CSIRO PUBLISHING

Pacific Conservation Biology, 2021, **27**, 284–295
https://doi.org/10.1071/PC20049

Screening of diseases in wild exotic birds on Tahiti Island – implications for French Polynesian conservation

Caroline Blanvillain [A,H], *Susana Saavedra* [B], *Tehani Withers* [A], *Jan Votýpka* [C,D], *Karine Laroucau* [E], *Steeve Lowenski* [F] *and David Modrý* [D,G]

[A]Société Ornithologique de Polynésie Française, Manu, BP 7023, Taravao, Tahiti.
[B]INBIMA Invasive Bird Management, PO Box 6009, 38008 S/C de Tenerife, Spain.
[C]Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, 128 44 Prague 2, Czech Republic.
[D]Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic.
[E]ANSES Agence nationale de sécurité sanitaire de l'environnement et du travail, Laboratoire de santé animale, Unité Zoonoses Bactériennes, 14, rue Pierre et Marie Curie, F94706 Maisons-Alfort, France.
[F]UMR1161 Virologie, ANSES Agence nationale de sécurité sanitaire de l'environnement et du travail, 14, rue Pierre et Marie Curie, 94706 Maisons-Alfort, France.
[G]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno Palackeho, 1-3 612 42 Brno, Czech Republic.
[H]Corresponding author. Email: cblanvillain@manu.pf

Abstract. In order to identify potential infectious disease threats to the native avifauna of French Polynesia, an evaluation was performed on the health status of four wild non-native species of birds on the island of Tahiti: common myna, red-vented bulbul, rock dove, and zebra dove. From six locations, a large sample set (151–349 individuals) was tested for several viruses and bacteria, and a small sample set (22–40 birds), because of its proximity to the last remaining population of the critically endangered Tahiti monarch, was checked for more pathogens. Disease-specific screening methods were used. None of the following viruses were found: Newcastle disease virus, avian influenza virus, West Nile virus in 159, 189 and 204 sera; 349 birds examined for poxvirus lesion; avian metapneumovirus and avian adenovirus in 38 and 38 sera; avian polyomavirus in 28 cloacal swabs. The prevalence of bacteria and avian malaria was: *Salmonella* Heidelberg (5% from 21 × 10 pooled samples of intestinal contents), *Chlamydia* spp. (8% on 196 cloacal swabs) including *Chlamydia psittaci* (3%), *Plasmodium relictum* – haplotype GRW04 (2% on 205 DNA), *Haemoproteus* spp. (25% on 205 DNA). In the limited sample set, *Klebsiella pneumoniae*, *Bordetella avium* and *Riemerella columbina* were isolated with a prevalence of 3% each in 40 tracheal swabs. The potential role of introduced birds as vectors of zoonosis in French Polynesia and the crucial finding of *Plasmodium relictum* with several ubiquitous and dangerous pathogens on Tahiti Island should be given the appropriate attention by local authorities and conservationists.

Keywords: avian diseases, biological invasion, eastern Polynesia, endemic bird, invasive species, Oceania.

Received 4 June 2020, accepted 3 January 2021, published online 23 February 2021

Introduction

The introduction of non-native organisms to islands has greatly impacted island biodiversity across the world (Diamond and Veitch 1981; Lowe *et al.* 2000), and the direct impact of invasive species on native fauna has been widely reported in French Polynesia (Blanvillain *et al.* 2003; Ghestemme *et al.* 2019). Due in large part to the advent of non-native species, half of the terrestrial avifauna of French Polynesia has disappeared (Steadman 2006) and of the 40 extant species, 20 are currently threatened by extinction (BirdLife International 2019).

Invasive animal and plant species are not the only exotic organisms that can be introduced to island ecosystems; pathogens may also represent an insidious threat to native island species. The movement of exotic organisms, either intentional or unintentional, is likely to contribute to an increasing emergence of infectious diseases in wild populations (Parker *et al.*

2006), and yet pathogens have been largely ignored by conservation biologists (Altizer *et al.* 2001; Friend *et al.* 2001). A careful review of the literature indicates that pathogenic organisms have been involved in the decline and extinction of several endemic species on oceanic and land-bridge islands (Diamond and Veitch 1981; Wikelski *et al.* 2004). Due to their long-term isolation, island birds are immunologically naïve and may be fatally susceptible to diseases that are considered benign to continental birds (Warner 1968). Although such impacts have been studied in New Zealand (Alley 2002; Howe *et al.* 2012), Hawai'i (Atkinson *et al.* 1995), Galapagos (Harmon *et al.* 1987; Wikelski *et al.* 2004), Cuba (Soares *et al.* 2017), and Mauritus (Swinnerton *et al.* 2005; Bunbury *et al.* 2007), very little has been done on the impact of introduced diseases on French Polynesia's avifauna (Beadell *et al.* 2006). Cunningham (1996) asserts that a failure to address disease risks has the potential to negatively impact conservation programs.

The island of Tahiti is one of Polynesia's largest islands, and it is the main point of entry to French Polynesia for many willing travellers and unwilling passengers. Tahiti is the only home of the Tahiti monarch (*Pomarea nigra*), a critically endangered species with a low breeding potential (Blanvillain *et al.* 2018). Its recovery program is challenging due to predation, competition, and habitat destruction by several exotic species, including two bird species: the red-vented bulbul (*Pycnonotus cafer*) and the common myna (*Acridotheres tristis*) (Blanvillain *et al.* 2003). A further 10 introduced birds are now well established in Tahiti (Thibault and Cibois 2017) including two Columbidae species, the rock dove (*Columbia livia*) and the zebra dove (*Geopelia striata*), that are constantly growing in numbers but are not yet considered to be a threat. Disease screening in introduced wild bird species is crucial in order to assess the potential risks against the Tahiti monarch recovery program and for other endemic birds of French Polynesia.

A limited range of infectious pathogens has been previously identified in the poultry industry in the Society Islands and Marquesas Islands in French Polynesia (Antras 2000, 2007, Table 1 in Supplementary Material). Among them, the ubiquitous avian metapneumovirus, poxvirus, *Mycoplasma*, *Pasteurella* and *Salmonella* could represent a threat to endemic birds. Asymptomatic or lentogenic strains of Newcastle diseases were also detected. A plethora of other pathogens, such as avian influenza virus, West Nile fever virus, columbid herpes virus, avian adenovirus, avian polyomavirus, *Mycobacterium avium* and *Chlamydia psittaci*, which may be a threat for the local native avifauna, remained undetected or have never been surveyed in the domestic or wild bird populations of French Polynesia (Table 2 in Supplementary Material).

In French Polynesia, the blood protozoan parasite *Plasmodium relictum* has already been detected in the northern Marquesan reed-warbler (*Acrocephalus percernis*) on Nuku Hiva Island (Marquesas Archipelago) and in wild exotic birds living on Moorea Island in the Society Archipelago (Beadell *et al.* 2006). However, the situation in Tahiti remains unknown. *Plasmodium relictum* is the most common and cosmopolitan agent of avian malaria and may be lethal for highly susceptible hosts that have evolved in the absence of *Plasmodium*, such as penguins in cold mosquito-free areas (Fix *et al.* 1988) and native Hawaiian birds (Atkinson *et al.* 1995). Introduced bird pox and avian malaria were identified as major threats for Hawai'i's endemic avifauna and are particularly contagious because of their transmission by introduced mosquitoes (Warner 1968; Atkinson *et al.* 1995; van Riper *et al.* 2002). In French Polynesia, *Culex quinquefasciatus*, *Culex sitiens* and *Culicoides belkinii* as well as many other hematophagous insects may represent suitable vectors of blood parasites, especially *Culex annulirostris*, *Aedes polynesiensis* and *Aedes vexans nocturnus* as they principally feed on birds (Bossin 2017). Widely distributed tropical species *C. quinquefasciatus* was introduced into French Polynesia in the 19th century and is now distributed in several archipelagos. *Culex sitiens* was described in the late 20th century in French Polynesia, and *C. annulirostris* may be a native species (Belkin 1962; Rivière 1988; Richard and Cao-Lormeau 2019). While *C. quinquefasciatus* is very well known as a vector of avian malaria, our knowledge about the other mosquito species is rather limited. However, at least two studies demonstrate the possible involvement of *C. sitiens* in the transmission of *Plasmodium juxtanucleare* within birds (Bennett *et al.* 1966; Chen *et al.* 2015; Atkinson *et al.* 2016).

Here we present results from disease screening conducted on a large range of avian pathogens in four wild exotic bird species, sampled at several different locations around Tahiti. The main objective was to obtain baseline data on pathogens that could pose a risk to the conservation of the local endemic avifauna of Tahiti and elsewhere in French Polynesia, in the case of spontaneous, accidental or intentional avian transfer between islands. We also aimed to collect data that could demonstrate the potential impact of introduced birds on human and avian health, in order to raise awareness among stakeholders and decision makers that may be involved in invasive bird control at local and international levels.

Materials and methods

The research reported in the present manuscript has been conducted in an ethical and responsible manner, in full compliance with all relevant local codes of experimentation and legislation. In the absence of a local ethics committee, we used the 'Guidelines to the Use of Wild Birds in Research Standard or Code of Practice' (Gaunt *et al.* 1997). Trapping, mist netting activities and animal handling procedures were developed under agreement with the local animal welfare organisation (Fenua Animalia) and local government. Bird manipulation, anaesthesia and euthanasia were performed by a veterinary doctor. Two exotic columbid species, the zebra dove and the rock dove, and two exotic passerines, the common myna and the red-vented bulbul, were targeted species as they are invasive, present in increasing numbers on Tahiti Island and potentially in contact with local endemic bird populations. The two passerines belong to the '100 of the World's Worst Invasive Alien Species' (Lowe *et al.* 2000). These birds were trapped on Tahiti Island between December 2012 and January 2013. Our aim was to collect 10 specimens of each species at six different locations (i.e. 240 birds).

Capture sites were selected across the island (Fig. 1; Table 1 for coordinates): Paea and Punauuia districts (Site 1), because of their geographical proximity to the last remaining territories of the Tahiti monarch, located in three remote valleys (Blanvillain *et al.* 2018); the international airport of Faa'a (Site 2) due to its potential as a pathway to bring new diseases into Tahiti Island;

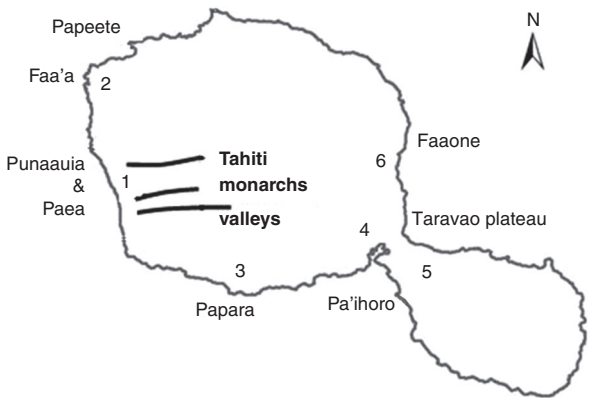

Fig. 1. Capture sites on Tahiti Island.

Papara District (Site 3) near a poultry farm that experienced *Salmonella enterica* Enteritidis outbreaks a few months before our study; the engineered landfills of Pa'ihoro (Site 4) because it attracts large flocks of introduced birds; Taravao plateau (Site 5), located in the smaller, south-eastern part of Tahiti, also near a poultry farm previously contaminated with *Salmonella enterica* Enteriditis; and finally the Faaone District (Site 6), located on the east coast of the main island, near poultry farms.

Some of the targeted bird species were very scarce at some locations but very abundant at others. For smaller species, such as red-vented bulbul and zebra dove, it was necessary to collect more specimens to obtain enough serum to perform all the analysis. Captures were performed using decoy traps or mist-nets. The specifically developed decoy traps used were 50 cm sided square mesh boxes, with a central decoy holding space and four catching compartments (Saavedra *et al.* 2012). Capture depended on individuals triggering a door release mechanism. Mist-netting was performed with a mesh size of 16 mm. The birds were caught in the morning and processed in the afternoon, the day of capture. Birds were killed by cervical dislocation after anaesthesia, a technique considered acceptable (Gaunt *et al.* 1997).

The following samples were collected from each bird: (1) blood (see below); (2) intestinal contents, pooled for 10 birds of the same species and trapped in the same location; and (3) tracheal ($n = 3$) and cloacal ($n = 1$) swabs. Samples collected were screened for a range of infectious pathogens relevant for avian and human health. Pathogens investigated included viruses (Newcastle disease virus, avian influenza virus, West Nile virus, avian pox virus), bacterial pathogens (Chlamydiaceae, *Salmonella* and *Mycobacterium avium*), and vector-borne protozoans causing avian malaria (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*). In addition, 7–10 specimens of each targeted introduced bird species caught in Paea and Punaauia districts were tested for a larger panel of diseases, due to their proximity to the Tahiti monarch's valleys and their potential impact on this species. Indeed, the red-vented bulbul, the common myna and the zebra dove were observed in the Tahiti monarch's territories whereas the four targeted introduced species sleep in common roost sites. Additional scanning included: viruses like avian metapneumovirus, avian polyomavirus and avian adenovirus, as well as bacterial and fungus

Table 1. Sample sites, showing the number of individuals caught, pooled (×10) fresh intestinal contents (IC) and set of swabs (SW) for four introduced bird species

ZD, zebra dove; RD, rock dove; RVB, red-vented bulbul; CM, common myna

Locality	Site no.	Coordinates	ZD	ZD IC	ZD SW	RD	RD IC	RD SW	CM	CM IC	CM SW	RVB	RBV IC	RVB SW	Total birds	Total IC	Total SW
Paea and Punaauia districts	1	17°39′50.65″S, 149°35′45.33″W	19	1	10	10	1	10	12	1	10	21	1	10	62	4	40
Faa'a district	2	17°33′30.99″S, 149°36′38.22″W	0	0	0	13	1	13	19	1	13	0	0	0	32	2	26
Papara district	3	17°45′28.08″S, 149°30′32.93″W	15	1	10	0	0	0	13	1	10	18	1	10	46	3	30
Pa'ihoro	4	17°43′48.46″S, 149°20′10.15″W	0	0	0	33	3	30	17	1	10	24	1	10	74	5	50
Plateau district (Taravao)	5	17°46′34.36″S, 149°15′20.10″W	0	0	0	0	0	0	31	1	10	11	1	10	42	2	20
Faaone district	6	17°40′51.59″S, 149°20′10.15″W	47	3	30	0	0	0	16	1	10	20	1	10	83	5	50
Total			81	5	50	56	5	53	108	6	63	94	5	50	349	21	216

Table 2. Results of pathogen screening showing the number (no.) and the type of sample tested, the methodology used and the no. of positive/total tests for the specific pathogens in four introduced bird species

ZD, zebra dove; RD, rock dove; RVB, red-vented bulbul; CM, common myna

Disease/*Pathogen*	No. and type of sample	Analysis (Norm)	ZD	RD	CM	RVB	Total
Large sample set (Sites 1–6)							
Avian Influenza virus (*Orthomyxovirus*)	189 sera	AGID (NF U 47–013)	0/44	0/54	0/74	0/48	0/189
Newcastle disease virus (*Paramyxovirus*)	151 sera[A]	HI (NF U 47–011)	0/44	0/39	0/61	0/45	0/151
West Nile fever virus (*Flavivirus*)	204 sera	ELISA[E]	0/34	0/50	0/82	0/38	0/204
Bird pox virus (*Poxvirus*)	1 lesion sampled on 349 birds examined[B]	Staining	0/0/81	0/0/56	0/1/108	0/0/94	0/1/349
Salmonella	21 pools of 10 fresh intestinal contents	Bacterial culture + PWB buffer (NF U 47–101)	0/5x10	0/5x10	1/6x10	0/5x10	1/21x10
Chlamydiaceae	196 cloacal swabs	PCR	1/42	8/53	1/60	5/51	15/196
Avian tuberculosis	1 lesion sampled on 210 necropsies[C]	Staining	0/1/50	0/0/50	0/0/60	0/0/50	0/1/210
Pasteurella spp.	8 lesion swabs sampled on 210 necropsies	Bacterial culture + MALDI TOF	0/1	0/1	0/5	0/1	0/8/210
Escherichia coli	8 lesion swabs sampled on 210 necropsies	Bacterial culture + MALDI TOF	1/1	0/1	0/5	0/1	1/8/210
Avian malaria (*Plasmodium* spp.)	205 ADN (blood in ethanol)	Nested PCR	0/47	0/49	4/59	0/50	4/205
Haemoproteosis (*Haemoproteus* spp.)	205 ADN (blood in ethanol)	Nested PCR	0/47	49/49	1/59	2/50	52/205
Leucocytozoon spp.	205 ADN (blood in ethanol)	Nested PCR	0/47	0/49	0/59	0/50	0/205
Limited sample set (Site 1)							
Avian metapneumovirus (*Pneumovirus*)	38/40 sera[D]	ELISA	0/10	0/10	0/9	0/9	0/38
Avian adenovirus (*Adenovirus* type 1)	38/40 sera[D]	AGID	0/10	0/10	0/9	0/9	0/38
Avian polyomavirus (*Papovavirus*)	28 cloacal swabs	PCR	0/7	0/7	0/7	0/7	0/28
Avian *Mycoplasma*	39 tracheal swabs	PCR	0/10	0/9	0/10	0/10	0/39
Pasteurella spp.	40 tracheal swabs	Bacterial culture + MALDI TOF	0/10	0/10	0/10	0/10	0/40
Escherichia coli	40 tracheal swabs		0/10	0/10	0/10	0/10	0/40
Bordetella avium	40 tracheal swabs		1/10	0/10	0/10	0/10	1/40
Klebsiella pneumoniae	40 tracheal swabs		0/10	0/10	0/10	1/10	1/40
Riemerella columbina	40 tracheal swabs		0/10	1/10	0/10	0/10	1/40
Enterococcus faecalis	40 tracheal swabs		0/10	0/10	0/10	1/10	1/40
Staphylococcus intermedius	40 tracheal swabs		0/10	1/10	0/10	0/10	1/40
Aspergillus fumigatus	40 tracheal swabs		0/10	4/10	0/10	2/10	6/40

[A]Eleven insufficient sera and 27 haemagglutination were also observed.
[B]One negative lesion was collected in one CM.
[C]One negative lesion was collected in one ZD.
[D]Two insufficient sera.
[E]The laboratory used was accredited for equine serum.

pathogens that may be contagious because they are present in the respiratory tract (*E. coli*, *Pasteurella*, *Bordetella*, *Klebsiella*, *Riemerella*, avian mycoplasma, *Aspergillus fumigatus* …).

The blood samples were kept at ambient temperature for 2 h until completely coagulated and placed overnight in the refrigerator at 3°C to obtain a good clot retraction. When blood haemolysis was observed, a centrifugation step (at room temperature, 500*g* for 10 min) was included. The resulting serum samples and swabs were stored frozen until assayed. One drop of fresh blood was preserved in an Eppendorf vial, with absolute ethanol, and frozen. A rapid necropsy was performed on each bird to collect digestive tract content. For Bird pox virus and *M. avium*, pathognomonic lesions were checked in all individuals caught. Lesions detected were photographed; representative tissues were fixed in 10% formaldehyde solution and routinely processed for evaluation by a histopathologist, cut at 4 µm and stained with Haematoxylin, Eosin and Safran staining. A lesion's swab was also performed. Birds presenting important internal lesions detected during necropsies were also sampled using lesion swabs. The content of the intestinal tract was mixed in plastic bags grouping 10 conspecific birds of each location on the same sampling day. Samples were cooled down and transferred fresh to the laboratory. According to the diagnostics, samples were submitted to specialised laboratories; methods of analysis, sample and pathogens screened, are summarised in Table 2.

Virus detection

The avian influenza agar gel immunodiffusion test was used for avian influenza virus (Woernle 1966) in accordance with the French AFNOR norm NF U 47–013. The method used is similar to that described by Beard (1970). The laboratory used was accredited COFRAC NF U 47–013 (agreement no. 1–6172).

Screening of sera for flavivirus antibodies was conducted using an ELISA kit (Ayadi *et al.* 2017) designed to detect antibodies against the structural premembrane and envelope proteins of West Nile Virus (ID Screen® West Nile Competition, IDvet, France). The laboratory used was accredited for equine serum. For Newcastle disease virus (paramyxovirus – PMV), the haemagglutination inhibition (HI) test was used for assessing antibody levels in birds (Brown *et al.* 1990). aPMV-1 antibodies were detected by a HI test in accordance with the French AFNOR norm NF U.47–011. Sera from species other than chickens may sometimes cause agglutination of chicken red blood cells. The laboratory used was accredited COFRAC NF U 47–011. For Bird pox virus, pathognomonic lesions were checked in all individuals caught. Lesions detected were fixed in 10% Formaldehyde solution, dehydrated, embedded in paraffin sectioned and photographed. Slices of less than 5 mm of thickness were cut out and included the boundary between the diseased and normal cells. Bollinger inclusions were checked after colouration using Haematoxylin and Eosin staining (Fischer *et al.* 2008), Gram staining (Gram 1884) and Periodic Acid Schiff. Typical lesions consist in hyperplastic epithelium demonstrating ballooning degeneration and intracytoplasmic eosinophilic prominent viral inclusion bodies (Bollinger bodies). For Avian metapneumovirus (AVP) (pneumovirus), an ELISA test was performed. The indirect method was used: microtitre plates are first coated with APV antigen; the test serum is then added, followed by an enzyme labelled anti-chicken conjugate. Enzyme activity was directly proportional to the antibody concentration in the test serum and APV-specific antibody was detected by the colour change seen by a spectrophotometer (Cook 2000). For Avian polyomavirus, the polymerase-chain reaction was used (Phalen *et al.* 1991). For Avian adenovirus, agar gel immunodiffusion test was used as for avian influenza virus (the method is the same as NFU 47–013).

Bacteria detection

For *Salmonella*, bacteriological analyses were performed in accordance with the protocol for *Salmonella* detection in food and animal feedstuffs. For this, 25 g of samples were individually pre-enriched with 225 mL of buffered peptone water broth (Fluka, Sigma Aldrich, France) as prescribed by the French AFNOR norm NF U 47–101. For *M. avium*, pathognomonic lesions were checked in all individuals caught. Lesions detected were photographed; slices of less than 5 mm thickness were cut out, fixed in 10% Formaldehyde solution, including as far as possible the boundary between diseased and normal areas. Acidoalcooloresistant bacteria were checked after colouration using Fite faraco staining in granulomas (Fite 1940). For Chlamydiaceae, genomic DNA from cloacal swabs was extracted using a Qiamp DNA mini kit (Qiagen, France) then analysed using a Chlamydiaceae-specific real-time polymerase chain reaction (PCR) based on the 23 S rRNA fragment (Ehricht *et al.* 2006). All samples with a quantitative cycle above 40 were considered negative. DNA samples that were positive for the 23 S of Chlamydiaceae upon qPCR analysis were further analysed to determine the chlamydia species using *C. psittaci*-, *C. gallinacea*- and *C. avium*- specific qPCR systems (Ménard *et al.* 2006; Zocevic *et al.* 2013; Laroucau *et al.* 2009, 2015). For Avian Mycoplasma, a PCR using ARN 16 S was performed (Fan *et al.* 1995). A culture of bacteria and fungi from a tracheal swab was performed using standard bacteriological/fungal procedures, as used at the Institute Pasteur. The representative bacterial or fungal colonies were characterised with a Bruker MALDI TOF (Matrix Assisted Laser Desorption Ionisation Time of Fly) mass spectrometer.

Blood parasite detection

Haemosporidian parasites in blood samples were detected via nested PCR targeting cytochrome *b* (Hellgren *et al.* 2004). This method enables distinguishing between *Plasmodium*/*Haemoproteus* and *Leucocytozoon* infections. Parasite presence was evaluated through electrophoresis; each sample was tested three times to reduce the number of false–negative results. All positive samples were sequenced using amplification primers. Sequences were edited and checked (Geneious software; ver. 10.0.6) for double peaks, indicating mixed infections. The haplotypes were assigned to known haemosporidian lineages using the MalAvi database (Bensch *et al.* 2009).

Data analysis

For each disease, the prevalence was established with its 95% confidence interval using

$$\varepsilon = 1.96 \times \sqrt{p \times (1 - p)/n},$$

where ε = margin of error, p = prevalence, n = sample size, range = $(p - \varepsilon)$–$(p + \varepsilon)$.

Results

Birds caught

In total, 349 birds were caught during the project (Table 1). All of them were screened for poxvirus lesion, and one lesion that could correspond to avian pox was collected; 189, 189 and 204 complete serum samples were extracted from birds caught all around Tahiti Island, and 38 × 2 from the Punaauia district only. In addition, 21 fresh intestinal contents (pooled from 10 birds) and several sets of swabs (40 tracheal, 196 + 28 cloacal) were collected, including eight lesion swabs and one lesion sample from the 210 necropsies and 205 drops of blood from different birds were collected in alcohol (Tables 1, 2).

Viral diseases

None of the seven viruses tested for were detected (Table 2). One bird was suspected of carrying bird pox lesions but this diagnosis was not confirmed by histology. Nematodes, intrafollicular yeast and bacterial granuloma (g–) were identified.

Bacterial and fungal diseases

Our results are summarised in Table 2. One of 21 pools of 10 intestinal contents revealed to be *Salmonella*-positive. Prevalence was 0.05 (range = 0.02–0.07; n = 210) if we consider the four introduced species; 0.17 (range = 0.26–0.77; n = 60) in common myna only. It was infected with *Salmonella* Heidelberg and collected at Site 3 (Papara District), near a poultry farm that was previously positive to *Salmonella* Enteritidis on eggs. Chlamydiaceae was detected in 15 cases, corresponding to a prevalence of 0.08 (range = 0.06–0.09; n = 196). *Chlamydia psittaci*, the

agent responsible for the ornithose-psittacosis, and *C. avium*, a recently described species, were detected only in rock doves, with a prevalence of 0.11 (range = 0.25–0.19; $n = 53$). All six *C. psittaci*-positive birds were located in the engineered landfills of Pa'ihoro and in the Paea and Punaauia districts. *Chlamydia gallinacea* was detected in four red-vented bulbuls, located in Paea and Punaauia, Papara districts and in Pa'ihoro, and in one common myna specimen caught in the Papara District.

From 210 necropsies, only one avian tuberculosis' pathognomonic lesion was suspected. It was an intestinal coccidiosis with fungus granulomas and also contained intraluminal cestodes. None of the 40 individuals sampled in the Paea and Punaauia districts were affected by *Pasteurella* or *Escherichia coli*. However, from 210 necropsies, one sick zebra dove, collected in Papara, was positive for *E. coli*, with a strain different from O1K1, O2K2 and O78K80, the strain considered as pathogenic in bird species (Dho-Moulin and Fairbrother 1999). The prevalence was 0.0046 (range = 0.0045–0.0048; $n = 210$). This strain was not typed by the laboratory. With respect to the additional pathogens tested in the Paea and Punaauia districts, one zebra dove was infected by *Bordetella avium*, with a prevalence of 0.03 (range = 0–0.07; $n = 40$). One red-vented bulbul was carrying *Klebsiella pneumoniae* with a prevalence of 0.03 (range = 0–0.07; $n = 40$). *Riemerella columbina* was isolated in one rock dove in Paea and Punaauia, with a prevalence of 0.05 (range = 0–0.14; $n = 20$) in columbid species. The 39 PCR tests on tracheal swabs were negative for *M. galliseptica* and *M. synoviae*.

Fungus diseases

Aspergillus fumigatus was isolated from two red-vented bulbuls and four rock doves with a prevalence of 0.15 (range = 0.4–0.26; $n = 40$).

Blood parasites

The causative agent of avian malaria, *P. relictum* (haplotype GRW04, according to the MalAvi database) was found in Tahiti. It was detected by nested PCR in four common myna specimens. Its overall prevalence is 6.8% (range = 0.00–0.13; $n = 59$) in common myna, 2.0% if all four introduced species are considered (range = 0.00–0.04; $n = 205$). Infected mynas were located in Faone, Pa'ihoro and Taravao plateau. The second identified blood parasite genus was *Haemoproteus* with a prevalence of 0.25 (range = 0.22–0.28; $n = 205$) in all species but a 100% prevalence in rock doves ($n = 49$). Rock doves were caught in Paea and Punaauia districts, Faone and Pa'ihoro locations only. It was also found in two red-vented bulbuls with a prevalence of 4% (range = 0.00–0.09; $n = 50$) (one in Paea and Punaauia Districts, one in Papara District) and in one common myna caught in Papara District with a prevalence of 2% (range = 0.00–0.05; $n = 59$). The most dominant haplotype (HAECOL) was found in 51 cases (rock dove, common myna, and red-vented bulbul), while the haplotype CCF2/H032 was identified only in one red-vented bulbul. Information about the detected lines was sent to the MalAvi database.

Distribution of diseases around Tahiti Island

Chlamydia psittaci was found mostly in the Pa'ihoro engineered landfill (site 4) whereas other Chlamydia were more widespread; *S. enterica* Heidelberg and *E. coli* were detected only at the Papara district (site 3), around a very active poultry farm. *Plasmodium relictum* was restricted to the south part of Tahiti (sites 4–6) whereas the distribution of *Haemoproteus* was confirmed in all districts in the northern part of the island (sites 1–4), but rock doves were collected only in sites 1, 2 and 4 (see Table 3 in the Supplementary Material).

Discussion

Although none of the seven viruses tested for were detected, several potential bacterial pathogens and two vector-borne protozoans that cause avian haemosporidiosis were found during our study on wild introduced birds living in Tahiti: *Salmonella*, Chlamydiaceae, *E. coli*, *P. relictum*, *Haemoproteus* spp. in the large sample set; *Riemerella*, *Bordetella* and *Klebsiella* in the small sample set.

Reliability of results

The absence of a positive result is not an indication that these pathogens do not exist within the species sampled (Cunningham 1996). According to Rosner (2010), approximately 54 individuals should be analysed to reliably indicate that a population is negative for the presence of the relevant pathogen but 25 individuals could be sufficient if sampling is very difficult to achieve. The FAO recognises that 191 individuals should be sampled in order to detect a disease with a prevalence of 5% if sensibility and specificity of the test used are of 98% (Sergeant 2019). This suggests that our data give reliable results for pathogens with a high prevalence, over 2%, 5%, 10%, 15%, depending on the specificity and sensibility of the tests used and the result considered (large versus limited sample sets: species versus multispecies levels).

Virus

Although none of the viruses tested for were detected, some of them may be present, but with a low prevalence. For prevalence <2%, the 95% upper bound confidence interval for the real prevalence (P) of the disease can be computed from the equation $n = 3/P$, where n is the sample size (Arya *et al.* 2012). This suggests that the 95% upper bound for the real prevalence could be 1.6% for avian influenza, 1.5% for West Nile fever, 1.9% for Newcastle Disease. This 95% upper bound could be 7.9% for avian metapneumovirus and avian adenovirus and 10.7% for the avian polyomavirus, much more than the 2% limit, but with a probability <5% to have no positive result in the 28/38 samples tested with such prevalence rates. However the prevalence of avian metapneumovirus in French Polynesia was only 1.2% in 640 captive chickens (Antras 2000), so it is very unlikely for it to be found at a higher prevalence in free-ranging birds whereas avian polyomavirus remains extremely rare in wild birds (Phalen 1998). No data are available on the adenovirus type 1 in French Polynesia but three adenovirus sequences of pigeons (including this type) were detected in 9 of 15 feral rock dove collected in Australia, demonstrating that these viruses have been introduced to Australia and should now be considered enzootic (Vaz *et al.* 2020). It is unlikely that avian influenza and West Nile fever, which affect many species, could remain at a low prevalence in respect to their high

contagiousness. Those two viruses are probably absent from French Polynesia. Newcastle disease was also not detected. The asymptomatic strain found previously could correspond to vaccination residual (Antras 2000, 2007). If bird pox remained undetected, the 95% upper bound for its real prevalence is 0.8% and several pathognomonic lesions were observed in uncaught red-vented bulbul and zebra doves (including in the Paea and Punaauia districts) outside the sampling period. Those lesions were also observed in 2014 in zebra doves living in Ua Pou (Marquesas Archipelago) and in 2013 on several Polynesian ground doves (*Alopecoenas erythroptera*) living in Rangiroa Island (Tuamotu Archipelago) (C. Blanvillain, pers. obs.). This disease is therefore present in French Polynesia, but with a low prevalence. It remains a potential risk for the Polynesian avifauna because of its impact on Hawai'i's endemic avifauna and its transmission by mosquitoes (Kligler *et al.* 1929; Warner 1968; van Riper *et al.* 2002). Moreover, the episode of bird pox coincided with the extinction of the Rangiroa Polynesian ground dove (Blanvillain *et al.*, unpubl. data). In Hawai'i, only two of the three strains of pox virus found in the islands threaten native forest birds and native passerines do not appear to be highly susceptible to fowlpox (Jarvi *et al.* 2008; Atkinson *et al.* 2012).

Bacteria

Chlamydiosis has never previously been reported in French Polynesia. Our data show that 15% of rock dove, 10% of red-vented bulbul, 2% of zebra dove and 2% of common myna were carrying Chlamydiaceae on the island of Tahiti. Intermittent shedding reported for chlamydia (Sachse *et al.* 2015) suggests a higher prevalence. *Chlamydia psittaci* is primarily associated with disease in parrots (Psittaciformes) and pigeons (Columbiformes). Mass mortality events have also occurred in wild gulls (*Larus* spp.), waterfowl (Anseriformes) and poultry (Galliformes). Other bird species are less commonly infected but can act as reservoir hosts of disease for humans and birds. Infections in passerine birds (Passeriformes) are rarely recorded and mortality is usually low in these birds while this organism is an important zoonosis (Andersen and Vanrompay 2000). To date, no clinical signs have been reported in chickens carrying *C. gallinacea* and in most of the *C. avium* pigeon carriers. However, *C. avium* seems to cause respiratory disease in psittacines and in some pigeons (Sachse *et al.* 2012; Pisanu *et al.* 2018). Mynas infected with *Salmonella* were collected in Papara. As for the previous bacterial disease, only direct contamination is reported. We propose treating these two diseases as low risk for the Tahiti monarch whereas they could potentially affect endemic pigeons (that may drink in open water) or even parrots, particularly if *C. psittaci* is also present or reaches one of the last Polynesian islands containing *Vini* spp., such as Rimatara, Ua Huka or Rangiroa (Thibault and Cibois 2017). All the other bacterial diseases screened in the large sample set (*S. enteritica pullorum,* avian tuberculosis, avian mycoplasmose, colibacillosis and pasteurellosis) should be considered as low risk diseases because of their direct contamination pathway, absence or low prevalence. Three bacterial diseases were detected in the Paea and Punaauia districts. Riemerellosis could also be transmitted by mosquitoes and fly bites, but it is a species-specific disease and was detected only in rock dove (Vancanneyt *et al.* 1999). Its potential transmission to local endemic pigeons, such as the grey-green fruit-dove (*Ptilinopus purpuratus*) in Tahiti should be investigated. In contrast, *Bordetella avium* is widespread in many North America species of wild birds from parrots to Passeriformes, sometimes with a high prevalence (Raffel *et al.* 2002). Its pathogenicity is demonstrated in many species and it is transmitted via water or litter contamination where it can remain virulent for 1–6 months in litter and up to 7 weeks in water. This mode of contamination reduces the risk of Tahiti monarch being affected because this arboreal species was rarely observed to drink in open water. *Klebsiella pneumoniae* is a ubiquitous germ, found in many species from passerine to psittacine birds (Davies *et al.* 2016). Contagion is direct; therefore its risk is also reduced.

Fungus

The aspergillosis has a worldwide distribution and is ubiquitous in nature. It is contracted as the result of inhalation of spores or by oral ingestion. The disease usually develops when that individual has a compromised immune system, or is stressed, so it is not considered to be a contagious disease (Raper and Fennell 1965). Recently, this aspergillosis killed 7 of the 200 remaining kakapo, a critically endangered parrot living in New Zealand (Anon. 2020) but its susceptibility was related to the ground-dwelling habits of the species.

Blood parasites

The crucial finding of the aetiologic agent of avian malaria, *P. relictum*, on Tahiti Island, albeit in a very small number of tested individuals, is serious and should be given the appropriate attention. Its distribution seems restricted to the south part of the island so far, whereas the Tahiti monarch lives on the west coast of Tahiti. Its introduction to the remote Hawaiian Islands has been implicated in the widespread decline and the possible extinction of many species within the endemic avian radiation of honeycreepers (Warner 1968; van Riper *et al.* 1986). While mortality in introduced bird species was negligible, mortality in many of those endemic species ranged from 50 to 90% (Jarvi *et al.* 2001). However, several other island birds seem to tolerate this infection (Valkiūnas *et al.* 2018) and *P. relictum* has not had the same impact on Hawai'i's monarch flycatcher, the elepaio, as it has on native honeycreepers (Vanderwerf *et al.* 2006). There is evidence based on the wide altitudinal range of the three species of elepaio in Hawai'i and the persistence of this species in low-elevation valleys on Oahu, where prevalence of infection with *P. relictum* is close to 100% (Vanderwerf *et al.* 2006). This suggests that monarch flycatchers may have some natural tolerance/resistance to the disease. Mortality during malaria infection also possibly reflects long isolation from malarial parasites, 4 million years ago in the case of the honeycreepers (Fleischer and McIntosh 2001; Beadell *et al.* 2006), but only 1.5–1.9 million years ago in the case of the elepaio (Vanderwerf 2007). Isolation is also a characteristic of Polynesian endemic avifauna. Monarch species living in Marquesas were isolated for 4 million years ago from continents (Cibois *et al.* 2004) whereas the Society Island monarch lineage has been isolated for less than 2 million years ago (Thibault and Cibois 2017). Several other genera present in French Polynesia have been isolated for several million years, such as *Aechmorhynchus*

Ptilinopus, *Ducula* and *Alopecoenas* (Jønsson *et al.* 2011; Cibois *et al.* 2012, 2014, 2017). The dispersal of the ancestor of *Vini peruviana* to Polynesia probably occurred in the early Pleistocene, between 2.5 and 1.8 million years ago (Schweizer *et al.* 2015). Warblers and kingfishers colonised French Polynesia less than 1 million years ago (Cibois *et al.* 2011; Andersen *et al.* 2015). Northern Marquesan reed-warblers are still numerous on Nuku Hiva despite *P. relictum* being detected in their blood (Beadell *et al.* 2006). However, two species of warblers have already gone extinct recently in French Polynesia; including one species on Moorea Island where *P. relictum* is present, and introduced pathogens remain on the suspect list (Cibois *et al.* 2011). Currently, only four lineages (SGS1, GRW4, GRW11, and LZFUS01) have been linked to *P. relictum* based on morphological characters of their blood stages (Hellgren *et al.* 2015). Our finding of the haplotype GRW4 in common myna confirms the previous statement that within the oceanic zoogeographic region, only worldwide distributed GRW4 occurs (Beadell *et al.* 2006; Hellgren *et al.* 2015; Valkiūnas *et al.* 2018). In Australia Clark *et al.* (2015) found no evidence of GRW04 infecting Australian native species. This suggests a poor ability to infect native species (Medeiros *et al.* 2013), a limited vector distribution (Santiago-Alarcon *et al.* 2012), or a more worrying explanation is that native birds do not survive when infected with GRW04 (Clark *et al.* 2015). The *Haemoproteus* haemoparasites have traditionally been considered incidental and relatively non-pathogenic vertebrate blood parasites (Padilla and Parker 2008). However, several *Haemoproteus*-induced deaths have been reported over the past 40 years, mainly among psittacines of Australia kept in European aviaries (Olias *et al.* 2011). This raises an important issue concerning the lack of knowledge about the pathogenicity and transmissibility of particular haemosporidian parasites. We are the first to report *Haemoproteus* spp. in French Polynesia (Clark *et al.* 2014). Whereas *Haemoproteus* spp. CCF2 lineage infected non-closely related bird species (Garcia-Longoria *et al.* 2019), the haplotype HAECOL1 was considered to be specific to Columbiformes (Earle and Little 1993). According to the MalAvi database (2020), this haplotype has been detected in India also from *Garrulax delesserti* and *Alcippe poioicephala* (both bird species belong to Timaliidae) (Gupta *et al.* 2019); thus our findings do not represent the first record of this pigeon/dove *Haemoproteus* species from non-columbiform bird hosts.

Zoonosis

We are the first to report the potential role of introduced birds as vectors of zoonosis in French Polynesia, particularly with our results about Salmonellosis, Chlamydiosis and Klebsiellosis. Chlamydiosis has never previously been reported in French Polynesia. Although the zoonotical potential of *C. psittaci* is clearly established, the pathogenicity of *C. avium* and *C. gallinacea*, two newly described species, has yet to be investigated (Sachse *et al.* 2014, 2015). Their excretion in faeces raises a public health problem because of the proliferation of the four introduced bird species sampled in Tahiti Island around homes, schools, restaurants and in the engineered landfills of Pa'ihoro. Mynas infected with *Salmonella* were collected in Papara, they were carrying *Salmonella* Heidelberg, responsible for severe human health issues in the USA (Gieraltowski *et al.* 2016). Carriers may rapidly spread the organism via waterways, when visiting human habitation or in open restoration space. *Klebsiella pneumoniae* in humans is an opportunistic pathogen showing multiple antibiotic resistance strains, becoming a problem for public health (Davies *et al.* 2016).

Level of endangerment of those pathogens for native birds

The aim of this study was to establish a baseline disease monitoring in order to anticipate new health problems emerging for the Tahiti monarch recovery program and possibly other endemic birds living on this island and elsewhere in French Polynesia. The pathogens found on Tahiti Island can be ranked according to their level of threat. Ubiquitous and potentially severe diseases transmitted by insects, because they are particularly contagious, are the most dangerous ones. They are followed by ubiquitous diseases transmitted by direct contagion. In French Polynesia, avian malaria and bird pox are present and should be considered as the more dangerous diseases as they could be transmitted by insects already present in French Polynesia (Beadell *et al.* 2006; Bossin 2017): *P. relictum* can be transmitted only by culicine moquitoes (Beadell *et al.* 2006), most likely *C. quinquefasciatus* or other indigenous species of *Culex* in Tahiti. *Culicoides* are potential vectors of lineage CCF2/HO32, but not *Haemoproteus columbae* (HAECOL). *Haemoproteus columbae* and other columbiform species of *Haemoproteus* are transmitted by ectoparasitic hippoboscid flies (Santiago-Alarcon *et al.* 2012). Any blood-feeding arthropod can transmit avian pox (van Riper and Forrester 2007). They are followed by ubiquitous and severe pathogens such as *Salmonella* sp., Chlamydiaceae, *E. coli*, *Bordetella*, and *Klebsiella* but their direct contagion pathways and their low prevalence reduce the risk of infecting the Tahiti monarch or other endemic bird species. Indeed, direct contamination is less likely to affect wild birds than poultry or captive birds. Moreover, the four exotic bird species studied have the habit of sleeping in common roost sites (C. Blanvillain, pers. obs.). This behaviour may favour direct and interspecific infections between them. In contrast, the Tahiti monarch is territorial (the other birds species are territorial or does not aggregate with the four introduced species studied during night). However, due to the invasive potential of the four exotic species sampled in our study, direct pathogens may one day be in contact with native birds and interisland dispersion of birds and diseases is at risk.

Recommendation

Detection of *P. relictum*, a blood parasite highly dangerous for native avian populations, deserves more attention and warrants further investigation into the effects of avian malaria on isolated avifauna outside of Hawai'i. This pathogen and the bird-pox lesions observed in several introduced wild birds in Tahiti, suggest that myna and bulbul control, performed in Paea and Punauia districts since 2012 in order to protect the Tahiti monarch (Blanvillain *et al.* 2020), could also have protected this species from more insidious disease contamination. The present bird control, which focuses on common myna and red-vented bulbul only, should probably be extended to several other exotic species and more communes around Tahiti in order to preserve public health and other endemic bird species such as the grey-green fruit-dove, still common but restricted to Tahiti and

Moorea Island. Introduced birds living on islands that are the last refuge of naive taxa should be sampled and tested for several diseases identified during our study, such as birdpox and chlamydia, in order to prevent acute mortality. Considering all threats present on Tahiti for the Tahiti monarch, including diseases, the establishment of a second population on another island would serve as a 'back up' scenario, and could ensure species survival. There are many examples of translocation efforts in eastern Polynesia that have been successful (Blanvillain *et al.* 2001; McCormack 2007; Robertson and Saul 2007). But translocation may also transfer hidden pathogens (Parker *et al.* 2006). If other endangered land bird species are living in the island candidates for this future translocation, avian malaria and bird pox should be checked in Tahiti monarchs and in the 'future' island before this translocation. As bird pox has an incubation period of 4–14 days and avian malaria presents intermittent shedding, it may be important to quarantine the monarchs as part of the translocation protocol or to verify the absence of those two pathogens in monarchs or in birds living within their territories beforehand. Several other pathogens, such as parasites (*Trichomonas*, ticks) or faecal bacteria (Yersiniose and campylobacterioses) were not included in our study. Blood smears prepared, fixed and stained with Giemsa for general diagnosis of blood parasites might have revealed whether filarial worms or avian trypanosomes are present or detected haemosporidian parasites that might not have amplified. They could also have been used to confirm if more than one morphological species of *Haemoproteus* is present. They should be included in any preliminary samples collected from introduced birds living within monarch territories in order to improve preparation for any future translocation. Screening or treatment against ectoparasites or internal parasites such as nematodes, trematodes, coccidiosis, capillariosis, should be carefully weighted. More data and preparation are needed to reduce both the risk for the land birds living in the 'future' island and the number of procedures required for monarchs, meaning that ideally only a faecal and physical exam, a blood smear, baseline haematology (Parker *et al.* 2006) and some limited treatments should be necessary for any future translocation.

Conclusion

Our results confirm that common myna and other introduced species are a source of harmful parasites and diseases. The potential role of introduced birds as vectors of zoonosis in French Polynesia and the risks towards native birds suggest that stakeholders should improve: their efforts to reduce the numbers of introduced birds in key areas; biosecurity measures to prevent interisland bird transfers; and public awareness. These results show that diseases may also be a serious issue in other countries in the Pacific and should be dealt with, for the protection of their endangered and vulnerable avifauna.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by the Minister of Agriculture (2013) and the ERD Funds under grant project OPVVV number 16_019/0000759. We acknowledge Valérie Roy and Valérie Antras (Service du Développement Rural) for their help in setting up the sampling protocols and for their assistance; the Tahitian chicken farmers (Paul, Renée and Georges); the TEP and Pa'ihoro engineered landfill employees (notably Jonny); Marc Desclaux (ADT) in Faa'a Airport. We also acknowledge the IFREMER centre of Vairao (Tahiti), and Rémy Tanguy, Rachid Aaziz, Fabien Vorimore, Philippe Branaa, the Malardé institute and Bio Chêne Vert for their support and encouragement during the project. Finally, we thank Liv Grant, Valérie Roy and Jacques Boubee for their critical reading of the manuscript.

References

Alley, M. R. (2002). Avian wildlife diseases in New Zealand: current issues and achievements. *New Zealand Veterinary Journal* **50**, 118–120. doi:10.1080/00480169.2002.36287

Altizer, S., Foufopoulos, J., and Gager, A. (2001). Conservation and diseases. *Encyclopedia of Biodiversity* **2**, 109–126.

Andersen, A. A., and Vanrompay, D. (2000). Avian chlamydiosis. *Revue scientifique et technique (International Office of Epizootics)* **19**, 396–404.

Andersen, M. J., Shult, H. T., Cibois, A., Thibault, J. C., Filardi, C. E., and Moyle, R. G. (2015). Rapid diversification and secondary sympatry in Australo-Pacific kingfishers (Aves: Alcedinidae: *Todiramphus*). *Royal Society Open Science* **2**, 140375. doi:10.1098/RSOS.140375

Anon. (2020). Aspergillosis threatens endangered kakapo, "the world's fattest parrot". Available at https://www.aspergillus.org.uk/blog/aspergillosis-threatens-endangered-kakapo-"-world's-fattest-parrot". [accessed 20 May 2020].

Antras, V. (2000). Enquête Zoo-sanitaire en Polynésie française. Tahiti, Polynésie française. Service du Développement Rural, Papeete.

Antras, V. (2007). Surveillance sanitaire de la population aviaire *Gallus gallus* de Polynésie française en 2007. *Bulletin épidémiologique, santé animale et alimentation* **43**, 14–16.

Arya, R., Antonisamy, B., and Kumar, S. (2012). Sample size estimation in prevalence studies. *The Indian Journal of Pediatrics* **79**, 1482–1488. doi:10.1007/S12098-012-0763-3

Atkinson, C. T., Woods, K. L., Dusek, R. J., Sileo, L. S., and Iko, W. M. (1995). Wildlife disease and conservation in Hawaii: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected iiwi (*Vestiaria coccinea*). *Parasitology* **111**, S59–S69. doi:10.1017/S003118200007582X

Atkinson, C. T., Wiegand, K. C., Triglia, D., and Jarvi, S. I. (2012). Reversion to virulence and efficacy of an attenuated canarypox vaccine in Hawai'i 'Amakihi (*Hemignathus virens*). *Journal of Zoo and Wildlife Medicine* **43**, 808–819. doi:10.1638/2011-0196R1.1

Atkinson, C., Utzurrum, R., Seamon, J., Schmaedick, M., LaPointe, D., Apelgren, C., Egan, A. and Watcher-Weatherwax, W. (2016). Effects of climate and land use on diversity, prevalence, and seasonal transmission of avian hematozoa in American Samoa. Technical Report HCSU-072. Hawai'i Cooperative Studies Unit, University of Hawai'i at Hilo, HI, USA.

Ayadi, T., Hammouda, A., Poux, A., Boulinier, T., Lecollinet, S., and Selmi, S. (2017). Evidence of exposure of laughing doves (*Spilopelia senegalensis*) to West Nile and Usutu viruses in southern Tunisian oases. *Epidemiology and Infection* **145**, 2808–2816. doi:10.1017/S0950268817001789

Beadell, J. S., Ishtiaq, F., Covas, R., Martim, M., Warren, B. H., Carter, T. A., Bensch, S., Graves, G. R., Jhala, Y. V., Peirce, M., Rahmani, A., Fonseca, D., and Fleischer, R. (2006). Global phylogeographic limits of Hawaii's avian malaria. *Proceedings of the Royal Society B: Biological Sciences* **273**, 2935–2944. doi:10.1098/RSPB.2006.3671

Beard, C. W. (1970). Demonstration of type-specific influenza antibody in mammalian and avian sera by immunodiffusion. *Bulletin of the World Health Organization* **42**, 779.

Belkin, J. N. (1962). The mosquitoes of the South Pacific (Diptera, Culicidae). Berkely, University of California Press.

Bennett, G. F., Warren, M., and Cheong, W. H. (1966). Biology of the Malaysian strain of *Plasmodium juxtanucleare* Versiani and Gomes,

1941. II. The sporogonic stages in *Culex* (*Culex*) *sitiens* Wiedmann. *The Journal of Parasitology* **52**, 647–652. doi:10.2307/3276421

Bensch, S., Hellgren, O., and Pérez-Tris, J. (2009). MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome *b* lineages. *Molecular Ecology Resources* **9**, 1353–1358. doi:10.1111/J.1755-0998.2009.02692.X

BirdLife International (2019). IUCN Red List for birds. Available at http://www.birdlife.org [accessed 6 March 2019].

Blanvillain, C., Thorsen, M., and Sulpice, R. (2001). Reintroduction of the Marquesan imperial-pigeon to Ua Huka Island. *Re-introduction News* **20**, 18.

Blanvillain, C., Salducci, J. M., Tutururai, G., and Maeura, M. (2003). Impact of introduced birds on the recovery of the Tahiti flycatcher (*Pomarea nigra*), a critically endangered forest bird of Tahiti. *Biological Conservation* **109**, 197–205. doi:10.1016/S0006-3207(02)00147-7

Blanvillain, C., Ghestemme, T., Withers, T., and O'Brien, M. (2018). Breeding biology of the Critically Endangered Tahiti monarch *Pomarea nigra*, a bird with a low productivity. *Bird Conservation International* **28**, 606–619. doi:10.1017/S095927091700048X

Blanvillain, C., Ghestemme, T., Saavedra, S., Yan, L., Michoud-Schmidt, J., Beaune, D., and O'Brien, M. (2020). Rat and invasive bird control to save the Tahiti monarch (*Pomarea nigra*), a critically endangered island bird. *Journal for Nature Conservation* **55**, 125820. doi:10.1016/J.JNC.2020.125820

Bossin, H. (2017). 'Principaux Moustiques Nuisibles Observés en Polynésie Française.' (Fiches de l'Institut Malardé: Papeete.)

Brown, J., Resurreccion, R. S., and Dickson, T. G. (1990). The relationship between the hemagglutination–inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. *Avian Diseases* **34**, 585–587. doi:10.2307/1591248

Bunbury, N., Barton, E., Jones, C. G., Greenwood, A. G., Tyler, K. M., and Bell, D. J. (2007). Avian blood parasites in an endangered columbid: *Leucocytozoon marchouxi* in the Mauritian pink pigeon *Columba mayeri*. *Parasitology* **134**, 797–804. doi:10.1017/S0031182006002149

Chen, T. H., Aure, W. E., Cruz, E. I., Malbas, F. F., Jr, Teng, H. J., Lu, L. C., *et al.* (2015). Avian *Plasmodium* infection in field-collected mosquitoes during 2012–2013 in Tarlac, Philippines. *Journal of Vector Ecology* **40**, 386–392. doi:10.1111/JVEC.12178

Cibois, A., Thibault, J. C., and Pasquet, E. (2004). Biogeography of eastern Polynesian monarchs (*Pomarea*): an endemic genus close to extinction. *Condor* **106**, 837–851. doi:10.1093/CONDOR/106.4.837

Cibois, A., Beadel, J. S., Graves, G. R., Pasquet, E., Slikas, B., Sonsthagen, S. A., Thibault, J. C., and Fleischer, R. C. (2011). Charting the course of reed-warblers across the Pacific islands. *Journal of Biogeography* **38**, 1963–1975. doi:10.1111/J.1365-2699.2011.02542.X

Cibois, A., Dekker, R., Pasquet, E., and Thibault, J. C. (2012). New insights into the systematics of the enigmatic Polynesian sandpipers *Aechmorhynchus parvirostris* and *Prosobonia leucoptera*. *Ibis* **154**, 756–767. doi:10.1111/J.1474-919X.2012.01260.X

Cibois, A., Thibault, J. C., Bonillo, C., Filardi, C. E., Watling, D., and Pasquet, E. (2014). Phylogeny and biogeography of the fruit doves (Aves: Columbidae). *Molecular Phylogenetics and Evolution* **70**, 442–453. doi:10.1016/J.YMPEV.2013.08.019

Cibois, A., Thibault, J. C., Bonillo, C., Filardi, C. E., and Pasquet, E. (2017). Phylogeny and biogeography of the imperial pigeons (Aves: Columbidae) in the Pacific Ocean. *Molecular Phylogenetics and Evolution* **110**, 19–26. doi:10.1016/J.YMPEV.2017.02.016

Clark, N. J., Clegg, S. M., and Lima, M. R. (2014). A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *International Journal for Parasitology* **44**, 329–338. doi:10.1016/J.IJPARA.2014.01.004

Clark, N. J., Olsson-Pons, S., Ishtiaq, F., and Clegg, S. M. (2015). Specialist enemies, generalist weapons and the potential spread of exotic pathogens: malaria parasites in a highly invasive bird. *International Journal for Parasitology* **45**, 891–899. doi:10.1016/J.IJPARA.2015.08.008

Cook, J. K. A. (2000). Avian rhinotracheitis. *Revue Scientifique et Technique, Office International des Epizooties* **19**, 602–613. doi:10.20506/RST.19.2.1233

Cunningham, A. A. (1996). Disease risks of wildlife translocations. *Conservation Biology* **10**, 349–353. doi:10.1046/J.1523-1739.1996.10020349.X

Davies, Y. M., Cunha, M. P. V., Oliveira, M. G. X., Oliveira, M. C. V., Philadelpho, N., Romero, D. C., *et al.* (2016). Virulence and antimicrobial resistance of *Klebsiella pneumoniae* isolated from passerine and psittacine birds. *Avian Pathology* **45**, 194–201. doi:10.1080/03079457.2016.1142066

Dho-Moulin, M., and Fairbrother, J. M. (1999). Avian pathogenic *Escherichia coli* (APEC). *Veterinary Research, BioMed Central* **30**, 299–316.

Diamond, J., and Veitch, C. R. (1981). Extinctions and introductions in the New Zealand avifauna: cause and effect? *Science* **211**, 499–501. doi:10.1126/SCIENCE.211.4481.499

Earle, R. A., and Little, R. M. (1993). Haematozoa of feral rock doves and rock pigeons in mixed flocks. *South African Journal of Wildlife Research* **23**, 98–98.

Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., and Sachse, K. (2006). Optimized DNA microarray assay allows detection and genotyping of single PCR amplifiable target copies. *Molecular and Cellular Probes* **20**, 60–63. doi:10.1016/J.MCP.2005.09.003

Fan, H. H., Kleven, S. H., Jackwood, M. W., Johansson, K. E., Pettersson, B., and Levisohn, S. (1995). Species identification of avian mycoplasmas by polymerase chain reaction and restriction fragment length polymorphism analysis. *Avian Diseases* **39**, 398–407. doi:10.2307/1591885

Fischer, A. H., Jacobson, K. A., Rose, J., and Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harbor Protocols* **5**, 4986. doi:10.1101/PDB.PROT4986

Fite, G. L. (1940). The fuchsin–formaldehyde method of staining acid-fast bacilli in paraffin sections. *The Journal of Laboratory and Clinical Medicine* **25**, 743–744.

Fix, A. S., Waterhouse, C., Greiner, E. C., and Stoskopf, M. K. (1988). *Plasmodium relictum* as a cause of avian malaria in wild-caught Magellanic penguins (*Spheniscus magellanicus*). *Journal of Wildlife Diseases* **24**, 610–619. doi:10.7589/0090-3558-24.4.610

Fleischer, R. C., and McIntosh, C. E. (2001). Molecular systematics and biogeography of the Hawaiian avifauna. *Studies in Avian Biology* **22**, 51–60.

Friend, M., McLean, R. G., and Joshua Dein, F. (2001). Disease emergence in birds: challenges for the twenty-first century. *The Auk* **118**, 290–303. doi:10.1093/AUK/118.2.290

Garcia-Longoria, L., Marzal, A., De Lope, F., and Garamszegi, L. (2019). Host–parasite interaction explains variation in the prevalence of avian haemosporidians at the community level. *PloS One* **14**, e0205624. doi:10.1371/JOURNAL.PONE.0205624

Gaunt, A. S., Oring, L. W., Able, K. P., Anderson, D. W., Baptista, L. F., Barlow, J. C., and Wingfield, J. C. (1997). 'Guidelines to the Use of Wild Birds in Research.' (Ornithological Council: Washington, DC.)

Ghestemme, T., Matohi, A., Blanvillain, C., Portier, E., Le Barh, M., and O'Brien, M. (2019). Catastrophic decline and subsequent conservation of the critically endangered Fatu Hiva monarch *Pomarea whitneyi* in Marquesas Islands. *Bird Conservation International* **29**, 598–615. doi:10.1017/S095927091900008X

Gieraltowski, L., Higa, J., Peralta, V., Green, A., Schwensohn, C., Rosen, H., Libby, T., Kissler, B., Marsden-Haug, N., Booth, H., Kimura, A., Grass, J., Bicknese, A., Tolar, B., Defibaugh-Chávez, S., Williams, I., Wise, M., and Salmonella Heidelberg Investigation Team (2016). National Outbreak of Multidrug Resistant Salmonella Heidelberg Infections Linked to a Single Poultry Company. *PLoS One* **11**, 0162369. doi:10.1371/JOURNAL.PONE.0162369

Gram, H. C. (1884). "Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten". *Fortschritte der Medizin* **2**, 185–189.

Gupta, P., Vishnudas, C. K., Ramakrishnan, U., Robin, V. V., and Dharmarajan, G. (2019). Geographical and host species barriers differentially affect generalist and specialist parasite community structure in a tropical skyisland archipelago. *Proceeding of the Royal Society B* **286**, 20190439

Harmon, W. M., Clark, W. A., Hawbecker, A. C., and Stafford, M. (1987). *Trichomonas gallinae* in columbiform birds from the Galápagos Islands. *Journal of Wildlife Diseases* **23**, 492–494. doi:10.7589/0090-3558-23.3.492

Hellgren, O., Waldenström, J., and Bensch, S. (2004). A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *Journal of Parasitology* **90**, 797–802. doi:10.1645/GE-184R1

Hellgren, O., Atkinson, C. T., Bensch, S., Albayrak, T., Dimitrov, D., Ewen, J. G., *et al.* (2015). Global phylogeography of the avian malaria pathogen *Plasmodium relictum* based on MSP1 allelic diversity. *Ecography* **38**, 842–50. doi:10.1111/ECOG.01158

Howe, L., Castro, I. C., Schoener, E. R., Hunter, S., Barraclough, R. K., and Alley, M. R. (2012). Malaria parasites (*Plasmodium* spp.) infecting introduced, native and endemic New Zealand birds. *Parasitology Research* **110**, 913–923. doi:10.1007/S00436-011-2577-Z

Jarvi, S. I., Atkinson, C. T., and Fleischer, R. C. (2001). Immunogenetics and resistance to avian malaria in Hawaiian honeycreepers (Drepanidinae). *Studies in Avian Biology* **22**, 254–263.

Jarvi, S. I., Triglia, D., Giannoulis, A., Farias, M., Bianchi, K., and Atkinson, C. T. (2008). Diversity, origins and virulence of Avipoxviruses in Hawaiian forest birds. *Conservation Genetics* **9**, 339–348. doi:10.1007/S10592-007-9346-7

Jønsson, K. A., Irestedt, M., Bowie, R. C. K., Christidis, L., and Fjeldså, J. (2011). Systematics and biogegraphy of Indo-Pacific ground-doves. *Molecular Phylogenetics and Evolution* **59**, 538–543. doi:10.1016/J.YMPEV.2011.01.007

Kligler, I. J., Muckenfuss, R. S., and Rivers, T. M. (1929). Transmission of fowl-pox by mosquitoes. *The Journal of Experimental Medicine* **49**, 649–660. doi:10.1084/JEM.49.4.649

Laroucau, K., Vorimore, F., Aaziz, R., Berndt, A., Schubert, E., and Sachse, K. (2009). Isolation of a new chlamydial agent from infected domestic poultry coincided with cases of atypical pneumonia among slaughterhouse workers in France. *Infection, Genetics and Evolution* **9**, 1240–1247. doi:10.1016/J.MEEGID.2009.08.005

Laroucau, K., Aaziz, R., Meurice, L., Servas, V., Chossat, I., Royer, H., de Barbeyrac, B., Vaillant, V., Moyen, J. L., Meziani, F., Sachse, K., and Rolland, P. (2015). Outbreak of psittacosis in a group of women exposed to *Chlamydia psittaci*-infected chickens. *Euro Surveillance* **20**, 21155. doi:10.2807/1560-7917.ES2015.20.24.21155

Lowe, S. J., Browne, M., Boudjelas, S., and De Poorter, M. (2000). 100 of the world's worst invasive alien species. A selection from the Global Invasive Species Database. *Aliens* **12**, 12.

MalAvi database (2020). MalAvi. A database for avian haemosporidian parasites. Version 2.4.7. Available at http://130.235.244.92/Malavi/fasta.html [accessed 9 November 2020].

McCormack, G. (2007). Lorikeet: Rimatara lorikeet ('Ura) reintroduction programme. Available at: http://www.atiu.info/attractions/birds/lorikeet/ [accessed 28 April 2010].

Medeiros, M. C., Hamer, G. L., and Ricklefs, R. E. (2013). Host compatibility rather than vector–host–encounter rate determines the host range of avian *Plasmodium* parasites. *Proceedings Royal Society B: Biological Sciences* **280**, 20122947. doi:10.1098/RSPB.2012.2947

Ménard, A., Clerc, M., Subtil, A., *et al.* (2006). Development of a real-time PCR for the detection of *Chlamydia psittaci*. *Journal of Medical Microbiology* **55**, 471–473. doi:10.1099/JMM.0.46335-0

Olias, P., Wegelin, M., Zenker, W., Freter, S., Gruber, A. D., and Klopfleisch, R. (2011). Avian malaria deaths in parrots, Europe. *Emerging Infectious Diseases* **17**, 950–952. doi:10.3201/EID1705.101618

Padilla, L. R., and Parker, P. G. (2008). Monitoring avian health in the Galapagos Islands: current knowledge. In 'Zoo and Wild Animal Medicine'. 6th edn. (Ed. M. E. Fowler, and R. E. Miller.) pp. 191–199. (Elsevier.)

Parker, K. A., Brunton, D. H., and Jakob-Hoff, R. (2006). Avian translocations and disease; implications for New Zealand conservation. *Pacific Conservation Biology* **12**, 155–162. doi:10.1071/PC060155

Phalen, D. N. (1998). Avian polyomavirus: my thoughts. *Am Fed Aviculture Watchbird* **25**, 28–39.

Phalen, D. N., Wilson, V. G., and Graham, D. L. (1991). Polymerase chain reaction assay for avian polyomavirus. *Journal of Clinical Microbiology* **29**, 1030–1037. doi:10.1128/JCM.29.5.1030-1037.1991

Pisanu, B., Laroucau, K., Aaziz, R., Vorimore, F., Le Gros, A., Chapuis, J. L., and Clergeau, P. (2018). Chlamydia avium detection from a ring-necked parakeet (*Psittacula krameri*) in France. *Journal of Exotic Pet Medicine* **27**, 68–74. doi:10.1053/J.JEPM.2018.02.035

Raffel, T. R., Register, K. B., Marks, S. A., and Temple, L. (2002). Prevalence of *Bordetella avium* infection in selected wild and domesticated birds in the eastern USA. *Journal of Wildlife Diseases* **38**, 40–46. doi:10.7589/0090-3558-38.1.40

Raper, K. B., and Fennell, D. I. (1965). 'The Genus Aspergillus.' (Williams and Wilkins: Baltimore)

Richard, V., and Cao-Lormeau, V.-M. (2019). Mosquito vectors of arboviruses in French Polynesia. *New Microbe and New Infect* **31**, 100569. doi:10.1016/J.NMNI.2019.100569

Rivière, F. (1988). Ecologie de Aedes (*Stegomyia*) *polynesiensis*, Marks, 1951 et transmission de la filariose de Bancroft en Polynesie. Doctoral dissertation, Paris 11.

Robertson H. A., and Saul, E. K. (2007). Conservation of Kakerori (*Pomarea dimidiata*) in the Cook Islands in 2006/07. Department of Conservation, Wellington. *DOC Research and Development Series* No. 296.

Rosner, B. (Ed.) (2010). 'Fundamentals of Biostatistics.' 7th edn. (Brooks/Cole: Boston.)

Saavedra, S., Ghestemme, T., and Blanvillain, C. (2012). French Polynesia: first control campaign for *Acridotheres tristis* and *Pycnonotus cafer* on Tahiti Island. *Pacific Invasives Initiative Newsletter* **6**, 4–5.

Sachse, K., Kuehlewind, S., Ruettger, A., Schubert, E., and Rohde, G. (2012). More than classical *Chlamydia psittaci* in urban pigeons. *Veterinary Microbiology* **157**, 476–80. doi:10.1016/J.VETMIC.2012.01.002

Sachse, K., Laroucau, K., Riege, K., Wehner, S., Dilcher, M., Creasy, H. H., *et al.* (2014). Evidence for the existence of two new members of the family Chlamydiaceae and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. *Systematic and Applied Microbiology* **37**, 79–88. doi:10.1016/J.SYAPM.2013.12.004

Sachse, K., Laroucau, K., and Vanrompay, D. (2015). Avian chlamydiosis. *Current Clinical Microbiology* **2**, 10. doi:10.1007/S40588-014-0010-Y

Santiago-Alarcon, D., Palinauskas, V., and Schaefer, H. M. (2012). Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Review* **87**, 928–964. doi:10.1111/J.1469-185X.2012.00234.X

Schweizer, M., Wright, T. F., Peñalba, J. V., Schirtzinger, E. E., and Joseph, L. (2015). Molecular phylogenetics suggests a New Guinean origin and frequent episodes of founder-event speciation in the nectarivorous lories and lorikeets (Aves: Psittaciformes). *Molecular phylogenetics and evolution* **90**, 34–48. doi:10.1016/J.YMPEV.2015.04.021

Sergeant, E. S. G. (2019). Epitools epidemiological calculators. Ausvet Pty Ltd. Available at http://epitools.ausvet.com.au [accessed 12 December 2019].

Soares, L., Marra, P., Gray, L., and Ricklefs, R. E. (2017). The malaria parasite *Plasmodium relictum* in the endemic avifauna of eastern Cuba. *Conservation Biology* **31**, 1477–1482. doi:10.1111/COBI.12995

Steadman, D. W. (2006). 'Extinction and Biogeography of Tropical Pacific Birds.' (University of Chicago Press: Chicago.)

Swinnerton, K. J., Greenwood, A. G., Chapman, R. E., and Jones, C. G. (2005). The incidence of the parasitic disease Trichomoniasis and its treatment in reintroduced and wild pink pigeons *Columba mayeri*. *Ibis* **147**, 772–782. doi:10.1111/J.1474-919X.2005.00466.X

Thibault, J. C., and Cibois, A. (2017). 'Birds of Eastern Polynesia. A Biogeographic Atlas.' (Lynx Edicions: Barcelona.)

Valkiūnas, G., Ilgūnas, M., Bukauskaitė, D., Fragner, K., Weissenböck, H., Atkinson, C. T., and Iezhova, T. A. (2018). Characterization of *Plasmodium relictum*, a cosmopolitan agent of avian malaria. *Malaria Journal* **17**, 184. doi:10.1186/S12936-018-2325-2

Vancanneyt, M., Vandamme, P., Segers, P., Torck, U., Coopman, R., Kersters, K., and Hinz, K. H. (1999). *Riemerella columbina* sp. nov., a bacterium associated with respiratory disease in pigeons. *International Journal of Systematic and Evolutionary Microbiology* **49**, 289–295. doi:10.1099/00207713-49-1-289

Vanderwerf, E. A. (2007). Biogeography of Elepaio: evidence from inter-island song playbacks. *The Wilson Journal of Ornithology* **119**, 325–333. doi:10.1676/06-015.1

Vanderwerf, E. A., Burt, M. D., Rohrer, J. L., and Mosher, S. M. (2006). Distribution and prevalence of mosquito-borne diseases in O'ahu 'Elepaio. *The Condor* **108**, 770–777. doi:10.1093/CONDOR/108.4.770

van Riper, C., van Riper, S. G., Goff, M. L., and Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**, 327–344. doi:10.2307/1942550

van Riper, C., van Riper, S. G., and Hansen, W. R. (2002). Epizootiology and effect of avian pox on Hawaiian forest birds. *Auk* **119**, 929–942. doi:10.1093/AUK/119.4.929

van Riper, C., and Forrester, D. (2007). 'Avian Pox. Infectious Diseases of Wild Birds.' (Wiley Blackwell: Oxford, UK.)

Vaz, F. F., Raso, T. F., Agius, J. E., Hunt, T., Leishman, A., Eden, J. S., and Phalen, D. N. (2020). Opportunistic sampling of wild native and invasive birds reveals a rich diversity of adenoviruses in Australia. *Virus evolution* **6**, veaa024. doi:10.1093/VE/VEAA024

Warner, R. E. (1968). The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *Condor* **70**, 101–120. doi:10.2307/1365954

Wikelski, M., Foufopoulos, J., Vargas, H., and Snell, H. (2004). Galápagos birds and diseases: invasive pathogens as threats for island species. *Ecology and Society* **9**, 1–5. doi:10.5751/ES-00605-090105

Woernle, H. (1966). The use of the agar-gel diffusion technique in the identification of certain avian virus diseases. *The Veterinarian* **4**, 17–28.

Zocevic, A., Vorimore, F., Vicari, N., *et al.* (2013). A real-time PCR Assay for the detection of atypical strains of Chlamydiaceae from pigeons. *PLoS One* **8**, 58741. doi:10.1371/JOURNAL.PONE.0058741