoo environments has been reported.[19] Additionally, they are not housed in biosecure aviaries, so staff could inadvertently play a role in disease transmission. Secondly, a prolonged pre-export quarantine period for disease screening is not a viable option. Trauma in pink pigeons is one of the leading causes of mortality.[68] Combined with the preference to export young birds, at a certain time of the year, there is a desire to keep the quarantine period short with minimal repeated handling. Extensive, repeated screening of birds may cause delays or injuries, which could result in failure of translocation or compromise welfare. Thirdly, by screening the population, intermittently shed parasites are more likely to be detected. This is of high importance given the negative fitness effects of inbreeding depression, meaning the recipient population likely has an elevated susceptibility to disease.[55,69,79] Finally, health screening the source population adds an increased evidence-base to the DRA, allowing production of a more targeted pre-export screening protocol.

The most frequently encountered pathogen was *T. gallinae*, with five positive samples detected by the TV pouch method. A similar method, the InPouch *Tritrichomonas foetus* culture pack (TF pouch) has been used previously in free-living pink pigeons.[15] The TV/TF pouch methods are more sensitive than wet-mount methods for detection of trichomonads,[9,54] with the TF pouch detecting over twice as many positive infections in pink pigeons.[15] The results from this health screen support this, with 4/5 samples being positive for trichomonads

only after incubation. The TV pouch is designed for human use, with medium selective for trichomonad growth whilst inhibiting yeasts, fungi and bacteria. After 24 hours of incubation five pouches had yeast overgrowth and two bacterial overgrowth. It is possible the inhibitory agents in the medium are not very effective against avian bacteria and yeasts. No trichomonads were found in pouches with yeast overgrowth, questioning whether this could have inhibited *T. gallinae* growth. Both pouches with bacterial overgrowth were positive for *T. gallinae*, but one only after 96 hours. It is possible that the presence of bacteria competing for the nutritive medium slowed protozoal growth in this pouch.

Genotyping of *T. gallinae* performed on samples from a diverse range of birds in the UK has identified both pathogenic and apathogenic strains.[3,20,49] Of the five positive *T. gallinae* cultures, genotyping was successfully performed on one sample, confirming a C strain, which is believed to be apathogenic.[3] The other positive cultures were bacterially contaminated or died, preventing further analysis. In future when genotyping, it would be advisable to retain subcultures of the original sample. If bacterial contamination is present, subculture using a different medium, supplemented with antibiotics could be considered. Investigation into the effects of yeast on trichomonad growth in the TV pouch would give further insight into the reliability of this screening method.

Trichomonosis can cause lesions in the digestive and upper respiratory tracts leading to starvation, asphyxiation and death. No visible lesions were seen in the positive birds, but two were in suboptimal body condition. The low body condition of these two birds, one recently fledged female and one breeding male, could have been related to their *T. gallinae* infection. However, other husbandry factors or non-infectious diseases leading to a reduction in food intake may also have contributed, such as poor care by foster parents during the neonatal

period, or aggression whilst paired for breeding, both of which have been reported in this species.[68,72] In a retrospective mortality review of 404 European captive pink pigeons only one death was attributed to trichomonosis.[68] This contrasts with a mortality review of the free-living population which reported 52% of deaths (22/43) attributed to this parasite.[17] It is possible that a more virulent *T. gallinae* strain exists in Mauritius, but the Mauritian pink pigeon population could also be less resistant to disease.[17,74] Genotyping of the *T. gallinae* present in Mauritius may further add to our understanding of the pathogenesis of this disease in the free-living population.

Yersinia spp. culture was performed to screen for *Y. pseudotuberculosis*. Within the Yersinia genus, only three species are recognised as pathogenic: *Y. enterocolitica, Y. pestis* and *Y. psuedotuberculosis*.[2] Clinical signs in birds include lethargy, anorexia, diarrhea, respiratory distress, emaciation and sudden death.[30] *Y. pseudotuberculosis* is seasonal, with most clinical cases occurring in winter.[30,68] Screening took place from January to April, when this bacteria would most likely be present. *Y. pseudotuberculosis* is most reliably cultured from organs demonstrating lesions. Fecal culture may also be performed, however shedding can be intermittent. PCR may be a more reliable method for detecting carrier status, however this test was not available in the UK at the time of screening. Although three birds screened positive for *Yersinia* spp. these were not considered pathogenic. One report details *Yersinia kristensenii* as pathogenic in iron-treated mice, but there are no reports of it being a natural pathogen.[63]

Unsporulated coccidial oocysts were found in three birds. Sixteen named species of *Eimeria* have been reported in Columbiformes,[8] for which pathogenicity is debatable as coccidian parasites are found in healthy birds.[35] Under stressful periods coccidian oocysts can

be shed in large numbers and lead to clinical disease, with young birds being particularly susceptible. Evidence shows that exposure to coccidia can help produce immunity, which may be beneficial if later exposed to more pathogenic strains.[35] Apart from the recent identification of *Eimeria mauritiensis* in the pink pigeon, no coccidian parasites have so far been reported in Columbiformes on Mauritius,[8] despite the presence of four other Columbiform species.[16] Therefore, the Mauritian pink pigeon population may have had minimal exposure to coccidian parasites, resulting in poor immunity. The three positive individuals in this study showed no signs of clinical disease. However, translocated birds will undergo transportation, further quarantine and then be released into captive breeding facilities, all of which could increase stress levels and shedding of oocysts. They may have contact with immunologically naive squabs, and since coccidian parasites have a direct life cycle and rapid intracellular multiplication, this could be detrimental to the breeding colony. With the knowledge that individuals are asymptomatically carrying coccidian parasites and that pre-export screening may fail to detect infection due to intermittent shedding, prophylactic treatment prior to export should be considered.

Strongyle ova were found in three birds. In low numbers, these are not clinically significant, and there are many reports of avian species, including Columbiformes, positive on routine screening.[19,40,66] No clinical disease was seen, but transportation stressors could cause increased shedding and build-up of this parasite.

Hemoparasite screening was a priority as *L. marchouxi* infection has been found in Columbiformes, and has caused a reduction in survival of pink pigeons.[14,76] Hemoparasites have been noted to affect host populations by reducing reproductive success[51] and immune response,[52,78] and chronic persistent infection can cause long-term accumulative effects which

impair fitness.[5] All birds screened negative for hemoparasites via PCR and microscopic examination of blood films. PCR was used as well as microscopy as it has a higher sensitivity[43,80] and reduces the likelihood of missing a low-level parasitemia, which is often found in chronically infected birds.[10] False negatives can occur even with PCR due to parasite DNA being present at very low concentrations in DNA samples of the hosts, resulting in intermittent parasite amplification. To minimise the risk of false negatives, samples were screened at least twice. This study spanned over a 10-month period, but screening was performed predominantly during winter months. Previous reports suggest a correlation between hemoparasite infection and vector density,[47] which may suggest winter sampling, when vector numbers are low in the UK and Channel Islands, may not be representative of parasitemia during all seasons. However, recent studies suggest that hemoparasites can persist in winter, possibly due to relapse of infection, and in wild birds, infections may be even more severe at this time than in the breeding season.[28,42]

Pigeon adenovirus is an important infectious disease but was not screened due to lack of availability and limitations of commercial tests in the UK. Pigeon circovirus, pigeon herpesvirus, PPMV-1, APMV-1, *C. psittaci*, *M. avium* and *Salmonella* spp. were not detected during screening. Despite these tests being negative, results will be influenced by the sensitivity and specificity of testing methods and intermittent shedding of pathogens.

CONCLUSION

This health screen was performed to identify potential pathogens currently present in the EEP pink pigeon population. Together with information from a retrospective mortality review[68] and literature search, these findings will contribute to a DRA and creation of an

evidence-based, practical pre-export protocol for translocation of EEP pink pigeons to Mauritius. Although none of the potential pathogens detected were causing overt clinical signs, given the susceptibility of the free-living population to disease, these results are still valuable. Performing a similar health screen on the free-living Mauritian population would further our understanding of disease susceptibility and exposure of this population. Not only would this further guide the pre-export protocol, but as captive bred EEP pink pigeons will have different immunological challenges and pathogen exposure to that of the free-living population, it would help to determine effective preventative medicine protocols to safeguard the translocated animals.

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Table 1: Infectious diseases of Columbiformes that formed part of EEP captive pink pigeon (*Nesoenas mayeri*) health screening: tests, samples required and screening laboratory

Disease	Test	Sample required	Screening Laboratory	Number tested	Positive results
Pigeon circovirus	PCR[a]	Cloacal swab	Animal Genetics[e]	42	0
Pigeon herpesvirus	PCR[a]	Pharyngeal swab	Animal Genetics[e]	42	0
Pigeon paramyxovirus-1	HAIT[b]	100µl serum	APHA[f]	41	0
Avian paramyxovirus-1	HAIT[b]	100µl serum	APHA[f]	36	0
Mycobacterium avium	PCR[a]	Combined pharyngeal & cloacal swab	Animal Genetics[e]	42	0
Chlamydia psittaci	PCR[a]	Combined conjunctival, choanal & cloacal swab	Animal Genetics[e]	42	0
	Serology[c]	50µl of whole blood	In-house laboratory[g,h]	42	0
Salmonella spp.	Culture	Cloacal swab or 3-day pooled fecal	Greendale[i]	23	0
Yersinia spp.	Culture	Cloacal swab or 3-day pooled fecal	Greendale[i]	21	3

Trichomonas gallinae	Culture (TV pouch)[d]	Crop swab	In-house laboratory[g,h]	42	5
	Genotyping	Positive TV pouch	UEA[j]	5	1
Leucocytozoon spp., *Plasmodium* spp., *Haemoproteus* spp.	PCR[a]	100µl whole blood in 96% ethanol	UEA[k]	42	0
	Microscopy	Blood smear	In-house laboratory[g]	42	0
Endoparasites	Zinc sulphate flotation	3-day pooled fecal	In-house laboratory[g,h]	42	6
Ectoparasites	Visual examination	N/A	N/A	42	0
Pigeon poxvirus	Visual examination	N/A	N/A	42	0

[a]Polymerase chain reaction
[b]Hemagglutination-Inhibition test
[c]Immunocomb: Avian *Chlamydophila Psittaci* Ab. Test Kit (Biogal Galed Labs Asc Ltd., Kibbutz Galed, 1924000, Israel)
[d]InPouch® *Trichomonas vaginalis* culture kit (BioMed Diagnotics, Inc. 1388 Antelope Rd, White City, Oregon, 97503, USA)
[e]Animal Genetics: St Austell, Cornwall, PL25 3LB, UK
[f]Animal and Plant Health Agency, Weybridge, Surrey, KT15 3NB, UK
[g]Tests were performed at Bristol Zoo Gardens, Clifton, Bristol BS8 3HA, UK
[h]Tests were performed at Durrell Wildlife Conservation Trust, Les Augrès Manor, La Profonde Rue, Trinity, Jersey, Channel Islands, JE3 5BP
[i]Greendale Veterinary Diagnostics Limited, Woking, GU21 2EP, UK
[j]Testing performed by Dr K. M. Tyler, Norwich Medical School (BMRC), University of East Anglia, Norwich, NR4 7TJ, UK
[k]Testing performed by David S Richardson, Professor in Evolutionary Ecology and Conservation, School of Biological Sciences, Norwich Research Park, University of East Anglia, Norwich, NR4 7TJ, UK