J. Comp. Path. 2017, Vol. 157, 246–255

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

DISEASE IN WILDLIFE OR EXOTIC SPECIES

Clinical Pathology of the Vulnerable Gough Moorhen (*Gallinula comeri*)

M. P. Dagleish*, P. G. Ryan†, S. Girling‡, M. Ghazali‡ and A. L. Bond§

** Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Scotland, UK, † FitzPatrick Institute of African Ornithology, DST-NRF Centre of Excellence, University of Cape Town, Rondebosch, South Africa, ‡ Royal Zoological Society of Scotland, Edinburgh Zoo, 134 Corstorphine Road, Edinburgh, Scotland and § RSPB Centre for Conservation Science, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, England, UK*

Summary

The Gough moorhen (*Gallinula comeri*) is native to Gough Island, Tristan da Cunha, and listed as Vulnerable by the International Union for Conservation of Nature due to its restricted range and susceptibility to introduced predators. A planned ecosystem restoration by eradication of introduced house mice (*Mus musculus*) via aerially delivered rodenticide requires a reproductively balanced population of Gough moorhens to be held in captivity to avoid primary and secondary poisoning. To aid disease detection during the period of captivity, Gough moorhens ($n = 43$; 25 adult females and 18 adult males) were captured, measured and sampled to determine ease of sexing by morphometrics, to establish reference ranges for routine haematological and biochemical parameters and to identify any intestinal and haemoparasites as well as determine which faecal bacteria were present. Male Gough moorhens had significantly greater mean body mass ($P = 0.019$) and head and bill length ($P = 0.001$) compared with females, but the overlapping ranges showed genetic identification of sex was required for accurate determination. Plasma globulin and total protein concentrations were significantly greater in female compared with male birds ($P = 0.032$ and $P = 0.012$, respectively) and probably related to egg yolk production. No haemoparasites or gastrointestinal parasites were found in any bird and there were no sex-related differences in the haematology. Multiple bacterial taxa were isolated from the faeces of all birds including *Enterococcus* spp. ($n = 42$), *Klebsiella* spp. ($n = 40$), *Staphylococcus aureus* ($n = 33$), *Staphylococcus intermedius* ($n = 16$), *Escherichia coli* ($n = 41$) and *Pseudomonas* spp. ($n = 7$). No clinical or subclinical disease was found in any of the birds examined, suggesting they are suitable for short-term captivity but rapid on-island genetic-based sex determination will be essential to ensure a reproductively balanced population.

Keywords: biochemistry; Gough moorhen; haematology; parasitology

Introduction

The Gough moorhen (*Gallinula comeri*) is a flightless land bird native to Gough Island (40.3°S, 9.9°W), a 65 km^2 island that is part of the UK Overseas Territory of Tristan da Cunha in the central South Atlantic Ocean (Allen, 1892; Ryan, 2007). The population was estimated to be around 2,000–3,000 breeding pairs in the 1980s, but is poorly studied (Watkins and Furness, 1986). This species was introduced to Tristan da Cunha, where the Tristan moorhen (*Gallinula nesiotis*) was driven to extinction sometime in the late 19th century, likely by introduced feral cats (*Felis catus*), black rats (*Rattus rattus*) and habitat alteration (Elliott, 1953; Beintema, 1972; Bourne and David, 1981). Eight Gough moorhens were introduced to Tristan da Cunha in 1956, where they now number around 2,000 pairs (Ryan, 2007; Groenenberg *et al.*, 2008). The reason the Gough moorhen has flourished on Tristan da Cunha when the Tristan

Correspondence to: M. P. Dagleish (e-mail: mark.dagleish@moredun.ac.uk).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2017.08.003

moorhen could not survive is unknown, but it may be due to the eradication of cats from the island in the 1970s (Richardson, 1984). On Gough Island the Gough moorhen typically remains in dense tussock grass (*Spartina arundinacea*) and fern bush vegetation, occasionally climbing larger branches of *Phylica arborea* trees and entering seabird burrows (Watkins and Furness, 1986; Wanless and Wilson, 2007). Their nests are open cups low down in the dense vegetation (Watkins and Furness, 1986) and the eggs and hatchlings are presumed to be vulnerable to predation by house mice (*Mus musculus*) introduced to the island during the 19th century (Watkins and Furness, 1986; Gray *et al*., 2014). Its restriction to a very small range and vulnerability to accidental introduction of predators has resulted in the Gough moorhen being listed as Vulnerable on the International Union for Conservation of Nature Red List since 1994 (Birdlife International, 2017).

House mice also threaten other species on Gough Island, including the critically endangered Gough bunting (*Rowettia goughensis*), which is endemic to this island (Ryan and Cuthbert, 2008), and three seabirds that breed principally, if not solely, on Gough Island: the critically endangered Tristan albatross (*Diomedea dabbenena*), the endangered Atlantic petrel (*Pterodroma incerta*) and endangered MacGillivray's prion (*Pachyptila macgillivrayi*), among others (Davies *et al*., 2015; Dilley *et al*., 2015). Therefore, among the UK Overseas Territories, Gough Island is recognized as the highest priority for island restoration (Dawson *et al*., 2015).

Introduced rodents have been removed from >570 islands worldwide (Howald *et al*., 2007; Bellingham *et al*., 2010; Russell and Holmes, 2015), typically by spreading cereal pellets laced with rodenticide across the island by helicopter (Towns and Broome, 2003; Cuthbert *et al*., 2014) or using bait stations on smaller islands (Russell and Holmes, 2015). For endemic obligate terrestrially-foraging species, especially generalists such as the Gough moorhen, there is a real risk of primary poisoning (i.e. ingesting bait directly; Wanless *et al*., 2010; Bond *et al*., 2016; Oppel *et al*., 2016) and/or secondary poisoning (i.e. ingesting poisoned rodents or other species; Empson and Miskelly, 1999; Eason *et al*., 2002; Towns and Broome, 2003; Phillips, 2010). To mitigate this, a small, reproductively balanced population can be placed in captivity during the eradication operation until the rodenticide, brodifacoum, is broken down in the environment (Empson and Miskelly, 1999; Phillips, 2010; Rexer-Huber and Parker, 2011; Oppel *et al*., 2016). Bringing wild, free-ranging species into captivity, even for a limited period, carries several risks including increased transmission of infectious diseases, especially as the captive population density inevitably exceeds greatly that of the free-ranging animals (Goodman *et al*., 2012; Sainsbury and Vaughan-Higgins, 2012). Therefore, an understanding of basic clinical pathology and reference ranges can better prepare aviculturists managing the temporarily captive population should individuals display abnormal behaviour, have difficulty acclimating to captivity or present with clinical signs of disease in the period of captivity during the planned mouse eradication operation (Dagleish *et al*., 2017).

This study reports the health status of free-ranging Gough moorhens by undertaking the first evaluation of standard haematological and plasma biochemical parameters used in clinical pathology to define reference ranges and the presence of haemoparasites, and by examining faecal samples for gastrointestinal parasite burden and resident bacterial species, together with the presence of any potential pathogens.

Materials and Methods

Capture, Measuring and Sampling Procedures

Gough moorhens were captured from an area of tussock grass and scattered *Phylica* trees around the weather station (40.349°S, 9.880°W) ~30 ≤ 54 m above sea level. Birds were captured using walk-in traps baited with tinned sweet corn or occasionally house mice killed in snap traps.

Birds were held in cloth bags and weighed with a spring balance (±5 g) then examined for subjective assessment of body condition, head + bill measured using Vernier callipers (±0.1 mm) and marked with coloured nail polish on the frontal shield or non-toxic animal marker spray on the back feathers to denote the animal had been sampled (this species is not suitable for marking with leg rings [Rexer-Huber *et al*., 2012] and individual identification after sampling was not required). Subsequent to this, 0.5 ml of blood was sampled via the superficial planter metatarsal vein using a 2.0 ml syringe with a 25 gauge × 16.0 mm needle (BD Medical, Oxford, UK), placed immediately into 0.5 ml tubes containing lithium heparin anticoagulant (0011-25, Vetlab Supplies, Pulborough, UK) and mixed gently. The remaining blood was applied to FTA™ Classic Cards (Whatman™, GE Healthcare UK Ltd., Little Chalfont, UK) and air dried to enable sexing by DNA analysis (see below). Faeces voided during capture were collected and placed into 1.5 ml sterile plastic capped tubes and processed for faecal parasite egg content and bacteriological examination either within 30 min or stored chilled and processed within 6 h for bacteriology or 12 h for parasite egg examination.

Haematological and Blood Biochemical Analyses

Blood samples were processed immediately for determination of haematocrit, haemoglobin concentration, pH, pCO_2, pO_2, base excess, bicarbonate, total CO_2, sO_2, sodium, potassium, ionised calcium and glucose with a portable i-STAT 1 handheld analyzer using i-STAT CG8+ cartridges (Abaxis Europe GmbH, Griesheim, Germany) according to the manufacturer's instructions. Blood samples were also analyzed for total protein, albumin, globulin, aspartate aminotransferase (AST), bile acids, creatine kinase, uric acid, glucose, calcium, phosphate, potassium and sodium with a laboratory based VetScan VS2 using the Avian/Reptile Profile Plus cartridges (Abaxis Europe GmbH). Parameters examined by the VetScan VS2 were performed within 10 min of blood sampling.

Thin blood smears were prepared by placing 2 µl of anticoagulated blood at one end of a glass microscope slide and smearing with a clean slide. Slides were air dried, fixed for 1 min in methanol, air dried, stained with May–Grunwald/Giemsa (Kayla Africa Suppliers and Distributors, Johannesburg, South Africa) and mounted to allow estimation of leucocyte number (the mean of 10 fields counted using ×500 magnification, multiplied by three) and white blood cell differential counts, red and white blood cell morphology and presence of blood parasites by light microscopy.

Faecal Bacteriology and Parasite Evaluation

Identification of faecal bacteria was performed using the MultiChrome™ Veterinary Microbiology Culture System (Kacey™ Diagnostics, Ashville, North Carolina, USA), which isolates gram-positive from gram-negative bacteria using selective media and further differentiates bacteria by inciting genus- or species-specific colour reactions. Faecal samples were streaked onto microbiology plates using a sterile loop. The plates were incubated at 38°C in air and examined after 24 and 48 h by two observers (MPD and ALB).

Presence, number and morphology of parasite eggs within faeces were assessed by the modified McMaster's method (Whitlock, 1948). Faecal samples were added to a saturated solution of NaCl at a ratio of 1 g:14 ml (w/v) in a stomacher bag and mixed thoroughly to emulsify, prior to passing through a 0.15 mm wire mesh sieve. The filtrate was stirred thoroughly, drawn up in a Pasteur pipette and run carefully into either side of a McMaster counting chamber (Vetlab Supplies). All eggs within both grids were counted under light microscopy and the total number multiplied by 50 to derive the number of eggs per gram (epg) of faeces (maximum sensitivity 50 epg).

Sexing of Gough Moorhens

Gough moorhens exhibit a low level of sexual size difference (Watkins and Furness, 1986; Parker *et al.*, 2012) and as our measurements indicated a high proportion of males based on a published discriminant function (Parker *et al.*, 2012) we undertook genetic analyses to determine individual's sex. DNA was extracted from blood samples on FTA™ Classic Cards using QuickGene DNA whole blood kit (Kurabo Industries Ltd., Osaka, Japan) as per the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was carried out using forward primer, P8 (5′-CTCCCAAGGATGAGRAAYTG-3′) and reverse primer, P2 (5′-TCTGCATCGCTAAATCCTTT-3′), which amplified fragments from the genes *CHDIZ* and *CHDIW* (Griffiths *et al.*, 1998; Lee *et al.*, 2002; Parker *et al.*, 2012). The final reaction mix contained 1 µl of template DNA, 7 µl Maxima Hot Start PCR mastermix (ThermoFisher Scientific) and 1 µl of each forward and reverse primer at 10 µM. Thermocycling conditions were: 95°C for 5 min; 30 cycles of 48°C for 45 sec, 72°C for 45 sec and 95°C for 30 sec; 48°C for 1 min and 72°C for 10 min. PCR products were separated by electrophoresis in a 3% agarose gel stained with GelRed™ (Biotium Inc., Fremont, California, USA) for 50 min. Under UV exposure, gel visualization revealed the bird's sex as described by Lee *et al.* (2002).

Statistical Analyses

Differences in haematological and plasma biochemical parameters between males and females were examined using a series of general linear models in R 3.3.1 (R Core Team, 2016). We examined differences in blood sodium, potassium and glucose measured on both instruments using paired t-tests. Differences were considered significant when $P \leq 0.05$.

Results

Bird Numbers, Sex and Morphometric Data

In total, 43 Gough moorhens were sampled: 25 females and 18 males based on DNA sexing. While the timing of breeding is highly variable for this species, eggs are laid no earlier than late September and chicks recorded no later than March (Richardson, 1984; Watkins and Furness, 1986), so all individuals sampled were considered fully grown and either adults or approximately 1 year old. The mean mass and head + bill length of the male Gough moorhens were both significantly larger compared with females ($P = 0.019$ and 0.001, respectively; Table 1), but the male and female ranges of both measurements overlapped considerably (Table 1).

Haematology and Plasma Biochemistry

Results from both blood analyzers were obtained from all 43 birds, although occasional individual results are missing due to haemolysis, icterus or other unknown reasons affecting the samples, but the number is stated from which the mean and range is derived for each parameter (Tables 1 and 2). There were few significant differences in biochemical parameters between sexes (Tables 1 and 2). Females had higher concentrations of globulin compared with males and the difference was significant ($P = 0.032$), which also made the total protein significantly higher ($P = 0.012$) in females (Table 2). Results for mean blood sodium, potassium and glucose concentrations were slightly but significantly greater ($t_{40} = 10.65$, $P < 0.001$, $t_{40} = 2.52$, $P = 0.016$ and $t_{40} = 12.24$, $P < 0.001$, respectively) when analyzed with the i-STAT machine compared with the VetScan VS2 (Tables 1 and 2).

All 43 blood smears showed predominantly normal erythrocyte morphology, except seven birds had a polychromasia score of 1+ (six females, one male) and one female had a score of 2+ (Fig. 1A). Three birds (two females, one male) had occasional metarubricytes and/or rubricytes present (Fig. 1B); both females had slight anisocytosis and were part of the group with a polychromasia score of 1+. Leucocyte morphology was normal (Fig. 1C and D), except two birds (one female, one male) had occasional reactive lymphocytes present. There were no significant differences between sexes in the numbers of total or individual leucocytes (Table 3). No blood parasites were found.

Faecal Bacteriology and Parasite Evaluation

Samples for faecal bacterial culture were available from all 43 birds and various bacteria were present (n = number of birds positive after 24 and 48 h of culture, respectively); *Enterococcus* spp. ($n = 42$ and 42), *Klebsiella* spp. ($n = 32$ and 40), *Staphylococcus aureus* ($n = 29$ and 33), *Staphylococcus intermedius* ($n = 5$ and 16), *Escherichia coli* ($n = 41$ and 41) and *Pseudomonas*

Table 1
Morphometric measurements, blood and biochemistry parameters of Gough moorhens measured using the Abaxis i-STAT

Parameter	*Females*	*Males*	*Overall*	*Males versus females*
Mass (g)	429 ± 54 (350–535) (25)	469 ± 49 (370–545) (18)	446 ± 55 (350–545) (43)	**$F_{1,41} = 5.95$ $P = 0.019$**
Head + bill (mm)	60.8 ± 1.2 (58.7–63.1) (25)	63.5 ± 1.4 (59.6–65.3) (18)	61.9 ± 1.8 (58.7–65.3) (43)	**$F_{1,41} = 47.42$ $P < 0.001$**
pH	7.458 ± 0.067 (7.245–7.578) (24)	7.449 ± 0.063 (7.324–7.540) (18)	7.454 ± 0.065 (7.245–7.578) (42)	$F_{1,40} = 0.20$ $P = 0.66$
pCO_2 (mmHg)	28.3 ± 3.6 (22.9–39.1) (24)	27.6 ± 2.7 (23.4–31.3) (18)	28.0 ± 3.3 (22.9–39.1) (42)	$F_{1,40} = 0.52$ $P = 0.47$
pO_2 (mmHg)	82 ± 30 (54–177) (24)	84 ± 20 (60–132) (18)	83 ± 26 (54–177) (42)	$F_{1,40} = 0.03$ $P = 0.87$
Base excess (mmol/l)	−4 ± 3 (−10–2) (24)	−5 ± 3 (−10–0) (18)	−4 ± 3 (-10–2) (42)	$F_{1,40} = 1.42$ $P = 0.24$
HCO_3 (mmol/l)	20.1 ± 2.4 (16.8–24.8) (24)	19.2 ± 2.2 (16.2–23.6) (18)	19.7 ± 2.3 (16.2–24.8) (42)	$F_{1,40} = 1.58$ $P = 0.22$
TCO_2 (mmol/l)	21 ± 2 (18–26) (24)	23 ± 14 (17–79) (18)	22 ± 9 (17–79) (42)	$F_{1,40} = 0.75$ $P = 0.39$
sO_2 (%)	95 ± 3 (90–100) (24)	96 ± 2 (92–99) (18)	96 ± 3 (90–100) (42)	$F_{1,40} = 1.85$ $P = 0.18$
Sodium (mmol/l)	142 ± 2 (137–148) (25)	142 ± 2 (138–146) (18)	142 ± 2 (137–148) (43)	$F_{1,41} = 0.01$ $P = 0.97$
Potassium (mmol/l)	3.5 ± 0.4 (2.9–4.2) (25)	3.6 ± 0.3 (3.0–4.1) (18)	3.6 ± 0.3 (2.9–4.2) (43)	$F_{1,41} = 0.81$ P = 0.37
Calcium (ionized) (mmol/l)	1.23 ± 0.08 (1.11–1.36) (25)	1.20 ± 0.05 (1.08–1.30) (17)	1.22 ± 0.07 (1.08–1.36) (42)	$F_{1,40} = 1.79$ $P = 0.19$
Glucose (mmol/l)	15.2 ± 2.4 (11.3–21.1) (25)	16.5 ± 2.9 (12.1–22.2) (18)	15.7 ± 2.7 (11.3–22.2) (43)	$F_{1,41} = 2.56$ $P = 0.12$
Haematocrit (%)	32 ± 5 (21–40) (25)	35 ± 3 (29–41) (18)	33 ± 4 (21–41) (43)	$F_{1,41} = 3.32$ $P = 0.08$
Haemoglobin (mmol/l)	6.9 ± 1.0 (4.4–8.4) (25)	7.3 ± 0.7 (6.1–8.7) (18)	7.1 ± 0.9 (4.4–8.7) (43)	$F_{1,41} = 3.31$ $P = 0.08$

Values presented as mean ± SD (range) (n).
Bold values indicate significant differences between the sexes.

Table 2
Blood and biochemistry parameters of Gough moorhens measured using the Abaxis VetScan VS2

Parameter	*Females*	*Males*	*Overall*	*Males versus females*
AST (IU/l)	682 ± 294 (287–1,357) (24)	851 ± 522 (285–1,743) (16)	749 ± 403 (285–1,743) (40)	$F_{1,38} = 1.71$ $P = 0.20$
Creatine kinase (IU/l)	979 ± 901 (308–4,169) (23)	1,098 ± 737 (381–3,323) (17)	1,029 ± 827 (308–4,169) (40)	$F_{1,38} = 0.20$ $P = 0.66$
Uric acid (µmol/l)	569 ± 224 (136–990) (24)	465 ± 260 (156–1,014) (17)	526 ± 242 (136–1,014) (41)	$F_{1,39} = 1.86$ $P = 0.18$
Glucose (mmol/l)	14.7 ± 2.5 (11.1–20.7) (24)	16.1 ± 2.7 (11.2–21.4) (17)	15.2 ± 2.6 (11.1–21.4) (41)	$F_{1,39} = 3.00$ $P = 0.09$
Calcium (mmol/l)	2.50 ± 0.47 (3.82–2.06) (21)	2.29 ± 0.14 (2.58–1.96) (17)	2.41 ± 0.37 (3.82–1.96) (38)	$F_{1,36} = 2.97$ $P = 0.09$
Phosphorus (mmol/l)	0.82 ± 0.60 (0.30–2.71) (24)	0.56 ± 0.33 (0.11–1.39) (17)	0.71 ± 0.51 (0.11–2.71) (41)	$F_{1,39} = 2.62$ $P = 0.11$
Albumin (g/l)	23 ± 2 (19–26) (24)	22 ± 2 (18–26) (17)	22 ± 2 (18–26) (41)	$F_{1,39} = 0.78$ $P = 0.38$
Globulin (g/l)	22 ± 5 (13–32) (24)	19 ± 3 (14–24) (17)	21 ± 5 (13–32) (41)	**$F_{1,39} = 4.96$ $P = 0.032$**
Total protein (g/l)	45 ± 6 (33–54) (24)	41 ± 3 (36–46) (17)	43 ± 5 (33–54) (41)	**$F_{1,39} = 7.01$ $P = 0.012$**
Potassium (mmol/l)	3.3 ± 0.8 (1.7–4.4) (24)	3.3 ± 0.7 (2.0–4.2) (17)	3.3 ± 0.8 (1.7–4.4) (41)	$F_{1,39} = 0.00$ $P = 0.99$
Sodium (mmol/l)	137 ± 4 (127–145) (24)	138 ± 2 (135–141) (17)	137 ± 3 (127–145) (41)	$F_{1,39} = 1.77$ $P = 0.19$

Values presented as mean ± SD (range) (*n*).
Bold values indicate significant differences between the sexes ($P \leq 0.05$).

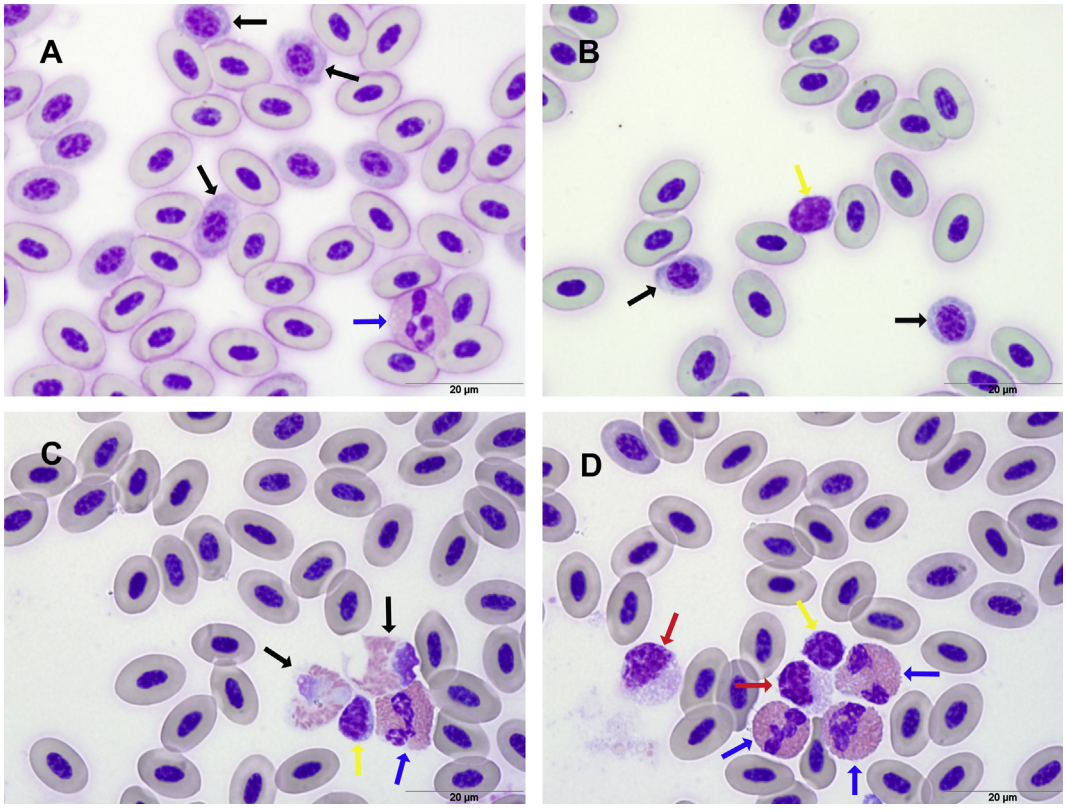

Fig. 1. Thin blood smears from Gough moorhens showing typical morphology of nucleated erythrocytes and selected leucocytes. May–Grunwald/Giemsa stain. Bar, 20 µm. (A) Note polychromasia in erythrocytes (black arrows) and an eosinophil which, due to the paucity of eosinophilic granules in the cytoplasm, was probably effete/degenerate (blue arrow). (B) Note two rubricytes (black arrows) and a lymphocyte (yellow arrow). (C) Note two heterophils (black arrows), an eosinophil (blue arrow) and a lymphocyte (yellow arrow). (D) Note three eosinophils (blue arrows), two monocytes (red arrows) and a lymphocyte (yellow arrow).

Table 3
White blood cell composition of Gough moorhens

Parameter	*Females (n = 25)*	*Males (n = 18)*	*Overall (n = 43)*	*Males versus females*
Total white blood cells ($\times 10^9$/l)	12.0 ± 5.9 (2.4–27)	12.4 ± 3.1 (5.1–20.3)	12.2 ± 4.9 (2.4–27)	$F_{1,41} = 0.10$ $P = 0.75$
Heterophils (%)	23 ± 8 (7–37)	27 ± 13 (12–67)	25 ± 11 (7–67)	–
Heterophils ($\times 10^9$/l)	2.93 ± 2.04 (0.38–7.33)	3.35 ± 1.60 (0.92–8.04)	3.11 ± 1.86 (0.38–8.04)	$F_{1,41} = 0.51$ $P = 0.48$
Lymphocytes (%)	69 ± 9 (54–90)	64 ± 14 (26–83)	67 ± 12 (26–90)	–
Lymphocytes ($\times 10^9$/l)	8.01 ± 3.82 (1.75–19.44)	7.96 ± 2.81 (3.12–14.01)	7.99 ± 3.40 (1.75–19.44)	$F_{1,41} = 0.01$ $P = 0.96$
Eosinophils (%)	1 ± 2 (0–6)	1 ± 2 (0–8)	1 ± 2 (0–8)	–
Eosinophils ($\times 10^9$/l)	0.18 ± 0.26 (0.00–0.83)	0.14 ± 0.18 (0.00–0.47)	0.16 ± 0.23 (0–0.83)	$F_{1,41} = 0.25$ $P = 0.62$
Monocytes (%)	7 ± 3 (2–15)	7 ± 4 (4–15)	7 ± 3 (2–15) (43)	–
Monocytes ($\times 10^9$/l)	0.83 ± 0.64 (0.07–3.02)	0.77 ± 0.47 (0.00–1.83)	0.80 ± 0.57 (0.00–3.02)	$F_{1,41} = 0.11$ $P = 0.74$
Basophils (%)	0 ± 0 (0–1)	0 ± 1 (0–1)	0 ± 1 (0–3)	–
Basophils ($\times 10^9$/l)	0.01 ± 0.03 (0.00–0.14)	0.04 ± 0.10 (0.00–0.33)	0.02 ± 0.07 (0.00–0.33)	$F_{1,41} = 1.38$ $P = 0.25$

Values are presented as the mean ± SD (range) (*n*).

spp. ($n = 5$ and 7). All birds had more than one bacterial taxon present and no fungal organisms were detected.

No parasite eggs were found in the faecal samples ($n = 43$). In light of this finding, samples of fresh subantarctic fur seal (*Arctocephalus tropicalis*) faeces were analyzed identically as a positive control and numerous parasite eggs were detected, suggesting the technique was working to the known sensitivity.

Discussion

This is the first study of the clinical pathology of Gough moorhens and to assess the presence of potential pathogens in this species. The results provide reference ranges that will be important for monitoring the health of birds that will be held in captivity, at unavoidably greater densities compared with natural conditions, during the planned eradication of house mice from Gough Island.

Gough moorhens appear to be free from both gastrointestinal helminths and haemoparasites, similar to previous findings in the only other endemic landbird found on Gough Island, the Gough bunting (Dagleish *et al.*, 2017). Evolution of a bird species totally resistant to parasites is unlikely, but parasite-free displaced wild bird species that have survived and flourished in a foreign environment are reported (Little and Earlé, 1994). Seasonal variation in parasite load or helminth fecundity is unlikely to account for this absence, which suggests that any helminths or haemoparasites present when the Gough moorhen evolved in its original habitat either required a vector to complete their life cycle, which is not present on Gough Island, or, if they had a direct life cycle the parasites were unable to maintain this in the environment these birds now inhabit. The lack of exposure to endoparasites could explain the very low levels of circulating eosinophils found in the Gough moorhens (Deem *et al.*, 2011). However, if parasites are present in the free-ranging birds, even at a very low density, captivity will inevitably increase endoparasite exposure with potentially detrimental consequences (Sainsbury and Vaughan-Higgins, 2012).

The significantly higher blood globulin concentrations in adult female moorhens was probably associated with reproduction and linked to the formation of increased maternal antibodies for the creation of the egg yolk (Okuliarova *et al.*, 2014). Gough moorhens breed throughout the austral spring and summer, but eggs can be present from late September (Richardson, 1984; Watkins and Furness, 1986) so the females would have been coming into breeding condition at the time of sampling, which would account for the higher blood globulin concentrations. The higher blood sodium, potassium and glucose concentrations given by the i-STAT compared with the VetScan VS2 analyzer, although significant, were very small. The different times between blood sampling to analysis may explain the discrepancy for sodium as it would diffuse from the plasma and into blood cells without opposition if the cellular transmembrane sodium pump mechanism was compromised in any way (Alberts *et al.*, 1994). This would be the case when blood is removed from the normal homeostatic mechanisms of the body and be affected by time from sampling to analysis. However, the difference is more difficult to explain for blood

potassium, which would normally leak out from compromised blood cells over time. As many of the cells in the blood sample would remain viable in the short time, the difference between glucose levels found by the two different analyzers may have been due to utilization to meet energy requirements. Conversely, all three values may be due to the greater sensitivity of the i-STAT analyzer due to its methodologies (ion-selective electrode potentiometry for potassium and sodium and amperometrically for glucose) compared with the VetScan VS2 analyser (spectrophotometric). However, as the differences are so small there is no justification for choosing one rather than the other so long as the same analyser is used when comparing samples.

The only abnormalities noted in the blood smears were mild polychromasia (occasional rubricytes and metarubricytes) in three individuals, with two of these showing slight anisocytosis. These abnormalities can occur in cases of erythrocyte regeneration where mild to moderate blood loss has occurred previously. However, it is also associated with splenic contraction during periods of increased sympathetic nervous system tone in some animals and so may be a normal feature of this species when physically restrained as has been reported in other Gruiformes (Hawkey *et al.*, 1983; Clark *et al.*, 2009).

The gastrointestinal tract of birds, especially generalist terrestrially-foraging species such as the Gough moorhen, will always have a large and frequently diverse population of bacteria and other microorganisms. Knowledge of which gut bacteria are present prior to captivity is critical despite many being essential commensals that play a role in protecting the host from pathogenic bacterial species as, under certain conditions, these may become pathogenic (McKenney and Kendall, 2016) and require specific pharmaceutical treatment. *Enterococcus* was the most common genus of bacteria isolated from the sampled Gough moorhens (98%) and has been isolated from a number of species of captive and wild birds. While the species of *Enterococcus* were not identified in this study, the possibility that enteropathogenic forms such as *E. hirae*, known to cause septicaemia in birds (Devriese *et al.*, 1991, 1992, 1995), are present in the Gough moorhen cannot be ruled out. *Escherichia coli* had a similarly high prevalence (95%) and has been isolated previously from a range of avian species, including apparently healthy passerines and waterfowl (Damare *et al.*, 1979; Brittingham *et al.*, 1988; Foster *et al.*, 1998). Avian strains of *E. coli* can be pathogenic and have been associated with disease in domestic poultry and wild finches where cytolethal distending toxin has been isolated (Foster *et al.*, 1998). As we were unable to strain-type our isolates this could be a potential risk.

Klebsiella spp., present in 93% of the Gough moorhens sampled, are common avian pathogens of the respiratory and gastrointestinal tract (Bangert *et al.*, 1988; Fudge, 2001; Hernandez *et al.*, 2003) and have the potential to cause significant morbidity and possible mortality. This genus is frequently associated with multidrug resistance (Giacopello *et al.*, 2016) and is a potential threat to captive Gough moorhens.

Staphylococcus spp. are uncommon in domestic birds, but have been linked with clinical disease associated with joint, tendon and bone infections and occasional septicaemia (Gross, 1978). As *S. aureus* was present in the faeces of 77% of Gough moorhens, this suggests a high prevalence despite being wild and free ranging, and the sympatric Gough bunting also has a high prevalence of *S. aureus* in its faeces (Dagleish *et al.*, 2017). This bacterium has been isolated in cases of septicaemia and associated with mortalities in immunocompromised birds due to concurrent disease or iatrogenic medication (i.e. glucocorticoids) (Wobeser and Kost, 1992). This may be significant when Gough moorhens are brought into captivity for the duration of action of the rodenticide, as captivity will be stressful and so potentially immunosuppressive. Awareness of the clinical signs of disease associated with *S. aureus* along with rapid identification of the bacterium and appropriate treatment will be essential.

Pseudomonas aeruginosa is another common avian pathogen, chiefly of the upper and lower respiratory tract, but cases of septicaemia and enteritis have been reported in psittacine birds (Brittingham *et al.*, 1988; Walker *et al.*, 2002; Rich, 2003). The faeces of 16% of Gough moorhens were positive for this bacterium and, as it is a significant pathogen, which, like *Klebsiella* spp., often demonstrates multidrug resistance, it may be significant during any period of captivity where stocking densities will be increased and cross contamination between enclosures is a possibility.

The lack of known avian bacterial enteropathogens including *Salmonella* spp., *Campylobacter* spp. or *Yersinia* spp. in the faeces from these birds (Pennycott *et al.*, 1998; Benskin *et al.*, 2009) was probably due to the MultiChrome™ Veterinary Microbiology Culture System (Dagleish *et al.*, 2017). *Salmonella* spp. appear as very small, translucent colonies masked easily by other coloured bacterial species and *Campylobacter* spp. require selective media and microaerophilic conditions, which were beyond the scope of this study.

In conclusion, the Gough moorhens sampled in this study were in good body condition with no obvious

signs of clinical or subclinical disease and there were no concurrent disease concerns with respect to bringing them into short-term captivity. However, the lack of sexual dimorphism will require rapid genetic analyses on Gough Island while individuals are being caught, and prior to distribution of the rodenticide, to ensure a reproductively-balanced captive population. The absence of gastrointestinal helminths and haemoparasites is helpful with respect to captivity, but it may render them susceptible to even low levels of parasitic challenge, as they may have lost any natural or age-related immunity (De Coster *et al*., 2010), especially if subjected to a relatively high and/or sustained pathogen challenge as may occur during captivity. The terrain and climate on Gough Island dictate that all aviaries will need to be in a single, easily accessible location (Rexer-Huber and Parker, 2011) and in the same area as aviaries containing Gough buntings (Dagleish *et al*., 2017), creating an artificially high population density with attendant health-related problems. Commensal bacteria can become pathogenic under circumstances of overcrowding or chronic stress (Wobeser and Kost, 1992; Pennycott *et al*., 1998) and, in addition to the diverse species of potentially pathogenic bacteria isolated from the faeces of Gough moorhens, it should be assumed that *Salmonella* and *Campylobacter* spp. are present also. Therefore, it is recommended that a wide range of therapeutic anthelmintic, antiprotozoal and antibiotic preparations, known to be safe for use in similar bird species, should be present throughout the habitat restoration programme of Gough Island.

Acknowledgments

We thank J. Bradley, D. Fox, C. Jones, R. Phillips, M. Risi and C. Taylor for assistance in the field, P. J. N. de Bruyn for providing the fur seal faecal samples, D. Bartley (Moredun Research Institute) for instruction and advice in faecal parasite evaluation techniques and loan of equipment, J. Finlayson (Moredun Research Institute) for staining of blood smears and preparation of equipment, P. Boso and colleagues (Greendale Veterinary Diagnostics Limited, UK) for interpreting the blood smears, C. Underwood (Moredun Research Institute) for histochemical advice, D. Brown (Royal Zoological Society of Scotland) for preparation of equipment and Abaxis UK Ltd. for the generous loan of both blood analyzers. Additional logistic support was provided by J. Kelly, A. Schofield and C. Stringer (RSPB), and the South African National Antarctic Programme. Finally, we thank the Administrator, Island Council and Conservation Department of Tristan da Cunha for permission to work on Gough Island.

This work was funded by the Royal Society for the Protection of Birds, Royal Zoological Society of Scotland, UK Department for Environment, Food and Rural Affairs (Defra) and the Scottish Government Rural and Environment Science and Analytical Services Division (RESAS).

Conflict of Interest Statement

The authors declare no conflict of interest with respect to publication of this manuscript.

References

Alberts B, Bray D, Lewis J, Raff M, Roberts K *et al*. (1994) Membrane transport of small molecules and ionic basis of membrane excitability. In: *Molecular Biology of the Cell*, 3rd Edit., Garland Publishing, New York, pp. 513–515.

Allen JA (1892) Description of a new gallinule, from Gough Island. *Bulletin of the American Museum of Natural History*, **4**, 57–58.

Bangert RL, Ward ACS, Stauber EH, Cho BR, Widders PR (1988) A survey of the aerobic bacteria in the feces of captive raptors. *Avian Diseases*, **32**, 53–62.

Beintema AJ (1972) The history of the Island Hen (*Gallinula nesiotis*), the extinct flightless gallinule of Tristan da Cunha. *Bulletin of the British Ornithologists Club*, **92**, 106–113.

Bellingham PJ, Towns DR, Cameron EK, Davis JJ, Wardle DA *et al*. (2010) New Zealand island restoration: seabirds, predators, and the importance of history. *New Zealand Journal of Ecology*, **34**, 115–136.

Benskin CM, Wilson K, Jones K, Hartley IR (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews*, **84**, 349–373.

BirdLife International (2017) Species factsheet: *Gallinula comeri*. http://datazone.birdlife.org/species/factsheet/gough-moorhen-gallinula-comeri downloaded on January 5th, 2017.

Bond AL, Risi MM, Jones CW, Ryan PG (2016) Potential for primary poisoning of a critically endangered endemic land bird during rodent eradication operations at Gough Island, Tristan da Cunha. *PeerJ Preprints*, **4**, e1744.

Bourne WRP, David ACF (1981) Nineteenth century bird records from Tristan da Cunha. *Bulletin of the British Ornithologists Club*, **101**, 247–256.

Brittingham MC, Temple SA, Duncan RM (1988) A survey of the prevalence of selected bacteria in wild birds. *Journal of Wildlife Diseases*, **24**, 299–307.

Clark P, Boardman W, Raidal S (2009) Gruiformes (bustards, button quail, coots and cranes.). In: *Atlas of Clinical Avian Hematology*, Wiley-Blackwell, Oxford, pp. 74–76.

Cuthbert RJ, Broome KG, Bradley J, Ryan PG (2014) Evaluating the effectiveness of aerial baiting operations for rodent eradications on cliffs on Gough Island, Tristan da Cunha. *Conservation Evidence*, **11**, 25–28.

Dagleish MP, Ryan PG, Girling S, Bond AL (2017) Clinical pathology of the critically endangered Gough bunting (*Rowettia goughensis*). *Journal of Comparative Pathology*, **156**, 264–274.

Damare JM, Hussong D, Weiner RM, Colwell RR (1979) Aerobic and facultatively anaerobic bacteria associated with the gut of Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*). *Applied Environmental Microbiology*, **38**, 258–265.

Davies D, Dilley BJ, Bond AL, Cuthbert RJ, Ryan PG (2015) Trends and tactics of mouse predation on Tristan Albatross *Diomedea dabbenena* chicks at Gough Island, South Atlantic Ocean. *Avian Conservation and Ecology*, **10**, 5.

Dawson J, Oppel S, Cuthbert RJ, Holmes N, Bird JP *et al.* (2015) Prioritizing islands for the eradication of invasive vertebrates in the United Kingdom Overseas Territories. *Conservation Biology*, **29**, 143–153.

De Coster G, De Neve L, Martín-Gálvez D, Therry L, Lens L (2010) Variation in innate immunity in relation to ectoparasite load, age and season: a field experiment in great tits (*Parus major*). *Journal of Experimental Biology*, **213**, 3012–3018.

Deem L, Parker PG, Cruz MB, Merkel J, Hoeck PE (2011) Comparison of blood values and health status of Floreana mockingbirds (*Mimus trifasciatus*) on the islands of Champion and Gardner-by-Floreana, Galápagos Islands. *Journal of Wildlife Disease*, **47**, 94–106.

Devriese LA, Chiers K, De Herdt P, Vanrompay D, Desmidt M *et al.* (1995) *Enterococcus hirae* infections in psittacine birds: epidemiological, pathological and bacteriological observations. *Avian Pathology*, **24**, 523–531.

Devriese LA, Cruz Colque JI, Haesebrouck F, Desmidt M, Uyttebroek E *et al.* (1992) *Enterococcus hirae* in septicaemia of psittacine birds. *Veterinary Record*, **130**, 558–559.

Devriese LA, Ducatelle R, Uyttebroek E, Haesebrouck F (1991) *Enterococcus hirae* infection and focal necrosis of the brain of chicks. *Veterinary Record*, **129**, 316.

Dilley BJ, Davies D, Bond AL, Ryan PG (2015) Effects of mouse predation on burrowing petrel chicks at Gough Island. *Antarctic Science*, **27**, 543–553.

Eason CT, Murphy EC, Wright GRG, Spurr EB (2002) Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. *Ecotoxicology*, **11**, 35–48.

Elliott HFI (1953) The fauna of Tristan da Cunha. *Oryx*, **2**, 41–56.

Empson RA, Miskelly CM (1999) The risks, costs and benefits of using brodifacoum to eradicate rats from Kapiti Island, New Zealand. *New Zealand Journal of Ecology*, **23**, 241–254.

Foster G, Ross HM, Pennycott TW, Hopkins GF, McLaren IM (1998) Isolation of *Escherichia coli* 186:K61 producing cyto-lethal distending toxin from wild birds of the finch family. *Letters in Applied Microbiology*, **26**, 395–398.

Fudge AM (2001) Diagnosis and treatment of avian bacterial disease. *Seminars in Avian and Exotic Pet Medicine*, **10**, 3–11.

Giacopello C, Foti M, Mascetti A, Grosso F, Ricciardi D *et al.* (2016) Antimicrobial resistance patterns of Enterobacteriaceae in European wild bird species admitted in a wildlife rescue centre. *Veterinaria Italia*, **52**, 139–144.

Goodman G, Girling S, Pizzi R, Meredith A, Rosell F *et al.* (2012) Establishment of a health surveillance program for the reintroduction of the Eurasian beaver (*Castor fiber*) into Scotland. *Journal of Wildlife Diseases*, **48**, 971–978.

Gray MM, Wegmann D, Haasl RJ, White MA, Gabriel SI *et al.* (2014) Demographic history of a recent invasion of house mice on the isolated Island of Gough. *Molecular Ecology*, **23**, 1923–1939.

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. *Molecular Ecology*, **7**, 1071–1075.

Groenenberg DSJ, Beintema AJ, Dekker RWRJ, Gittenberger E (2008) Ancient DNA elucidates the controversy about the flightless island hens (*Gallinula* sp.) of Tristan da Cunha. *PLoS One*, **3**, e1835.

Gross WB (1978) Staphylococcosis. In: *Diseases of Poultry*, 7th Edit., MS Hofstad, BW Calneck, CF Helmboldt, WM Reid, HW Yoder Jr., Eds., Iowa State University Press, Ames, pp. 313–316.

Hawkey C, Samour JH, Ashton DG, Hart MG, Cindery RN *et al.* (1983) Normal and clinical haematology of captive cranes (Gruiformes). *Avian Pathology*, **12**, 73–84.

Hernandez J, Bonnedahl J, Waldenstro MJ, Palmgren H, Olsen B (2003) *Salmonella* in birds migrating through Sweden. *Emerging Infectious Diseases*, **9**, 753–755.

Howald GR, Donlan CJ, Galván JP, Russell JC, Parkes J *et al.* (2007) Invasive rodent eradication on islands. *Conservation Biology*, **21**, 1258–1268.

Lee PLM, Brain PF, Forman DW, Bradbury RB, Griffiths R (2002) Sex and death: *CHD1Z* associated with high mortality in moorhens. *Evolution*, **56**, 2548–2553.

Little RM, Earlé RA (1994) Lack of avian haematozoa in the Phasianinae of Robben Island. *Ostrich*, **65**, 248–249.

McKenney ES, Kendall MM (2016) Microbiota and pathogen 'pas de deux': setting up and breaking down barriers to intestinal infection. *Pathogens and Disease*, **74**, ftw051.

Okuliarova M, Kankova Z, Bertin A, Leterrier C, Mostl E *et al.* (2014) Maternally derived egg hormones, antibodies and antimicrobial proteins: common and different pathways of maternal effects in Japanese quail. *PLoS One*, **12**, e112817.

Oppel S, Bond AL, Brooke M de L, Harrison G, Vickery JA *et al.* (2016) Temporary captive population and rapid population recovery of an endemic flightless rail after an aerial broadcast rodent eradication operation. *Biological Conservation*, **204B**, 442–448.

Parker GC, Rexer-Huber K, Parker KA, Ryan PG, Techow NMSM *et al.* (2012) Morphometric and genetic sexing in the Gough Moorhen *Gallinula comeri*. *Ringing and Migration*, **27**, 20–25.

Pennycott TW, Ross HM, McLaren IM, Park A, Hopkins GF *et al.* (1998) Causes of death of wild birds of the family Fringillidae in Britain. *Veterinary Record*, **143**, 155–158.

Phillips RA (2010) Eradications of invasive mammals from islands: why, where, how and what next? *Emu*, **110**, i–vii.

R Core Team (2016) *R: A Language and Environment for Statistical Computing*. Version 3.3.1 [computer program]. R Foundation for Statistical Computing, Vienna, Austria.

Rexer-Huber K, Parker G (2011) *Captive Husbandry of the Gough Island Bunting and Moorhen*. RSPB Research Report No. 42, Sandy, UK.

Rexer-Huber K, Parker GC, Ryan PG, Cuthbert RJ (2012) Ringing injuries in the Gough moorhen *Gallinula comeri*. *Ringing and Migration*, **27**, 53–56.

Rich GA (2003) Syndromes and conditions of parrotlets, *Pionus* parrots, poicephalus, and mynah birds. *Seminars in Avian and Exotic Pet Medicine*, **12**, 144–148.

Richardson ME (1984) Aspects of the ornithology of the Tristan da Cunha group and Gough Island, 1972–1974. *Cormorant*, **12**, 123–201.

Russell JC, Holmes ND (2015) Tropical island conservation: rat eradication for species recovery. *Biological Conservation*, **185**, 1–7.

Ryan PG (2007) *Field Guide to the Animals and Plants of Tristan da Cunha and Gough Island*. Pisces Publications, Newbury.

Ryan PG, Cuthbert RJ (2008) The biology and conservation status of the Gough bunting *Rowettia goughensis*. *Bulletin of the British Ornithologists Club*, **128**, 242–253.

Sainsbury AW, Vaughan-Higgins RJ (2012) Analyzing disease risks associated with translocations. *Conservation Biology*, **26**, 442–452.

Towns DR, Broome KG (2003) From small Maria to massive Campbell: forty years of rat eradications from New Zealand. *New Zealand Journal of Zoology*, **30**, 377–398.

Walker SE, Sander JE, Cline JL, Helton JS (2002) Characterization of *Pseudomonas aeruginosa* isolates associated with mortality in broiler chicks. *Avian Diseases*, **46**, 1045–1050.

Wanless RM, Cooper J, Slabber M, Ryan PG (2010) Risk assessment of birds foraging terrestrially at Marion and Gough Islands to primary and secondary poisoning by rodenticides. *Wildlife Research*, **37**, 524–530.

Wanless RM, Wilson JW (2007) Predatory behaviour of the Gough Moorhen *Gallinula comeri*: conservation implications. *Ardea*, **95**, 311–315.

Watkins BP, Furness RW (1986) Population status, breeding and conservation of the Gough Moorhen. *Ostrich*, **57**, 32–36.

Whitlock HV (1948) Some modifications of the McMaster helminth egg-counting technique and apparatus. *Journal of the Council for Scientific and Industrial Research*, **21**, 177–180.

Wobeser G, Kost W (1992) Starvation, staphylococcosis and vitamin A deficiency among mallards overwintering in Saskatchewan. *Journal of Wildlife Diseases*, **28**, 215–222.

[*Received, May 22nd, 2017*
Accepted, August 7th, 2017]