Molecular epidemiology of *Trichomonas gallinae* in European Turtle Doves (*Streptopelia turtur*)

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Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

School of Biology

March 2017

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

Firstly I would like to express my utmost gratitude to Simon Goodman, Jenny Dunn and Keith Hamer for their guidance throughout this PhD. I would also like to thank Tony Morris at RSPB for his continuous support.

I am thankful to the RSPB fieldwork assistants and volunteers, and University of Leeds masters student Laura Curtis who all worked unfathomably hard to collect data on the elusive Turtle Dove. To Jenny Dunn for managing to coordinate such a feat every season and to Tony Morris whose bursts of nest-finding related energy we all admired and were grateful for.

I am very grateful to the team at NBAF (NERC Biomolecular Analysis Facility, Sheffield), in particular Deborah Dawson, Helen Hipperson and Gavin Horsborough for their guidance throughout the genetic analysis. To Gavin for being persistently upbeat whilst working through issues with protocols, to Deborah Dawson for her continuous efforts in progressing the project and to Helen Hipperson for her efforts during bioinformatics analysis and quick responses during code-related panics.

I would also like to thank a much wider team at both the RSPB and Natural England who, during Turtle Dove workshops, provided feedback and ideas which considerably helped in developing the direction of this PhD.

Lastly, I would like to thank my family who always took a great interest in my research and provided unwavering support. There are no words to capture the magnitude of appreciation for Andy Thomson, who has unwittingly been on this journey also, experienced the highs and lows and above all, fed me three square meals a day during the final stages of writing-up.

The data collected for this PhD was the result of multiple contributors. RSPB and Natural England funded the work as part of a wider research program on the European Turtle Dove which is under the umbrella of Operation Turtle Dove. The wider research program focused on researching the ecology of the UK breeding population of Turtle Doves and trialling conservation solutions, in addition to increasing our understanding of Turtle Dove populations across the western Palearctic flyway. There were four fieldwork seasons in the UK (2010-2014), managed by Jenny Dunn and contributed to by various fieldworkers (see below for a full list of names). This PhD started in 2013, whereby I joined the team for the remaining years and also carried out the environmental transmission sampling. Overall, the data collected between 2011-2014 contributed to this study. Data collection also took place in 2015 which was managed by myself with the help of Laura Curtis, a masters student from the University of Leeds.

Data collected from outside the UK was also the product of multiple efforts. Chris Orsman from the RSPB had previously collected data from Burkina Faso. Jenny Dunn and I visited Senegal in 2014 and collected the data from that year. Subsequently, John Mallord and Chris Orsman from the RSPB visited and collected data from Senegal in 2015. The data from France 2014 was the result of my two week visit to a research team (Office National de la Chasse et de la Faune Sauvage) based in Chizé, France, whereby I joined Cyril Eraud to collect *T. gallinae* samples and had additional help from Hervé Lormée, Jean-Marie Boutin and Marcel Rivière with catching birds.

The genetic analysis of *T. gallinae* samples collected from years prior to the start of this thesis i.e 2011 and 2012 was conducted by Rosie Lennon and Jen Stockdale respectively. All samples collected from 2013-2015 were genetically analysed by myself and Sami Asad, a masters student from the University of Leeds, who analysed a subset of *T. gallinae* samples collected from species of Columbidae (UK 2013, n=87) with regard to amplifying the Fe hydrogenase gene and identifying strains.

All samples collected between 2014-2015 were analysed with the award of an NBAF grant to conduct the analysis at the University of Sheffield.

Fieldwork teams (2010-2014)		
Year	Main responsibilities	
2010-2012	All	Jenny Dunn, Jennifer Stockdale, Rosie Lennon, Jenny Bright, Judit Mateos, Erica Wells, Jacqui Weir, Catie Gutmann Roberts, Vivien Hartwell, Jake Frost, Rebecca Melville, Joanne Stonehouse, Tony Morris
2013	Bird catching & sampling	Jenny Dunn, Eliza Leat, Mauren Gibson, Rebecca Thomas, Tony Morris
	Radio-tracking & habitat data collection	Rebecca Andrews, Chris Pringles, Laura Wright, Holly Neville-Smith,
2014	Bird catching & sampling	Jenny Dunn, Alex Ball, Kerry Skelhorn, Rebecca Thomas, Tony Morris
	Radio-tracking & habitat data collection	Emma Cutten, Louis O'Neill, Connie Miller

Abstract

Disease is usually ignored as a potential driver of species decline. This is concerning since disease could have a greater impact on a species as it becomes vulnerable to other extinction risks. This thesis investigated *Trichomonas gallinae* infection in the UK's fastest declining farmland bird, the European Turtle Dove *Streptopelia turtur*. It employed molecular techniques to acquire data on parasite prevalence and identify strains, and trialled the application of Next Generation Sequencing technology to disease surveillance. Overall, 50 adult Turtle Dove samples from 2011-2015 were analysed and temporal variation in strain frequency was revealed. A degree of population structure in *T. gallinae* infecting different Turtle Dove populations (France 2014, n=40; Senegal, n=28) was apparent, along with some evidence of wide-ranging parasite dispersal, indirectly through their host. The potential risk of shared resources as a transmission route of *T. gallinae* was investigated with 226 food and 117 water samples screened for its presence. Evidence suggested *T. gallinae* was regularly present in both food and water resources. This has important implications for supplementary feeding being a conservation management tool. The reservoir of *T. gallinae* in the UK was reviewed by sampling potential hosts of Columbidae (n=166), Galliformes (n=13) and Passeriformes (n=90). The detection of strains other than the finch epidemic strain in free-ranging Passerines revealed a greater level of genetic heterogeneity than previously shown in other studies. There were no significant associations between *T. gallinae* strain infection or coinfection with haemosporidians and measures of reproduction, body condition or post-fledging survival in Turtle Doves however, sample sizes were small. Overall, this study increases our understanding of the epidemiology of *T. gallinae* both in the wider bird population and a species of Vulnerable conservation status. It demonstrates how *T. gallinae* infecting wild birds is a useful model for investigating aspects of host- parasite ecology and encourages further research with this system.

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List of abbreviations

AGE – Aqueous Garlic Extract

ATL – American Tegumentary Leishmaniasis

BAP – Biodiversity Action Plan

bTB – bovine tuberculosis

BTO – British Trust for Ornithology

Bd – Batrachochytrium dendrobatidis

CDV – Canine Distemper Virus

DEFRA – Department for Environment, Food and Rural Affairs

DFTD – Devil Facial Tumour Disease

DOC – Degree of Change

eDNA – environmental DNA

Fe-hyd – Iron hydrogenase

GLM – Generalised Linear Model

GLMM – Generalised Linear Mixed Model

ITS – Internal Transcribed Spacer

IUCN – International Union for the Conservation of Nature

LRT – Likelihood Ratio Test

MG – Mycoplasma gallisepticum

MHC – Major Histocompatibility Complex

MOTUs – Molecular Taxonomic Units

NGS – Next Generation Sequencing

OIE – World Organization for Animal Health

qPCR – quantitative Polymerase Chain Reaction

PBS – phosphate buffered saline

PCR – Polymerase Chain Reaction

RHD – Rabbit Haemorrhagic Disease

SARS – Severe Acute Respiratory Syndrome

SINV – Sinbis Virus

SQPV – Squirrel Pox Virus

STI – Sexually Transmitted Infection

WA – MCF – Wildebeest Associated Malignant Catarrhal Fever

TDNT – Turtle Dove nestling

WPNT – Woodpigeon nestling

Chapter 1

Introduction

1.1 Overview

Disease is a particular threat to declining species, with the risk of becoming a driver of population decline increasing as the species becomes more vulnerable to extinction due to other factors (Daszak, 2000, Heard et al., 2013). It has the potential to be a significant driver of biodiversity loss when interacting with other extinction threats such as invasive species or land-use change (Heard et al., 2013). Despite the current unprecedented rate of species declines, disease is still often overlooked as a contributing factor (Tompkins and Jakob-Hoff, 2011, Hayhow et al., 2016). Disease epidemics can cause local population crashes that an endangered species may struggle to recover from (Laurenson et al., 1998, Lampo et al., 2006, Carne et al., 2014). In addition, the impact of chronic infection is little understood (Friend et al., 2001); it may be responsible for more subtle but long-lasting depression of population size. Synergistic interactions between infection and other environmental stressors can also alter the outcome of infection, with fitness consequences for the host (Appleby et al., 1999, Lochmiller and Deerenberg, 2000, Clinchy et al., 2004, Navarro, 2004, Knowles et al., 2009, Christe et al., 2012, Clinchy et al., 2013). The impact of infection is further complicated by the presence of different strains of parasite, which can vary in virulence, and the possibility of coinfection between strains and different species of parasite (van Rooyen et al., 2013). These interactions may be synergistic or antagonistic, therefore masking the impact of single infections, and vary depending on the specific combination of strains and parasites present (Behnke et al., 2009, Fenton et al., 2010, Thumbi et al., 2013).

The European Turtle Dove *Streptopelia turtur*, hereafter referred to as the Turtle Dove, is the fastest declining breeding bird in the UK and has a Vulnerable conservation status (Birdlife International, 2015, Harris et al., 2016). Comparisons of ecological studies conducted in the 1960s and late 1990s reveal a reduction in breeding productivity between the two periods whilst other potential drivers of decline such as being hunted during migration, degradation of over- wintering habitat and variable food availability whilst over- wintering have also been highlighted (Marchant et al., 1990, Tucker and Heath, 1994, Boutin, 2001, Browne and Aebischer, 2004, Browne and Aebischer, 2005, Eraud et al., 2009, Eraud et al., 2013). There is potential for parasitic infection to interact with these stressors and increase extinction risk. A high prevalence of *Trichomonas gallinae* infection was revealed by a survey conducted in 2011, along with mortality of both adults and nestlings from the disease trichomonosis (Lennon et al., 2013, Stockdale et al., 2015). Furthermore, a high prevalence of blood parasites was also indicated by a survey conducted in 2011, although the sample size was small (Stockdale, 2012). Hence investigating the epidemiology and potential impacts of these infections is of paramount importance.

Molecular epidemiology is the application of molecular techniques to the study of epidemiology, which aims to determine the causation and dynamics of disease in a population. It allows reliable detection and precise identification of parasite strains based on DNA sequences. This information is imperative to understanding the complex interactions that occur within the host and the resulting impact on host populations. In this thesis I evaluate the potential impact of *T. gallinae* infection, which can cause the disease trichomonosis, on Turtle Doves and describe how disease surveillance is achieved through the application of molecular techniques. To further investigate the epidemiology of *T. gallinae*, a novel transmission route is assessed in terms of its risk to Turtle Doves and both recognized and suspected reservoir hosts are screened for infection with genetic variation used to infer transmission pathways. Data collected on the breeding ecology of Turtle Doves are analysed in respect to parasite and strain prevalence to inform of potential impacts of infection.

1.2 Disease as a conservation issue

Historically, disease was over-looked as a driver of extinction. The IUCN Red List has records of species extinctions which date back to 1500 but disease was only cited as a contributing factor from the mid- 1800s onwards (being involved in less than 4% of known extinctions from 1900), which coincides with the development of definitive tests for infectious diseases (Smith et al., 2006). Its contribution is mostly implied after a species has gone extinct as it is difficult to prove in retrospect (McCallum and Dobson, 1995, Smith et al., 2006, McCallum, 2012). There are very few cases whereby disease is the sole or main factor in species extinction. Other drivers of decline have normally reduced the population to a vulnerable level whereby disease was able to eradicate the remaining individuals. The Polynesian Tree Snail *Partula turgida*, was reduced to existing only in the form of captive populations by the introduction of the predatory snail, *Euglandina rosea*, into their Polynesian range (Mace et al., 1998) Extinction was caused by infection by a microsporidian parasite which eradicated the last remaining population held in captivity (Cunningham and Daszak, 1998, Coote and Loeve, 2003). Similarly, canine distemper (caused by the Canine Distemper Virus CDV) was close to causing the extinction of Black-footed Ferrets *Mustela nigripes*, as it wiped out the only remaining wild population and severely affected a captive breeding program (Thorne and Williams, 1988). A pathogen can be solely responsible for the rapid decline of a species, demonstrated by the virulent pathogen of amphibians, *Batrachochytrium dendrobatidis* (hereafter referred to as Bd) which emerged in the 1970s and has since caused the declines of hundreds of species world-wide and numerous extinctions (Skerratt et al., 2007, Berger et al., 2016). Disease can cause local population crashes in species of little conservation concern which may be alarming due to the initial high rates of mortality but recovery is possible with population abundance stabilizing at similar levels to those present before the outbreak (Hochachka and Dhondt, 2000). Long-term population depression caused by chronic infection has been hypothesized but is more difficult to demonstrate in wild populations as the impact has to be

disentangled from other drivers of decline (Friend et al., 2001). Furthermore, the impact of parasite infection depends on the ability to mount an effective immune response. At the level of the individual, this is influenced by nutritional status, stress and the presence of appropriate antibodies due to either a previous history of infection or those passed on maternally (Lyles and Dobson, 1993). Immunosuppression can occur when faced with immediate environmental stressors (Raberg et al., 1998). Predation pressure has been shown to induce chronic stress and down-regulate the immune response resulting in a higher prevalence and intensity of infection (Clinchy et al., 2004, Navarro, 2004, Clinchy et al., 2013). Tawny Owls *Strix aluco*, enduring low food availability have higher parasite loads and those that experienced low food availability whilst in the nest had higher parasite burdens as adults, revealing a long-term effect on infection (Appleby et al., 1999). Reproduction may also compromise immunity as it is an energetically demanding activity, with support from studies revealing an increase in reproductive effort being linked to increased parasitaemia (Lochmiller and Deerenberg, 2000, Knowles et al., 2009, Christe et al., 2012). Furthermore, seasonal variation in infection prevalence with an increase observed during the breeding season lends further support to the costs of reproduction negatively impacting immunity (Applegate, 1970, Applegate, 1971, Cosgrove et al., 2008).

1.3 Transmission routes

Understanding transmission routes provides the foundation for investigating the epidemiology of parasite infection. Transmission routes can be direct, from individual to individual, or via a medium such as food or water. Transmission of Bd occurs through contact with the infected skin of hosts and was proved with transmission experiments using captive- bred sibling frogs (Berger et al., 1998). The bacterium *Pasteurella haemolytica*, responsible for pasteurellosis, was proved to spread through direct contact after transmission experiments with unaffected Domestic Sheep and Rocky Mountain Bighorn Sheep *Ovis canadensis canadensis*, who succumbed to pneumonia after being infected (Onderka and Wishart, 1988). Spillover events, where contact between domestic animals and wildlife populations can result in a parasite being transferred, are thought to be largely responsible for the transmission of CDV. For example, the morbillivirus responsible for an epidemic in a lion *Panthera leo*, population in Serengeti National Park, Tanzania was identified as being closely related to one isolated from domestic dogs (Roelke-Parker et al., 1996). Transmission routes can be indirect via the environment. A parasites ability to persist in the environment or survive outside a host has very different implications for the impact on the host population (De Castro and Bolker, 2005). Theory suggests that in order to drive extinction, the parasite must be able to survive in the environment until it is transmitted (De Castro and Bolker, 2005). Parasites able to persist within this environmental context may require physiological adaptations. Protozoans may form a hardy, thick wall for protection during a life stage known as oocyst. Well-known species include *Cryptosporidium parvum* and *Giardia lamblia*, both responsible for severe diarrhoea in humans and animals which can lead to

dehydration and possibly death in those who are immuno-compromised (Current and Garcia, 1991, Adam, 2001). The oocyst stage of *C. parvum* is durable and resistant to treatment of contaminated water supplies by chlorination, causing problems in controlling the spread of this parasite (Carpenter et al., 1999).

Crowding within host populations or increased cross-species contact allows more opportunities for transmission and therefore facilitates the spread of infection (Altizer et al., 2006). Urbanisation and agricultural intensification have caused shifts in the ecology of wildlife populations that have altered exposure to transmission routes (Daszak, 2000). Removing foraging habitats may increase transmission of disease as individuals feed at higher densities on the fewer remaining resources and come into contact with a greater of range of species (Carrete et al., 2009). Crowding is further encouraged with the implementation of supplementary feeding as a conservation tool. It has been applied to the management of Red Deer *Cervus elaphus*, in the form of winter feeding (Smith, 2001). However, a range of viral, bacterial and protozoal parasites were detected in populations of Elk provided with supplementary feeding, with brucellosis in particular, occurring at a significant prevalence and causing abortions (Smith, 2001). A favourite past-time of many households, garden bird watching, is instigated through the provision of garden bird feeders but this practice has been linked to the spread of conjunctivitis in House Finches *Carpodacus mexicanus*, in the US and trichomonosis in garden birds in the UK (Hartup et al., 1995, Robinson et al., 2010). In some cases, the benefits of resource provisioning may outweigh the potential for disease transmission by improving nutrition in the host and actually benefiting host immunity (Becker et al., 2015). The case for resource provisioning ought to be evaluated within the context it is being employed with consideration of the wider impacts in that particular environment (Tollington et al., 2015).

1.4 Host range

Parasites are usually considered as either specialists with a narrow host range or generalists with a broad host range. In simple models of host-parasite dynamics, host-specific parasites that are directly transmitted increase with the number of susceptible and infectious hosts (Lafferty and Gerber, 2002). They are unlikely to cause extinction due to their density-dependent nature because if the host population crashes then the associated parasite is lost along with it (Dobson and Hudson, 1992, Lafferty and Gerber, 2002, Langwig et al., 2012). Host-specific parasites are theoretically only able to drive their hosts to extinction if transmission is frequency-dependent, meaning the transmission of the parasite does not depend on host density (McCallum, 2012). This is considered a possibility in the case of Tasmanian Devil Facial Tumour Disease (DFTD) which is an infectious cancer spread by biting, which occurs frequently during sexual encounters and quarrels over food (Pearse and Swift, 2006, McCallum, 2012). The dynamics of generalist parasites lack such restrictions, as transmission rate does not rely on the density of a single host species (Lafferty and Gerber, 2002). Species which are susceptible to infection and are able to maintain infection at

the population level are known as reservoir hosts (Haydon et al., 2002). Parasite prevalence can remain at high levels in reservoir hosts who suffer no ill-effects, despite the impact it has on susceptible host populations. The Squirrel Pox Virus (SQPV) in the UK is an example of this, whereby the parasite is maintained in circulation in the reservoir host of the Grey Squirrel *Sciurus carolinensis* who are resistant to the disease, whilst it drives the decline of the susceptible Red Squirrel *Sciurus vulgaris* (Sainsbury et al., 2000, Tompkins et al., 2002). There may be multiple reservoir host species, which has important consequences for the exposure of the parasite to the susceptible host population. A large number of American tegumentary leishmaniasis (ATL) cases in humans in Minas Gerais State of Brazil resulted in a study to determine the prevalence and genetic lineages of *Leishmania* sp. in suspected reservoir hosts of domestic dogs and small forest mammals (Quaresma et al., 2011). This revealed intense transmission patterns of the parasites and a high prevalence of infection in rodents and marsupials suggesting that these particular hosts were important in maintaining the parasite (Quaresma et al., 2011). Identifying reservoir hosts in the field allows the assessment of exposure risk and facilitates the design of effective control measures (Haydon et al., 2002).

1.5 Impact of infection on wildlife populations

It is often the impact of a parasite that first draws our attention and instigates research into whether it is accountable for observed cases of mortality or morbidity. A complication in establishing the cause of death in the affected individual is the likelihood of the carcass being scavenged, rendering recovery for necropsy difficult. This is often the case with small and even large animals (Prosser et al., 2008, Dunbar et al., 2000). When pasteurellosis was suspected as the cause of population declines and low survival of Pronghorn *Antilocapra americana* neonates, the neonates were monitored through radio-tagging (Dunbar et al., 2000). When the radio signal indicated mortality, the animal was recovered as soon as possible but the majority of carcasses consisted mostly of just a head and neck due to scavenging, rendering the identification of cause of death impossible (Dunbar et al., 2000). A definite diagnosis would have required observing lesions typical of septicemic pasteurellosis and isolation of the etiological agent, *Pasteurella* spp., by swabbing tissues other than the tonsils or naso-oropharyngeal area, where *Pasteurella* spp. can be found even in healthy animals (Onderka and Wishart, 1988, Dunbar et al., 1990).

If a population is already declining due to other factors, it can be difficult to disentangle the relative impacts of these and those of the parasite. An example is the Red Squirrel in the UK, whose declines were traditionally explained by competition with the introduced Grey Squirrel (Kenward and Holm, 1993, Kenward et al., 1998). When the SQPV was discovered to be highly pathogenic in the Red Squirrel, it was assumed to be responsible for observed cases of mortality in the wild where victims were observed with the same symptoms (Tompkins et al., 2002). Survival was possible however, and aided by optimal temperature, a continuous food supply, an absence of ectoparasites and lack of predation or

competition pressure (Tompkins et al., 2002). Whilst this is not representative of the natural environment of the Red Squirrel, it is suggestive of their population decline being due to a complex interaction of all these factors, which may also vary between different populations from different habitats. The impact of disease or sub-clinical infection can be much more subtle. It can indirectly affect survival by increasing susceptibility to predation or reducing competitive fitness (Murray et al., 1997, Laiolo et al., 2007, Descamps et al., 2011). There may also be a relationship between disease and fecundity, whereby it reduces breeding productivity, as revealed by American Kestrels *Falco sparverius* infected by the nematode, *Trichinella pseudospiralis*. Infected pairs take longer to lay their first egg, lay fewer eggs than uninfected pairs and the percentage of eggs laid that hatch is also reduced (Saumier et al., 1986). Coinfection by either different strains of the same parasite or by different parasites is an area of research that has not been explored to the same extent in wildlife as in humans, where the focus is on diseases of high risk within developing countries, such as mixed- species malaria infections or HIV and malaria (Mayxay et al., 2004, Ter Kuile et al., 2004). Research has been conducted experimentally on avian species, specifically focusing on coinfection between malaria parasites within the genera *Haemoproteus* and *Plasmodium*. Although it is recognised that different genera of haemosporidian parasites interact differently with their host and other co-infecting parasites, further investigation is warranted to establish the impact of coinfection on wild populations (van Rooyen et al., 2013).

1.6 Turtle Doves

Populations of Turtle Doves are undergoing severe declines and have become a cause of major conservation concern (Tucker and Heath, 1994). Turtle Doves are listed on the UK's Biodiversity Action Plan (BAP) and under DEFRA's Public Service Agreements (PSA) targets to reverse the long-term decline in abundance of farmland birds (Anon, 1998). They have recently been listed as having a conservation status of Vulnerable (Birdlife International, 2015). The Turtle Dove is a trans-Saharan migrant, spending the winter in sub-Saharan Africa with its breeding range extending from the western Palearctic to China, where Britain is on the north-western edge of its range (Figure 1.1) (Tucker and Heath, 1994, Eraud et al., 2013). Turtle Doves have undergone a 93% decline in abundance in the UK between 1995- 2014 (Figure 1.2) (Harris et al., 2016). Population declines have also been observed in other European countries, with the latest estimation being -78% between 1980- 2013 (PECBMS, 2015).

Murton et al., (1964; 1968) performed the first ecological study of Turtle Doves in Britain, when their numbers and geographical range were increasing (Murton et al., 1964). They were observed to feed on weed seeds, specialising on *Fumaria* and grass species from hay fields, waste lands and cultivated crops such as peas or wheat (Murton et al., 1964, Murton, 1968). Agricultural land has undergone intensification since the 1960s and a change in foraging behaviour was observed during the late 1990s with further changes possible since then (Browne and Aebischer, 2003a). Wheat and rape seeds now form the main components of

their diet, compared to weed seeds recorded previously, reflecting their dependence on food derived from farming and no longer using natural sites (Browne and Aebischer, 2003a). Although this dietary switch may appear initially beneficial, as the energy values are much higher (Browne and Aebischer, 2003a), the food source may be of poorer quality.

The habitat of Turtle Doves in the UK consists of scrub and hedges around farmland, ideally having weed-rich areas and low open vegetation cover (Browne and Aebischer, 2003a, Browne and Aebischer, 2004, Dunn and Morris, 2012). Turtle Doves nest within thorny bushes such as Hawthorn *Crataegus monogyna*, approximately 1-3m from the ground, have a clutch size of two with incubation lasting approximately 14 days followed by a nestling period of around 15 days (Calladine et al., 1997, Browne et al., 2004). The most recent study, conducted in the 1990s, has shown that Turtle Doves generally rear two broods, not including replacement clutches, with a 35% nest success rate in producing young (Browne and Aebischer, 2004). In the 1960s, Turtle Doves had a relatively long breeding season from May to late August/ September however they now migrate back earlier in autumn (Murton, 1968, Browne and Aebischer, 2003b, Browne and Aebischer, 2004). Overall, Turtle Doves are only producing half the number of young per pair than those in the study based on data collected in 1960s but failure rates of individual nesting attempts are not significantly higher indicating that a reduction in the average number of nest attempts per pair is behind the decline in breeding productivity (Browne and Aebischer, 2004, Browne and Aebischer, 2005). The lack of nesting habitat due to intense hedgerow management may have contributed to reduced breeding performance and earlier termination of the breeding season (Browne and Aebischer, 2003b, Browne and Aebischer, 2004, Browne et al., 2005). Turtle Doves are a migratory species and other factors that may be contributing to their reduction in numbers along their migration route cannot be ignored. They are a legal quarry species in Austria, France, Greece, Italy, Portugal and Spain although the timing and length of the hunting season and bag quotas require more rigorous definitions (Boutin, 2001). The degradation of over-wintering habitat, due to the cutting of acacia for charcoal and periods of drought, have also been highlighted (Marchant et al., 1990, Tucker and Heath, 1994, Browne and Aebischer, 2004). Over-winter food availability in the form of cereal production is positively correlated with survival rates in the Mali-Senegal area (Eraud et al., 2009). Combined with the evidence that Turtle Doves move between over -wintering sites, this supports the hypothesis that they rely on food supply over much larger areas than just within Mali and Senegal and are sensitive to agricultural changes (Eraud et al., 2009, Eraud et al., 2013). To summarize, a number of likely drivers of Turtle Dove population decline have been identified, including: a decrease in breeding productivity, being hunted during migration, degradation of over- wintering habitat and variable food availability whilst over-wintering. Another potential stressor has been revealed by the recent screening of UK Turtle Dove populations showing a high prevalence of infection by the protozoan parasite *Trichomonas gallinae* (Lennon et al., 2013).

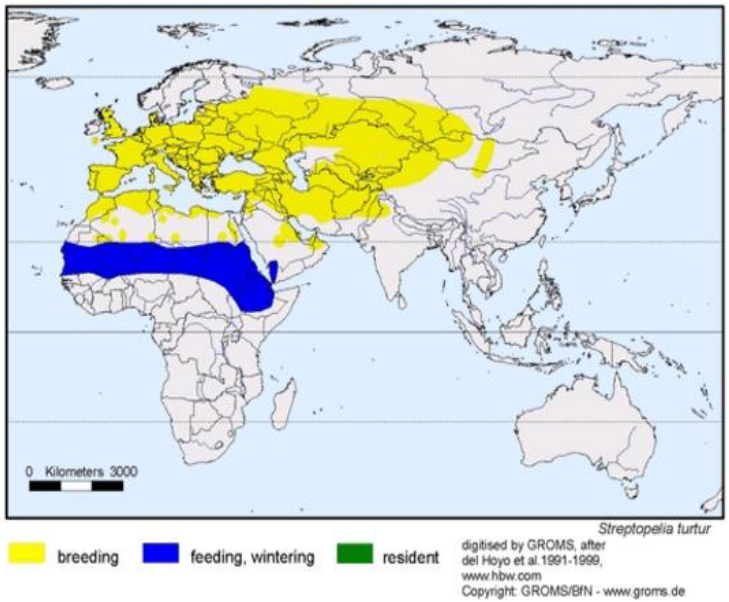


Figure 1.1: A distribution map showing the breeding and over-wintering grounds of Turtle Doves Streptopelia turtur. Taken from http://www.planetofbirds.com/Columbiformes-Columbidae-turtle-dove-streptopelia-turtur

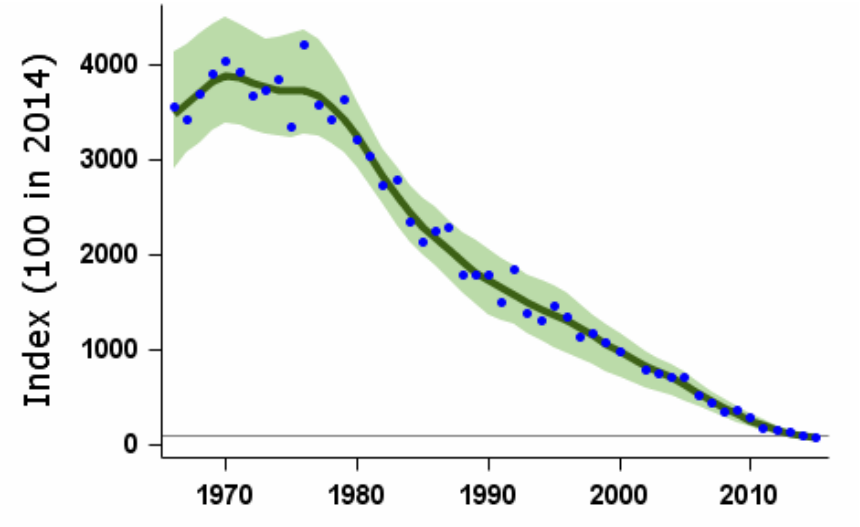
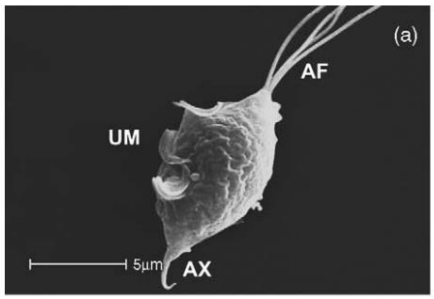


Figure 1.2: Common Birds Census (CBC)/ Breeding Bird Survey (BBS) data from 1966- 2015. Smoothed population index reveals severe declines in Turtle Dove abundance in the UK from the late 1970s onwards. Green shading represent 85% confidence limits. Taken from http://blx1.bto.org/birdtrends/species.jsp?s=turdo

1.7 Trichomonas gallinae

Trichomonas gallinae is an amitochondrial (lacks a mitochondrial organelle), microaerophilic protozoan parasite first recovered from the upper digestive tract of a chicken and a pigeon in 1878 (Stabler, 1947). It belongs to the family Trichomonadidae, a group of amitochondrial flagellated organisms. Species belonging to this family are mostly symbiotic, inhabiting a wide variety of invertebrates and vertebrates, the majority of which form a commensal relationship (Honigberg, 1963). A few species are pathogenic: *Trichomonas vaginalis*, which occurs in the genitourinary system of humans and causes the sexually transmitted infection (STI) trichomoniasis (Rein and Chapel, 1975) and *Tritrichomonas foetus*, which is urogenital and responsible for reproductive failure in bovines (Yule et al., 1989) but has also been identified as causing feline diarrhoeal disease (Levy et al., 2003). *Pentatrichomonas hominis* inhabits the large intestine of a number of mammalian hosts and although typically considered non-pathogenic, it has been linked to cases of diarrhoea in domestic cats and dogs (Gookin et al., 2007, Kim et al., 2010). *Trichomonas gallinae* is known to cause the disease commonly known as 'canker' in Columbiform and Galliform birds (Honigberg, 1963). This disease is also known as trichomonosis, which is the term this thesis will adopt. Trichomonosis is a clinical infection, with symptoms that range from excess salivation and inflamed mucosa to the production of yellow lesions in the upper digestive tract (Stabler, 1947). These may disappear after some days or continue to grow, preventing the bird from feeding and resulting in fatality (Stabler, 1947). Columbiforms are widely believed to be the natural host of *T. gallinae*, with the occurrence in Galliformes also widely recognized, in addition to reports in Falconiformes, Strigiformes, Psittacines and Passeriformes (Stabler, 1947, McKeon et al., 1997, Boal et al., 1998, Krone et al., 2005, Anderson et al., 2009, Park, 2011). The life-cycle of *T. gallinae* is direct, whereby the parasite is transmitted from host to host without the requirement of an intermediate host (Forrester and Foster, 2009). In species of Columbidae, transmission is from parent to offspring, via regurgitated crop milk but is also thought to occur via direct contact between infected and uninfected individuals whilst feeding or billing during courtship (Stabler, 1947, Kocan and Herman, 1971).



Taken from Tasca & Carlie, 2003

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Photo credit: Krysta Rogers

Photo credit: Rebecca Thomas

Figure 1.3: A) A scanning electron microscopy image of the trophozoite of T. *gallinae*. (AF: anterior flagella, UM: undulating membrane, AX: axostyle. Taken from Tasca & Carlie, 2003. B-D) Examples of trichomonosis lesions found in Columbids B) clinical signs used in diagnosis, photo obtained from http://www.irishwildlifematters.ie/animals/pigeons-conditions.html C) gross necropsy reveals lesions in oral cavity of a Band-tailed Pigeon, photo obtained from https://calwil.wordpress.com/tag/trichomoniasis/ and D) the oral cavity of a dead Turtle Dove nestling is dissected revealing trichomonosis lesions, photo taken during fieldwork season of 2013.

T. gallinae is slightly pear-shaped with features including four anterior flagella, an undulating membrane running most of the length of the body with a fifth flagellum along its margin and a lack of a free-trailing flagellum (Stabler, 1947). This species varies greatly in size, from 6.2 – 18.9µm long and 2.3 – 8.5 µm wide (Stabler, 1947). *T. gallinae* reproduces by binary fission and is a clonal, haploid organism (Stabler, 1941).Detection of *T. gallinae* and other pathogenic trichomonads of interest, particularly *T. vaginalis*, has traditionally been through wet- mount microscopy (Fouts and Kraus, 1980). A swab of the sample is taken and rotated in a drop of saline on a glass microscope slide followed by immediate observation for motile trichomonads (Fouts and Kraus, 1980). This process was improved by including a culture step before wet-mount identification which improved the sensitivity of the diagnostic test (Fouts and Kraus, 1980). Indeed, one of the earliest papers describing *T. gallinae* notes that it is easy to grow in artificial media (Stabler, 1947). However, wet-mount microscopy is insufficient as a means to distinguish between *Trichomonas* species due to their morphological resemblance (Hersh, 1985). The method of culture was relied upon for accurate species identification as the media, although containing similar ingredients, still varied slightly in order to support axenic growth of a particular *Trichomonas* species (Diamond, 1962).

Errors in identifying the correct *Trichomonas* species were still made. Large bowel diarrhoea in cats and the association with trichomonad parasites lead to the assumption that *Pentatrichomonas hominis* was the causative agent, as it is known to infect the intestines of a number of mammalian hosts (Romatowski, 1996, Gookin et al., 1999). This parasite still grew, albeit slowly, in media that typically support feline trichomonads (Levy et al., 2003). Gene sequence analysis allowed the identification of *Tritrichomonas foetus*, a trichomonad associated with the reproductive system in bovines, as being the etiological agent in the case of feline trichomonal diarrohea (Levy et al., 2003). The host or site within the host that the trichomonad is found to inhabit is not indicative of the species of Trichomonas. Early reports of *T. tenax*, considered a commensal of the human mouth, being recovered from the respiratory tract and in some cases being associated with clinical symptoms led to a review of the condition, known as pulmonary trichomonosis (Hersh, 1985). It concluded that these infections were opportunistic and resulted from the aspiration of the trichomonad from the mouth into the lungs whereby it was able to thrive in the already diseased environment (Hersh, 1985). Furthermore, there have been a number of cases of human trichomonads such as *T. vaginalis* and *Pentatrichomonas hominis* (Davaine, 1860) being recovered from sites within the human body not considered ‘normal’, which may also be the result of opportunistic infections (Hersh, 1985). These examples serve to demonstrate that upon the discovery of a trichomonad, a reliable process of identification ought to be followed, such as gene sequence analysis (Levy et al., 2003). An example of the potential outcome of an opportunistic infection was exemplified recently with the case of

trichomonosis becoming an emerging infectious disease in finches (Robinson et al., 2010). An outbreak in the UK caused declines in the breeding populations of Greenfinches *Chloris chloris* and Chaffinches *Fringilla coelebs* by 35% and 21% respectively (Robinson et al., 2010). The disease subsequently spread to Fennoscandia and central Europe (Peters et al., 2009, Neimanis et al., 2010, Robinson et al., 2010, Lawson et al., 2011b, Zadravec et al., 2012, Ganas et al., 2014). A clonal strain of *T. gallinae* (A1) was identified as the causative agent by both morphological and molecular analysis (Robinson et al., 2010, Lawson et al., 2011a). Global efforts in this area of research have revealed that it is not the sole etiological agent of trichomonosis outbreaks as another variant (A2) was isolated from the Pacific Coast Band-tailed Pigeon *Patagioenas fasciata monilis* population in California during epidemics (Girard et al., 2014b). Furthermore, a new species of *Trichomonas* (*T. stableri* n.sp.) was also detected during these epidemics confirming a non-clonal etiology of avian trichomonosis in Band-tailed Pigeons (Girard et al., 2014a). These discoveries would not have been possible without identifying the parasites using molecular techniques. It is becoming standard to include this type of analysis for the accurate and reliable identification of etiological agents of infection but expanding geographical and temporal monitoring is imperative for progress within this field.

1.8 Metabarcoding for parasite identification and molecular epidemiology

Metabarcoding is the combination of DNA sequence based identification and new, massively parallel, high-throughput sequencing techniques. It is conducted on a Next Generation Sequencing (NGS) platform which allows the sequencing of multiple individual molecules and has a much higher sequencing capacity than traditional Sanger sequencing, resulting in millions of reads per run (Taberlet et al., 2012). It has facilitated the jump from identifying single organisms to whole communities simultaneously (Cristescu, 2014). Conserved loci are adopted as a 'barcode' for species identification. The 16S rRNA gene is ubiquitous in bacterial genomes and used widely to differentiate bacterial species, with 99% sequence identity used to classify species or phenotypic clusters (Medini et al., 2008). A notable and debated example is that of the COI barcode, originally proposed as a universal barcode to be used across all species but since shown to be mostly appropriate for vertebrates (Hebert et al., 2003, Vences et al., 2005, Rubinoff et al., 2006, Bhadury et al., 2006, Eberhardt, 2010). The barcoding system provides a means to rapidly identify a species but it relies upon a database, containing reference sequences linked to the species that have been vouched for (Hajibabaei et al., 2007). NGS technology can be applied to the characterization of species composition from either environmental samples, also known as eDNA (Taberlet et al., 2012), or bulk samples containing whole organisms (Brandon-Mong et al., 2015). This thesis will focus on the application of the latter, which involves identifying organisms from within a certain taxonomic group where the DNA is of high quality, allowing the use of standard barcode markers which amplify relatively long fragments (Taberlet et al., 2012). This has been successful in assessing the diversity of benthic communities in both marine and freshwater

ecosystems, soil fungal communities and rainforest nematode diversity within soil, litter and canopy habitats (Porazinska et al., 2010, Hajibabaei et al., 2011, Schmidt et al., 2013, Leray and Knowlton, 2015). One test involving the construction of known bulk samples showed similar recovery rates of identified species when comparing morphology, Sanger sequencing and NGS but another test revealed NGS performing much better by recovering a wider range of taxa (Hajibabaei et al., 2011, Yu et al., 2012) and it is fast becoming the standard approach. The main limitations involve artefactual sequences being generated during PCR or sequencing. PCR-generated errors include point mutations and the formation of chimeric molecules (Kobayashi et al., 1999, Acinas et al., 2005). Amendments of the PCR protocol and employing a 99% similarity cut-off point when reporting sequence diversity have been suggested to minimize the impact of such errors (Acinas et al., 2005). There are now programs during bioinformatic analysis that can identify PCR chimera product and remove it (Coissac et al., 2012). Sequencing errors are due to the misreading of homopolymers whereby polymerase slippage during the elongation step can result in either insertions or deletions (Margulies et al., 2005, Huse et al., 2007). The end result is length variation in homopolymers with mostly shorter artefactual reads (Taberlet et al., 2012). It is recommended to discard low frequency reads, which are suspected to be artefacts, with the benefits outweighing the risk of underestimating biodiversity (Reeder and Knight, 2010, Brown et al., 2015). There are also a number of programs that deal with and remove noisy reads during bioinformatics analysis (Coissac et al., 2012). If not dealt with correctly, these errors may result in the misclassification of sequences, which could mislead estimates of genetic variability and the level of diversity that is recorded (Coissac et al., 2012). The costs and accessibility of NGS has thus far restricted its application to disease ecology but as costs are falling, NGS is becoming part of routine human disease surveillance and clinical diagnostics (Metzker, 2010, Boyd, 2013, Roetzer et al., 2013), so these technological advances are likely to be subsequently employed in livestock and wildlife health (Benton et al., 2015). Challenges exist relating to the storage and quality control of the volume of data that is produced by NGS, and the differences in bioinformatic protocols adopted by different research groups mean direct comparisons of results is difficult, but the potential of NGS to determine fine-scale epidemiological patterns and processes is vast (Pop and Salzberg, 2008, Metzker, 2010, Koser et al., 2012, Benton et al., 2015).

1.9 Thesis

The general aim of this thesis is to investigate the molecular epidemiology of *T. gallinae* with a focus on infection in Turtle Dove populations, and whether it may be a contributing factor to their decline. In Chapter 2 I establish the prevalence of *T. gallinae* infection in Turtle Dove populations and examine temporal and geographical variation in the strain composition by applying molecular techniques to parasite detection and identification. Furthermore, I test the application of NGS technology to disease surveillance of a free- ranging population. In Chapter 3 I investigate the risk that supplementary feeding and other shared resources pose

as a transmission route, particularly as it will inform future conservation management of Turtle Doves in the UK. In Chapter 4 I review the reservoir host population of *T. gallinae*, considering the recent shifts in the host range of *T. gallinae*, as a result of the spillover from species of Columbidae to Passerines (Robinson et al., 2010, McBurney et al., 2015). Exploring the genetic variation of *T. gallinae* strains in reservoir hosts in addition to environmental resources will increase our understanding of *T. gallinae* transmission. In Chapter 5 I examine data on the breeding success of Turtle Doves collected as sampled birds are radio-tracked over the breeding season. This leads to the discovery of their nests which are subsequently monitored to record measures of breeding productivity. In one year, post-fledging survival is also monitored by radio-tracking. This information is analysed in conjunction with the results from molecular analysis detailing *T. gallinae* infection status. Furthermore, coinfection with blood parasites is also considered. Blood samples are taken from the birds and simultaneously analysed with the *T. gallinae* samples by the application of NGS. This provides information on the lethal and sub-lethal impacts of coinfection.

Chapter 2

Prevalence and strain composition of *Trichomonas gallinae* infecting Turtle Dove populations.

2.1 Introduction

Disease surveillance is the ongoing monitoring, analysis and management of associated health risks of disease (OIE 2006). It involves gathering information to aid the understanding of disease threats and outbreaks whilst also providing an early warning system of emerging pathogens allowing prompt management interventions (Artois et al., 2009). A survey, on the other hand, assesses disease or pathogen presence at one point in time and has the potential to highlight a system that needs monitoring (OIE 2006). The techniques used for both purposes have evolved considerably over the last two decades. One limitation of traditional methods used in disease surveillance was the reliance on visible signs of disease in an individual or population. If the disease was not immediately recognisable, a description of the related syndrome would allow the temporal and spatial tracking of disease incidents (Fuchs and Weissenbock, 1992, Mayer et al., 1997). Surveillance that relies on morbidity may overlook infections whose clinical signs are subtle, short- lived or where variation is present in the host- pathogen interaction (Artois et al., 2009). The nature of the ecosystem may result in cases of mortality being missed, as immigration, recruitment and high scavenging rates could mask population declines (Prosser et al., 2008, George et al., 2015). One approach to diagnosing the disease requires identification of the pathogen which involves technological expertise and can be limited to specialist laboratories, for example detection of rabbit haemorrhagic disease (RHD) required electron microscopy of the liver when the animal had already succumbed to the disease (Delahay et al., 2008). The ability to identify the etiological agent, whilst the animal is alive and without the presence of clinical signs, would enable the detection of sub-clinical infection and pathogen carriers and contribute to effective monitoring of the pathogen (Kaandorp, 2004).

2.1.1 *Trichomonas gallinae*

Previous screenings of UK Turtle Doves *Streptopelia turtur*, which hold the conservation status of Vulnerable, revealed a high prevalence of infection (86%) by *Trichomonas gallinae* (Lennon et al., 2013, Birdlife International, 2015). Infection by *T. gallinae* has been linked to mortality in both adult and nestling Turtle Doves (Stockdale et al., 2015). Furthermore, this parasite is a cause of conservation concern in another vulnerable Columbid population, the Mauritian Pink Pigeon *Columba mayeri*, where it is responsible for decreased survival rates in adults and is a major mortality factor in squabs and fledglings (Bunbury et al., 2007, Bunbury et al., 2008). Therefore initiating disease surveillance of the Turtle Dove population was fully warranted.

Traditionally, detection of infection by *Trichomonas* species was via wet mount microscopy (McKeon et al., 1997). The further development of *T. gallinae* detection techniques was driven by the relevance of its sister taxa, *T. vaginalis*, to human health. *T. vaginalis* has high prevalence world-wide, being the highest reported non-viral STI (Van der Pol, 2007). The similarities between these species, morphologically and genetically, resulted in transferable knowledge in the application of diagnostic and treatment tools, such as use of the InPouch culture kit specific to *T. vaginalis* that is also the most sensitive detection method for *T. gallinae* (Bunbury et al., 2005). Presence or absence of the parasite is confirmed by polymerase chain reaction (PCR) and identification of the strain can be achieved by DNA sequencing. The genetic marker typically used for trichomonads is the ITS1/5.8S/ITS2 ribosomal region. This genomic region encodes the small subunit 5.8S rRNA and flanking internal transcribed spacers (ITS1 and ITS2), which are subsequently removed by RNA processing (Katiyar et al., 1995). The 5.8S rRNA sequence is relatively conserved, differing only by a few nucleotides between genera, whereas the non-coding ITS sequences are short but diverse, with minimal potential secondary structure, proving suitable for differentiation of evolutionary relationships at the inter-species level (Katiyar et al., 1995). It has become the unspoken species barcode, exemplified by the studies using it as a diagnostic tool to detect infection by the parasitic *Trichomonas* species (Gaspar da Silva et al., 2007, Gerhold et al., 2008, Sansano-Maestre et al., 2009, Martinez-Herrero et al., 2014). Furthermore, it has revealed strain variation within *T. gallinae* and phylogenetic analysis has suggested some interesting evolutionary relationships between the strains, with some appearing more closely related to other *Trichomonas* species (Gerhold et al., 2008, Grabensteiner et al., 2010). To further improve strain resolution, the hydrogenosomal Fe- hydrogenase (Fe-hyd) gene has been recently developed for intra specific variation, although it is a single copy gene and known to be difficult to amplify (Lawson et al., 2011a, Sansano-Maestre et al., 2016). It has allowed inferences on epidemiological processes in other avian systems, such as hypothesized spillover events (McBurney et al., 2015).

There are variations in the strains of *T. gallinae* as some are associated with being non-pathogenic or moderately pathogenic and others are associated with virulence (Stabler, 1954, Lawson et al., 2011a). In this case, a strain is a genetic variant within a species. Experimental trials have been performed with strains of *T. gallinae* and demonstrated that highly virulent strains exist which ultimately cause death and that there may be a genetic basis behind this pathogenicity (Stabler and Kihara, 1954, Honigberg et al., 1971, Narcisi et al., 1991). Protection against the virulent strain may be possible with acquired immunity from a previous infection with a non-pathogenic strain (Stabler, 1948). Recently, a virulent strain has been repeatedly isolated from free-ranging bird populations. Raptors in Spain displaying clinical signs of trichomonosis were infected with one particular genotype (Sansano-Maestre et al., 2009). The pathogen responsible for the trichomonosis outbreak in British finches was identified as a clonal strain of *T. gallinae* (Lawson et al., 2011a). Furthermore, a British Woodpigeon suffering

clinical signs of trichomonosis was infected with a strain that clustered with this virulent genotype (Lennon et al., 2013). Although these findings provide evidence that virulent strains of *T. gallinae* may be differentiated genetically, the clinical virulence of strains can only be specifically addressed when other disease agents that cause similar symptoms are ruled out. Whether genetic differentiation corresponds to variable virulence would have to be further tested with animal trials (Grabensteiner et al., 2010).

In order to identify potential ecological drivers of infection, I used data collected from screening Turtle Dove populations from different countries (UK, France, Senegal, Burkina Faso) and in some cases, over multiple years (UK = five years, Senegal = two years) to determine whether there was a variation in prevalence of *T. gallinae* infection or in the genetic strains of *T. gallinae* infecting these populations. The application of a NGS platform in multiple strain detection was tested with considerations of whether this system should be implemented in routine disease surveillance of wildlife populations.

2.2 Methods

2.2.1 Sample collection

In order to collect samples from Turtle Doves in the UK, between one and two temporary bait sites were set up at seven farms in Essex (Abbotts Hall Farm: 51°79'N, 0°84'E; Cobbs Farm: 51° 98'N, 0° 71' E; Flambirds/ Stow Maries aerodrome: 51° 67'N, 0°63'E; Limesbrook farm: 51°77'N, 0°77'E; Perry Green Farm: 51°86'N, 0°61'E; Sunnymead Farm: 51°85'N, 0°98'E and Upp Hall Farm: 51°88N', 0°73'E;), four farms in Norfolk/ Cambridgeshire (Ouse Bridge Farm: 52°56'N, 0°32'E, Manor Farm: 52° 25'N, 0°11'E, Hobbs Lot Farm: 52°26'N, 0°15'E, Orwell Pit Farm: 52°25'N, 0°15'E) and three farms in Hampshire (Damers Farm: 50°98'N, 01°92'W; Kings Farm: 50°98'N, 01°92'W and Martin Down barn: 50°96'N, 01°92'W) (Figure 2.1). The farms in Essex and Cambridgeshire are located in typical agricultural areas of the UK, surrounded by other arable farms and small towns or villages. The farms in Wiltshire are also arable but set within semi-natural habitat as they border a National Nature Reserve of chalk downland with areas of managed scrub land and grassland. All farms had regular sightings of Turtle Doves for at least two years prior to sample collection. Between May and August, whoosh nets were used to catch Turtle Doves using these bait sites (Redfern and Clark, 2001). All Turtle Doves were swabbed using a sterile viscose swab that was inserted into the oral cavity and used to swab the mouth cavity, oesophagus and crop. The swab was inoculated into an individual InPouch TF culture kit (Biomed Diagnostics, Oregon), sealed and incubated at 37°C for three to seven days in order to culture the parasite. All birds were ringed using standard BTO metal rings, aged and sexed with a range of biometrics taken, such as wing length, head to bill and tarsus length (Redfern and Clark, 2001).

Figure 2.1: A map of the sampling locations in the UK.

Sample collection in France was undertaken at two different locations along the French Atlantic coast: Chizé Forest (46°12'N, 0°42'W) and Oléron Island (45°93'N, 01°28'W). Samples from these Turtle Dove populations represent a different European breeding population and are from different habitats to the UK. Chizé Forest covers ~3435 hectares and is a mixture of mature deciduous and coniferous woodland with areas of scrub. Chizé Forest is surrounded by areas of arable farmland and small towns. Oléron Island encompasses a wide variety of habitats such as farmland, small woodland areas and villages. Birds were caught using baited potter traps during a two week period (24th May- 7th June 2014) and sampled using the same methods as in the UK.

In order to compare the prevalence of *Trichomonas* infection with populations on wintering grounds, Turtle Doves were caught in Oursi, Burkina Faso (14°68'N, 0°46'W) from November 2012- April 2013. The capture site is located in the Sahel Reserve, on the northern shore of a seasonal lake bordered by a variety of Acacia sp. The surrounding habitat is desert with dune systems running from east to west. Another wintering population of Turtle Doves was sampled at a site in Senegal (14°38'N, 16°80'W) in 2014 and 2015. This site is an enclosed area that has been protected from grazing allowing semi- mature woodland to form, consisting mostly of acacia bushes. Small allotments growing fruit and vegetables are regularly maintained within the site. A small reservoir was present during 2014 but a particularly dry season during 2015 resulted in the reservoir drying out. Outside of this area, the landscape is arid with small settlements scattered locally and low densities of livestock allowed to graze freely. Birds were caught using mist nets in 2014 and a combination of whoosh nets and mist nets in 2015. Sampling was performed following the same methods as the UK. Samples from Burkina Faso and Senegal 2015 had to be stored for a period before importation and subsequent processing. Samples from Burkina Faso were stored in refrigerated conditions (4°C) for approximately 18 months before being imported. These conditions were not ideal, as storage in -20°C was recommended, therefore sample degradation is likely. In order to address some of the logistical issues that may be encountered with collecting samples from abroad, two different methods were compared for Senegal samples in 2015: ethanol storage and Whatman FTA card storage. After the samples were incubated for 7 days within the InPouch, a few drops of the culture media was transferred to a FTA card using a disposable pipette and allowed to air dry. The remainder of the culture media was transferred to 1.5ml Eppendorf tubes, leaving enough room for an equal volume of absolute ethanol to be subsequently added to each tube.

2.2.2 Permits and ethical approvals

Statement on ethics committee approval

In the UK, catching and ringing was carried out under a British Trust for Ornithology licence held by Jenny Dunn and sampling for parasites was carried out under licence from the Home Office (PPL 70/7641) which was approved by the University of Leeds ethical review board. In France, catching and ringing took

place as part of the research of Office National de la Chasse et de la Faune Sauvage. Capturing and sampling for parasites was approved by Le Prefet de la Region d'Ile-de-France, Prefet de Paris. Ringing and sampling procedures carried out in Africa were identical to those approved under HO legislation in the UK and a permit for this was granted by the Director of Wildlife and Hunting in Burkina Faso and Direction des Eaux, Forêts, Chasses et de la Conservation des Sols in Senegal. Samples were imported under licence from DEFRA.

2.2.3 Parasite isolation

Parasites were isolated following the protocol of Riley et al., (1992), modified as follows: ~2.5ml of culture was centrifuged at 3200rpm for 5 minutes, the resulting pellet was washed with 1ml of phosphate-buffered saline (PBS) by centrifugation and then re-suspended in 200µl of PBS. These samples were then stored at -20°C.

2.2.4 DNA extraction

The methodology concerning the parasite samples collected from the UK between 2011 and 2012 is described as per Lennon et al., (2013) and Stockdale et al., (2014) respectively. Two different DNA extraction methods were used (Table 2.1): DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions or a modified ammonium acetate method (Nicholls et al., 2000). The latter comprised of the following: the parasite pellet had 250µl digestion buffer (20mM EDTA, 50mM Tris, 120mM NaCl, 1% SDS, pH 8.0) and 50µg of Proteinase K (Sigma- Aldrich, UK) added to it whereby it was digested at 37°C overnight (Nicholls et al., 2000). After digestion, 300µl of 4M ammonium acetate was added and the samples were vortexed every 10 minutes for a period of 30 minutes at room temperature. The samples were centrifuged at 13000rpm for 10 minutes and the supernatant kept by transferring to another Eppendorf, discarding the pellet. 1ml of 100% ethanol was added to the supernatant, vortexed and spun at 13000rpm for 10 minutes to precipitate the DNA. The supernatant was discarded and 500µl of 70% ethanol was added to the DNA pellet before being spun at 13000rpm for 5 minutes in order to wash the pellet. The supernatant was discarded and the pellet air- dried for 1-2 hours. The DNA pellet was dissolved in 20µl- 50µl low TE buffer, depending on the size of the pellet, in a water bath at 65°C. The extracted DNA was stored at -20°C.

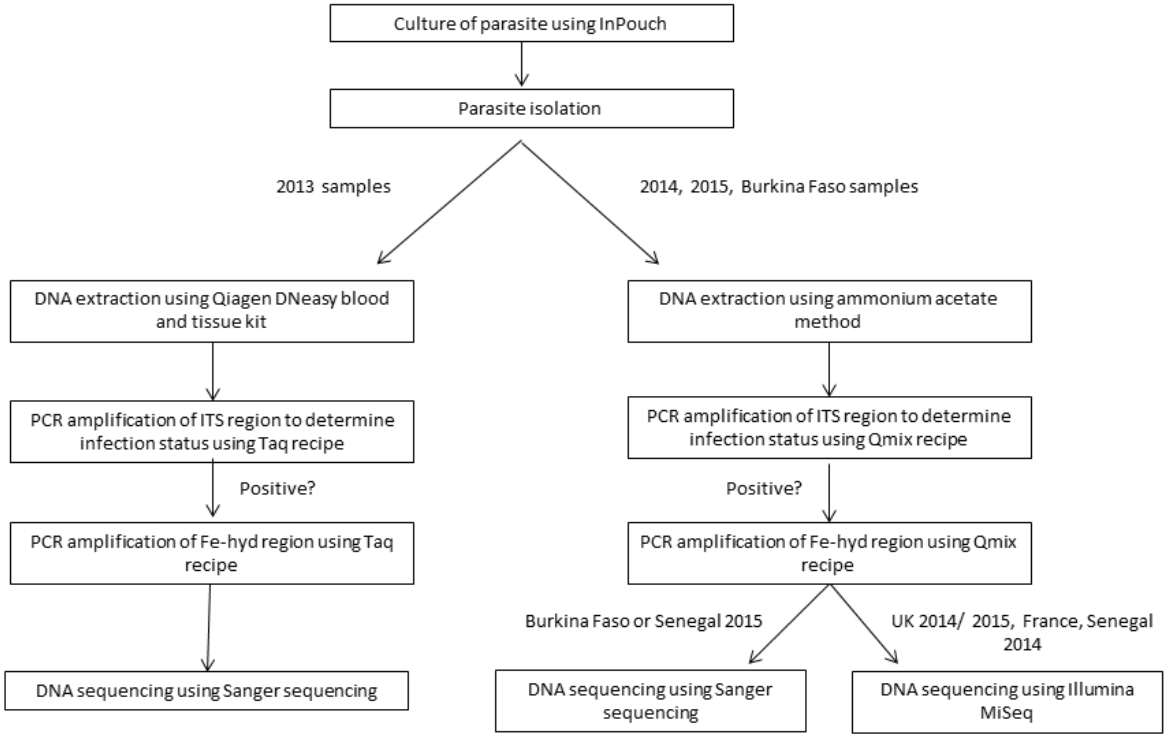


Figure 2.2: Flowchart demonstrating the methodology for the sample flow.

Table 2.1: Method of genetic analysis used for each dataset. The lab work conducted on the 2014- 2015 samples was courtesy of the NERC Biomolecular Analysis Facility (NBAF), Sheffield which is funded by the Natural Environmental Research Council, UK.

			Method of analysis		
Country	Year	DNA extraction	PCR recipe		Sequencing
			ITS	Fe-hyd	
UK	2013	DNeasy blood and tissue kit	Taq	Taq, amplified full length gene	Sanger sequencing (Beckman Coulter Genomics)
	2014	ammonium acetate method	Qmix	Qmix, 4 overlapping primer pairs	Illumina MiSeq
	2015	ammonium acetate method	Qmix	Qmix, 4 overlapping primer pairs	Illumina MiSeq
France	2014	ammonium acetate method	Qmix	Qmix, 4 overlapping primer pairs	Illumina MiSeq
Burkina Faso	2012/2013	ammonium acetate method	Qmix	Did not run	Sanger sequencing
Senegal	2014	ammonium acetate method	Qmix	Qmix, 4 overlapping primer pairs	Illumina MiSeq
	2015	ammonium acetate method	Qmix	Did not run	Sanger sequencing

2.2.5 PCR amplification of the ITS 1/ 5.8S/ ITS 2 ribosomal region

Two different PCR recipes were used, depending on when samples were collected (Table 2.1), which involved the same set of primers, TFR1 [f] (5'- TGCTTCAGTTCAGCGGGTCTTCC -3') and TFR2 [r] (5 '- CGGTAGGTGAACCTGCCGTTGG -3'), to target a 400bp length of the ITS1/ 5.8S/ ITS2 ribosomal region of the genome (hereafter referred to as the ITS region). The PCR reaction involving Go Taq Hot Start Polymerase (Promega, UK) followed the protocol of Robinson et al., (2010) and comprised of the following: 1X PCR buffer (Promega, UK), 2mM Magnesium chloride ($MgCl_2$) (Promega, UK), 0.2mM dNTP mix (Promega, UK), 0.5µM forward and reverse primer (Sigma- Aldrich, UK), 1.25U/µM of Go Taq Hot Start Polymerase (Promega, UK) and a volume of molecular grade water to bring the total PCR reaction volume to 49µl whereby 1µl of DNA was then added. A negative control of molecular grade water and a positive control of T. *gallinae* were included in every run of PCR. A positive control of T. *gallinae* was provided by Alrefaei et al. (University of East Anglia, finch epidemic strain, Type A). PCR thermal cycling was performed as follows: 5 minutes denaturation at 94°C, then 35 cycles of 45 seconds at 94°C, 30 seconds at 63°C and 45 seconds at 72°C, followed by 5 minutes at 72°C for a final elongation. A Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA) was used to run the PCR reactions. The PCR products were electrophoresed through a 1.5% agarose gel, which was stained with Gel Red, in 1X TBE buffer and visualised by UV light. The alternative PCR recipe consisted of 0.8X Qiagen Multiplex PCR Master Mix (Qmix) (Qiagen, Hilden, Germany), 0.5µM forward and reverse primer (Sigma-Aldrich, UK) and a volume of sterile double distilled water (ddH_20) to bring the total PCR reaction to 9µl whereby 1µl of DNA was then added. A negative control of molecular grade water and a positive control of *T. gallinae* were included in every run of PCR. A touchdown PCR thermal cycling program was performed as follows: 15minutes at 95°C, 11 cycles of 1 minute at 94°C, 30 seconds at 66°C (decreasing by 1°C every cycle until 56°C), 1 minute at 72°C, then 24 cycles of 1 minute at 94°C, 30 seconds at 55°C and 1 minute at 72°C with a final elongation step of 10 minutes at 72°C. A Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA) or DNA Engine Tetrad 2 (Bio-Rad Laboratories Inc, CA, USA) was used to run the PCR reactions. The PCR products were electrophoresed through a 1% agarose gel, which was stained with Gel Red or ethidium bromide, in 1X TBE buffer and visualised by UV light. The presence of an amplicon at the expected product size indicated the presence of *T. gallinae* infection. If the result was negative, the sample was run a second time to confirm.

2.2.6 PCR amplification of the Fe- hydrogenase region

Two different methods to amplify the 1000bp region of the hydrogenosomal Fe-hydrogenase gene (hereafter referred to as the Fe-hyd region) were used, depending on when the samples were collected (Table 2.1). The first used primers TrichhydFOR [f] (5'- GTTTGGGATGGCCTCAGAAT- 3') and TrichhydREV [r] (5'- AGCCGAAGATGTTGTCGAAT-3') The PCR reaction consisted of: 1x PCR Buffer

(Promega, UK), 3mM $MgCl_2$ (Promega, UK), 0.25 µM dNTP mix (Promega, UK), 0.25 µM forward and reverse primer (Invitrogen, UK), 5U/µM Go Taq Hot Start Polymerase (Promega, UK) and a volume of molecular grade water to bring the total PCR reaction volume to 49µl whereby 1µl of DNA was then added. A negative control of molecular grade water and a positive control of *T. gallinae* were included in every run of PCR. PCR thermal cycling was performed as follows: 5 minutes denaturation at 94°C, then 35 cycles of 45 seconds at 94°C, 30 seconds at 53°C and 45 seconds at 72°C, followed by 5 minutes at 72°C for a final elongation. A Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA) or DNA Engine Tetrad 2 (Bio-Rad Laboratories Inc, CA, USA) was used to run the PCR reactions. The PCR products were electrophoresed through a 1.5% agarose gel, which was stained with Gel Red, in 1X TBE buffer and visualised by UV light.

The TrichhydFOR and TrichhydREV primers could not be used on samples which were to be run on the Illumina MiSeq as maximum read lengths are limited to 550bp. Therefore, two sets of four primer pairs each (Primer sets A and B) were designed that divided the 1000bp region into four 300-400bp overlapping sections (Table 2.2). These primers were designed using Primer 3 (version 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/) based on a consensus sequence of all the available Fe-hyd sequences on GenBank (accessed 13/04/2015), using the search terms '*Trichomonas gallinae*' and 'Fe- hydrogenase' (n=27). The primers were tested on 10 samples known to be positive with *Trichomonas* infection after the ITS region was successfully amplified. A positive control was also included. The PCR reaction used the same concentration of reagents as detailed with the Qmix PCR and the thermal cycling program was the same as that used with the TrichhydFOR and TrichhydREV primers. The PCR products were electrophoresed through a 1.5% agarose gel, which was stained with ethidium bromide, in 1X TBE buffer and visualised by UV light. Primer set B was chosen as the last primer pair of primer set A amplified multiple non- specific bands.

Table 2.2: Primer sets tested for amplification of the Fe-hyd gene.

Primer set	Name	Forward primers	Reverse primers	Length (bp)
A	Fe-hyd1	ACGGAAAGTGGCTTTCTCC	GTGTGCCACCGAATGTTG	387
	Fe-hyd2	CATGCCATCAGACTCGACAC	GGAAACCATCTTGCCTGTTG	357
	Fe-hyd3	AGGGCAAGAAGGTCACAGTC	TCTTGATGAGGGAGGAAAGC	345
	Fe-hyd4	AAGGATCCAAAGGCTGTCTTC	AGATCTGGCCAGCACCAG	394
B	Fe-hyd1	GCCACGATGAAACATGCTC	ACCGACTGGGCAATAGAGTG	326
	Fe-hyd2	CACATCCGCCATCATCTTC	GCAGATTGTAAGGTCAGCA	349
	Fe-hyd3	TTGGCTACAAGGAGGGTACAG	CGAGGAGCTTTGGAAGGTAG	302
	Fe-hyd4	TTGGGTTAACTACGTTGAGCAG	GAAGCCGAAGATGTTGTCG	325

2.2.7 Sanger Sequencing

In the case of one set of samples (Table 2.1), the positive PCR products were purified using Wizard SV Gel & PCR Clean-Up System (Promega, UK) and sequenced by Beckman Coulter Genomics (Takeley, Essex, UK). All products were sequenced in both directions. When testing new sets of primers or checking the PCR protocol was amplifying the target DNA region, a subset of samples were sequenced using the Sanger method for confirmation. Samples from Burkina Faso and Senegal 2015 (Table 2.1.) were also sequenced using this Sanger method which was conducted as follows: samples were purified using ExoSAP-IT ®(Sigma-Aldrich, UK) (Bell, 2008), by adding 2µl of ExoSAP-IT to 6 µl of PCR product and incubating for 15 minutes at 37°C followed by 15 minutes at 80°C to inactive the enzymes. A separate sequence PCR was performed on a 96-well skirt plate for each forward and reverse reaction using a reagent mix composed of: 0.875x BigDye® Sequencing Buffer (Applied Biosystems, CA, USA), 0.125x BigDye® Terminator v3.1 Ready Reaction Mix (Applied Biosystems, CA, USA), 0.33µM primer used in original PCR and a volume of sterile ddH_20 to bring the total volume to 8µl whereby 2µl of purified DNA (ExoSAP-IT®) was then added. A sequencing control was included on each plate, consisting of: 0.75X BigDye® Sequencing Buffer (Applied Biosystems, CA, USA), 0.25X BigDye® Terminator v3.1 Ready Reaction Mix (Applied Biosystems, CA, USA), 2X -21 M13 Control Primer (forward) (Applied Biosystems, CA, USA) and a volume of sterile ddH_20 to bring the total reaction to 8µl whereby 2µl of pGEM® -3Zf (+) double- stranded DNA Control Template (Promega, UK) was added. The thermal cycling program was performed as follows: 60 s at 96°C then 39 cycles of 10 seconds at 94°C, 5 seconds at 50°C and 4 minutes at 60°C. A Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA) or DNA Engine Tetrad 2 (Bio-Rad Laboratories Inc, CA, USA) was used to run the PCR reactions. The PCR products were stored at 4°C until purification. An ethanol/ EDTA/ sodium acetate precipitation method was used for purifying the extension products. This involved a reagent mix containing 2µl of 125mM EDTA, 2µl of 3M sodium acetate (pH 5.2), 10µl ddH_20 and 52.5µl 95% ethanol. This reaction mix was then added to each well containing a PCR product and incubated in the dark for 15 minutes. The plate was centrifuged at 4,000rpm for 15 minutes, tapped upside down to remove most of the liquid and centrifuged again at low speed (190g) for 30 seconds to dry. Each pellet was washed by adding 66.5µl of 70% ethanol and spinning the plate for 5 minutes at full speed (4,000rpm). Removing the liquid was repeated in the same manner as previously described. The plate was allowed to dry briefly (approximately 1 minute) before 10µl of Formamide (Applied Biosystems, CA, USA) was added to each well and the plate denatured for 3 minutes at 95°C. The plate was placed on ice until it was ready to load onto the ABI3730 DNA Analyser (Applied Biosystems, CA, USA).

2.2.8 Library preparation for Illumina sequencing

Each sample underwent a tailed PCR reaction that amplified the target region and added Illumina sequencing primer sites to the amplicons. The 25 µl PCR reagent

mix consisted of 10µl Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 2.5µl of each forward and reverse primer (3µM) tailed with Illumina sequencing primer sites (F- 5' -3'; TCTACACGTTCAGAGTTCTACAGTCCG-ACGATC and R 5'-3'; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT) (Sigma- Aldrich, UK), 9µl of sterile double distilled water (ddH_20) and 1µl of DNA. The PCR thermal cycling programs were identical to those used in the initial PCR's for the ITS and Fe-hyd regions. The PCR products were electrophoresed through a 1% agarose gel, stained with ethidium bromide, in 1X TBE buffer and visualised by UV light. The intensity of the amplicon was used to estimate PCR product concentration in order to pool amplicons for each sample in similar concentrations. A second tailed PCR was performed to add unique identifier sequences (Fi5 and Ri7 primers in unique combination for each sample) and Illumina sequencing sites to the amplicon products. The 10 µl PCR reagent mix comprised of 5µl Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1µl of each Fi5 and Ri7 primer (1µM) (Integrated DNA Technologies, Belgium) and 4µl of pooled products for each sample from the previous PCR. The thermal cycler conditions were as follows: 15 minutes at 95°C, then 10 cycles of 10 seconds at 98°C, 30 seconds at 65°C and 30 seconds at 72°C, finishing with 5 minutes at 72°C. Each sample was quantified on a FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) using the QuantiFluor® dsDNA system (Promega, UK) following the manufacturer's instructions. Samples from each column on a 96- well plate were pooled together in equal concentrations, resulting in 12 samples to subsequently purify. A volume of EB buffer (Qiagen, Hilden, Germany) was added to each pooled product to increase the overall volume to 50µl and allow the purification process to work efficiently.

The Agencourt AMPure XP system (Beckman Coulter, UK) was used for purification. The magnetic bead mix was vortexed to re-suspend them and a 0.5x concentration was added to the pooled product, mixed and incubated at room temperature for 15 minutes. The tubes were placed on a magnetic rack to separate the beads from the solution. Once the liquid was clear, the supernatant was transferred to a new tube and the beads discarded. An AMPure XP bead concentration of 0.6x was then added to the supernatant, mixed and incubated at room temperature for 15 minutes. The tubes were placed on a magnetic rack and once the liquid was clear, the supernatant was removed and discarded. The bead pellet was washed with 200µl of 80% ethanol twice, and then air-dried at room temperature for a few minutes. The samples were removed from the magnetic rack and eluted in 15ul of nuclease- free TE (10mM Tris- Acetate, 1mM EDTA, pH 8.0) before being placed back on the magnetic rack. Once the beads were separated from the solution, the solution was transferred to a new tube and 1.5µl of 10mM Tris/ 0.05% Tween 20 (pH 8.0), was added.

In order to confirm that each step of the library preparation for Illumina sequencing had worked, two samples representing the results of the 1st PCR, 2nd PCR and 2nd PCR post- purification were run on the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) to determine whether DNA fragments of the expected length were present after each stage. The Agilent High Sensitivity DNA

kit was used according to the manufacturer's instructions (Agilent Technologies, CA, USA). Once the protocol was confirmed, subsequent libraries were checked on the Agilent 4200 Tapestation (Agilent Technologies, CA, USA) to check peak amplicon size.

Quantification of each pooled product was performed using qPCR. Triplicate dilutions of 1:100, 1:1000 and 1:10000 of the unknown libraries were produced by serial dilution. The reagent mix consisted of 6µl of KAPA SYBR® FAST qPCR Master Mix plus primers (KK4835, KAPA Biosystems, UK) and 2µl sterile ddH_20. This was made up to 10µl total volume with 2µl of each dilution. Included on each 96-well plate were the kit standards and no template controls, both in triplicate. The thermal cycling program used was 5 minutes at 95°C, then 35 cycles of 30 seconds at 95°C and 45 seconds at 60°C. A StepOnePlus Real- Time PCR system (Applied Biosystems, CA, USA) was used to run the qPCR. The concentration of each plate library was calculated using their CT scores in a linear regression of CT vs. log concentration (pM) based on the standards. Each sample was normalised to 4nM and equal volumes of each were pooled to create the final library for sequencing. The library was sequenced using 250 paired-end reads on a MiSeq desktop sequencer (Illumina, San Diego, CA).

A subset of samples (n=28) were sequenced using both sequencing methods for purposes of validation.

2.2.9 Sequence analysis

Sequences returned from Sanger sequencing were manually assessed for sequencing errors, trimmed and the forward and reverse sequences aligned in BioEdit (Hall, 2005). Each sequence was searched for in the NCBI-BLAST database (Altschul et al., 1997) to determine the closest sequence match. If the sequence was of poor quality (less than 70% query cover) it was removed from the analysis. There are multiple approaches to analysing NGS data which may result in a different number of MOTUs by the end of the analysis pipeline. The method chosen could therefore affect downstream ecological analysis and result in different conclusions being drawn. In order to address this, two different approaches were tested and the end results compared. The first approach is used consistently in metabarcoding, particularly in diet analysis, and clusters the sequences based on a similarity threshold after a number of steps which are designed to remove probable artefacts. Within this method, the parameters chosen at each step may further inflate or limit the resulting number of MOTUs therefore a few were tested here. The second approach (jMHC) was initially designed to genotype multigene families such as the major histocompatibility complex (MHC) and distinguish between true alleles and artefacts based on their sequencing depth within an amplicon (Lighten et al., 2014). It is suspected that the latter approach may be more suitable due to the similarities between MHC and the ITS region in being present in multiple copies which may vary within an individual. Both similar and divergent alleles may be present within a sample and

clustering based on sequence similarity is not a suitable technique to address this (Stuglik et al., 2011).

The initial steps in filtering out poor quality sequences remained the same for both approaches. The bioinformatics analysis pipeline for NGS data was run from a Linux platform on the University of Sheffield's High Performance Cluster (HPC). The sequences were first demultiplexed into sample files according to the MID tag sequence by the Illumina MiSeq software. Trimmomatic v0.36 (Bolger et al., 2014) was used to remove Illumina adapter sequences, low quality bases in the leading or trailing ends and remove low quality sequences that did not meet the minimum Phred quality score of 20 or the minimum length of 100bp. Paired end reads were aligned using FLASH 1.2.11 (Magoc and Salzberg, 2011) on a 177bp region of overlap for the ITS region and 213bp for Fe-hyd1, 189bp for Fe-hyd2, 238bp for Fe-hyd3 and 216bp for Fe-hyd4. Those that did not meet the minimum length of 250bp were discarded. For the clustering technique, the 'trim seqs' command in MOTHUR (Schloss et al., 2009) was used to de-multiplex sequences according to the gene amplified, using 100% matches to the primer pair sequences, which is recommended to prevent inflation of the resulting number of MOTU's (Clare et al., 2016). The effect of allowing one mismatch was also tested. The files containing each gene sequence per sample were de-replicated using the 'derep fulllength' command in USEARCH (Edgar, 2010), with only sequences represented at least 10 times within the sample being kept. Potential chimeric sequences spuriously created during PCR were removed using the 'uchime denovo' command in USEARCH (Edgar et al., 2011). A number of different clustering thresholds were tested (97%, 98% and 99%), performed by the 'cluster fast' command in USEARCH (Edgar, 2010). The effect that the presence of singletons (sequences that only appeared in one sample) had under the different clustering thresholds was also examined. The final sets of MOTUs were identified using the BLAST nucleotide database, accessed using the 'blastn' command in the blast+ package (Altschul et al., 1997).

For the MHC technique, the fasta files resulting from the previous alignment step were the input files for the jMHC program (Stuglik et al., 2011). Sequences for each primer pair were used to extract the relevant sequences and the output file gave sequence variant depths quantified among amplicons (Stuglik et al. 2011). An approach known as the DOC (Degree Of Change) was used to distinguish between biologically accurate sequences and artefacts based on sequencing depth (Lighten et al., 2014). This relies on the assumption that an obvious reduction in sequencing depth occurs between actual sequences and artefacts. This can be represented by an inflection point occurring on a linear graph between variant number and cumulative frequency (Figure 2.3). The variants present after this inflection point are treated as artefacts and discarded. Due to the variable and occasionally poor sequence read depth in this study, a sequence also had to be present in at least 50 copies within an amplicon to be retained. The calculations were performed in the custom Excel macro (Appendix S2, Supporting Information, Lighten et al., 2014). The ITS sequences were searched for in the

NCBI-BLAST database (Altschul et al., 1997) to determine the closest sequence match.

2.2.10 Phylogenetic analysis

In order to visualize evolutionary relationships between the ITS sequences discovered in this study, a number of reference sequences were downloaded from GenBank that represented T. *gallinae* strains based on the ITS region (Table 2.3). The nomenclature for strains was adopted from Chi et al., (2013) in order to promote consistency of use. Nomenclatures for strains that have since been reported have followed that of the authors that reported them. An alignment of all the strain representatives from GenBank in addition to those identified in this study was performed with ClustalW (Thompson et al., 1994) in Bioedit (Hall, 2005) under the default settings (gap opening penalty: 0, gap extension penalty: 0). The sequences were trimmed to 309bp and a neighbour – joining tree constructed with genetic distance measured by the maximum composite likelihood method and branch reliability tested using a bootstrap of 1000 replicates using MEGA6 (Tamura et al., 2013). In order to check the topology of this phylogenetic tree, another was constructed using the minimum evolution method with genetic distance measured using maximum parsimony and branch reliability being tested with a bootstrap of 1000 replicates, also using MEGA6 (Tamura et al., 2013).

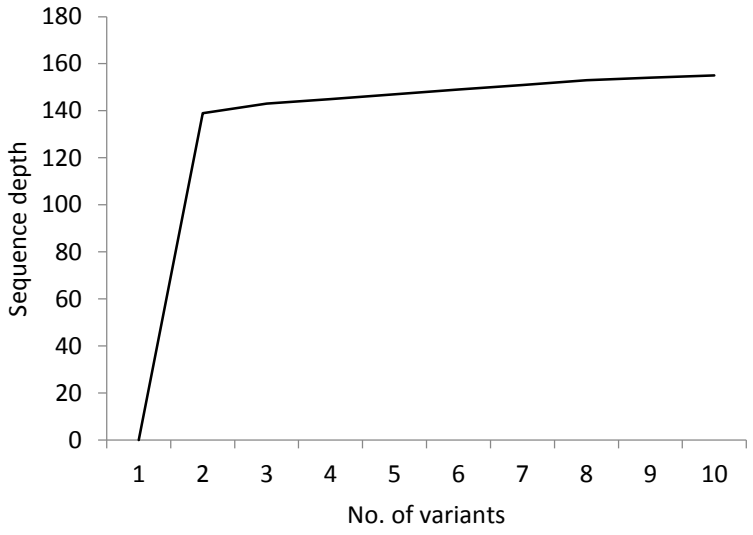


Figure 2.3: A graph to demonstrate 'Depth of Change' with the cumulative sequence depth for ten most abundant variants in one sample. An inflection point can be seen at two variants and subsequent variants are treated as artefacts and discarded.

2.2.11 Fe-hyd fragment analysis

The Fe-hyd region was amplified from samples from new geographical populations (i.e. France and Africa) therefore it was expected that new variants would be discovered. In order to facilitate Fe-hyd strain identification, samples with all four fragments of the Fe-hyd sequence from all datasets (i.e. datasets for subsequent chapters) were overlapped to form the full length sequence. These sequences were de-replicated using the 'derep_fulllength' command in USEARCH (Edgar, 2010) and Fe-hyd MOTU's were identified. Those that 100% matched a sequence in GenBank were identified as that sequence. New sequences were assigned MOTU numbers. Attempts were made to identify partial Fe-hyd sequences by aligning them with full length MOTU's and sequences downloaded from GenBank (Table 2.4). Alignment was performed with ClustalW (Thompson et al., 1994) in Bioedit (Hall, 2005) under the default settings (gap opening penalty: 0, gap extension penalty: 0). The sequences were trimmed to the length of the unidentified fragment and a neighbour–joining tree constructed with genetic distance measured by the maximum composite likelihood method and branch reliability tested using a bootstrap of 1000 replicates in MEGA6 (Tamura et al., 2013). If an unidentified sequence grouped with a recognized strain (either from GenBank or full length sequences from this study) and that group had good support (>50), it was identified as that strain. All identifications were confirmed by performing alignments of the well supported groups to check the sequences were identical. Individual fragments could not be resolved to Fe-hyd subtype level and overlapping fragments 1 and 2 were too conserved to allow identification within Type A, Type C and Tcl-1 strains. Overlapping fragments 3 and 4 were required to distinguish between Fe-hyd subtypes. It was also possible to identify Fe-hyd strains based on three overlapping fragments. Samples with multiple copies of fragments 3 and 4 were not identified as the combination of fragments could not be determined. It is likely these sequences were the result of contamination as only one case of coinfection was identified by the ITS region, therefore they were discarded.

Table 2.3: Representative ITS sequences of the most widely reported *T. gallinae* strains from GenBank used for phylogenetic analyses.

Host species	Origin	ITS strain type	GenBank	Reference
Greenfinch	UK	A	GQ150752	Lawson et al., (2011)
Broad- winged Hawk (*Buteo platypterus*)	Florida, USA	B	EU215368	Gerhold et al., (2008)
Rock Pigeon (*Columba livia*)	Colorado, USA	C	EU215362	Gerhold et al., (2008)
Common Ground Dove	Texas, USA	G	EU215359	Gerhold et al., (2008)
Columbid	Australasia	GEO	JQ755287	Peters & Raidal (unpub.)
White- winged Dove (*Zenaida asiatica*)	Texas, USA	H	EU215360	Gerhold et al., (2008)
Racing Pigeon (*Columba livia domestica*)	Austria	II	FN433474	Grabensteiner et al., (2010)
Racing Pigeon	Austria	III	FN433473	Grabensteiner et al., (2010)
Mourning Dove (*Zenaida macroura*)	Texas, USA	J	EU215365	Gerhold et al., (2008)
Band- tailed Pigeon (*Patagioenas fasciata*)	California, USA	K	EU215367	Gerhold et al., (2008)
Cooper's Hawk (*Accipiter cooperii*)	Arizona, USA	L	EU215366	Gerhold et al., (2008)
European Turtle Dove	Spain	Tcl – 1	KF993705	Martinez- Herrero et al., (2014)
Canary (*Serinus canaria domestica*)	Austria	V	FN433477	Grabensteiner et al., (2010)
Bearded vulture (*Gypaetus barbatus*)	Czech Republic	VI	FN433478	Grabensteiner et al., (2010)
Human	USA	T. tenax	U86615	Felleisen (1997)
Columbid	Australasia	WQR	JQ75578	Peters & Raidal (unpub.)
Human	China	*T. vaginalis*	AY871048	(Xiao et al., 2006)

Table 2.4: Representative Fe-hyd sequences of reported *T. gallinae* strains downloaded from GenBank used for phylogenetic analyses.

Host	Origin	Fe-hyd strain type	GenBank	Reference
Greenfinch	UK	A1	JF681136	Lawson et al., (2011)
Sparrowhawk (*Accipiter nisus*)	UK	A1.1	KC529660	Chi et al., (2013)
Woodpigeon (*Columba palumbus*)	UK	A1.2	KC962158	Chi et al., (2013)
Woodpigeon	UK	A1.3	KC529661	Chi et al., (2013)
Madagascar Turtle Dove (*Streptopelia picturata*)	Seychelles	A2	JF681141	Lawson et al., (2011)
Rock Pigeon	North America	C1	Identical to AF446077	Lawson et al., (2011)
Woodpigeon	UK	C2	KC529664	Chi et al., (2013)
Booted Eagle	Spain	C2.1		Sansano et al., (2016)
Collared Dove (*Streptopelia decaocto*)	UK	C3	KC529663	Chi et al., (2013)
Woodpigeon	UK	C4	KC529662	Chi et al., (2013)
Woodpigeon	Spain	C5		Sansano et al., (2016)
Booted Eagle	Spain	C6		Sansano et al., (2016)
Collared Dove	Spain	C7		Sansano et al., (2016)

Examination of relationships between strain presence and variables such as year and country sampled was performed using a binomial General Linear Model (GLM) in R version 3.3.2 (R Core Team, 2016) with the response variable being the presence or absence of a strain. The significance of each term in the model was tested using likelihood ratio tests (LRT). Pairwise comparisons were conducted with all levels within the factor of country (France, Senegal and UK). Burkina Faso was not included as the sample size was too small (n=4). Senegal was removed for the analysis with Type A as the response variable as there was no variation in its presence in this country. The variance in frequency of DNA haplotypes between populations was examined using AMOVA in Arlequin (Excoffier et al., 2005) so that the information in the full DNA sequence could be taken into account.

2.3 Results

2.3.1 Prevalence and DNA sequences

The prevalence of *T. gallinae* infection in the Turtle Dove populations sampled and the number of DNA sequences recovered from positive samples is detailed in Table 2.5. Prevalence of *T. gallinae* infection in adult Turtle Doves from all populations and in all years sampled was very high, reaching 100% in most cases. All cases of infection in adult Turtle Doves were sub- clinical. It is expected that the prevalence of infection in Burkina Faso is an underestimate due to the parasite samples being refrigerated (instead of frozen) for a year prior to DNA extraction. Overall, 51 samples (total N=107) were Sanger sequenced to identify the ITS type (UK 2013, Burkina Faso 2012/2013, Senegal 2015) and four samples were Sanger sequenced to identify the Fe-hyd type (UK 2013). DNA sequence lengths for the ITS region varied from 136 – 287bp depending on the quality of the sequence. The length of the Fe-hyd sequences varied from 793bp to 859bp. All of these samples matched previously reported sequences in GenBank with 100% similarity and 99% - 100% overlap. The remaining 56 samples (total N: 107) had the ITS region sequenced via the Illumina platform on different runs (Run 1: 6 samples, Run 2: 47 samples, Run 3: 28 samples and Run 4: 1 sample). Of these 56 samples, the Fe-hyd region was only successfully amplified for 36 of them and also sequenced on the Illumina MiSeq. The runs varied in sequencing depth (Table 2.6), with an overall trend of an increase in the number of reads with each run performed. This is likely to be a result of optimizing the NGS library preparation protocol to increase amplicon yield.Each run contained 10% duplicates (i.e 10 duplicates per 96 well plate). Note that this includes all samples from all the datasets contributing to this thesis. Genotype repeatability for re-sequenced individuals was high (100%) within the same sequencing run. Repeatability between different runs was not tested. The DNA sequence length for the ITS region was 323-326 bp and therefore longer than Sanger sequences of the same region. The DNA sequence for Fe-hyd1 was 287bp, Fe-hyd2 was 311bp, Fe-hyd3 was 262bp and Fe-hyd4 was 284bp. Taking into consideration the overlapping

sections between Fe-hyd fragments, the full length sequence was 915bp and also longer than Sanger sequences of the same region.

Table 2.5: The number of Turtle Doves samples tested and found positive for *T.gallinae* infection. The number of DNA sequences obtained for each population/ year are also shown. (Birds from Burkina Faso were caught during the winter of 2012- 2013 therefore the data are collated).

	Year	Prevalence	Total N sampled	N ITS seqs	N Fe-hyd seqs
UK	2013	96%	23	18	4
	2014	90%	10	7	5
	2015	100%	4	3	2
France	2014	100%	78	40	23
Burkina Faso	2012/2013	89%	19	11	0
Senegal	2014	100%	11	6	4
	2015	100%	45	22	2
TOTAL				107	40

Table 2.6: Range of sequencing depths for samples from each Illumina run before and after sequence processing, and for the gene regions amplified.

Run No.	Raw sequencing depth (no. reads) per sample	Post- processing (no. reads)	ITS amplicon (no. reads)	Fe-hyd amplicons (no. reads)
Run 1	~4700 – 120, 000	~8 – 19,0000	~2 – 5000 reads	~ 1 - 7000
Run 2	~1300 – 17,000	~1000 – 15,000	~1 - 2800	~ 1 - 2200
Run 3	~ 260 – 260,000	~8000 – 220,000	~ 46 – 22,000	~1 – 7,000
Run 4	~ 128,000	~120,000	96	~16 – 80,000

Table 2.7: Number of ITS MOTUs as a result of a combination of different parameters during the clustering step of the NGS data sequence analysis pipeline. NB: this dataset includes all of the samples analysed for this thesis. Combining them was required for this stage as the aim was to differentiate between real sequences and artefacts.

Singletons	Absent		Present	
No. of primer mismatches	0	1	0	1
Clustering (perc identity)				
100%	1299	2039	1930	3020
99%	51	72	103	143
98%	28	34	43	52
97%	12	15	18	24

Table 2.8: Results of sequencing validation. Results in bold are where results differed between the two methods. 'New' indicates potentially new sequence but were not confirmed with re-sequencing.

Year	Country	Sanger sequencing result	Illumina MiSeq result
2014	UK	Type C	Type C
		Type A	Type A
		Type A	Type A
		Type C	**Type A**
		Type A	Type A
2014	France	GEO	GEO
		Type C	Type C
		Type C	Type C
		Type C	Type C
		Tcl-1	Tcl-1
		Tcl-1	Tcl-1
		Type C	Type C
		GEO	GEO
		Tcl-1	Tcl-1
		Type C	Type C
		Tcl-1	Tcl-1
		Type III	Type III
		Type C	Type C
		Tcl-1	Tcl-1
2014	Senegal	Type C	Type C
		GEO	GEO
		Tcl-1	Tcl-1
2015	Senegal	GEO	GEO
		GEO	GEO
		GEO	GEO
		GEO	GEO
		New	**Tcl-1**
		GEO	GEO

Two methods to identifying sequence variants were tested: clustering and the jMHC approach. Different parameters within the clustering approach were also tested, with the impact on resulting number of MOTUs shown in Table 2.7. Excluding singletons and disallowing a primer mismatch when de-multiplexing greatly reduces the number of MOTUs. It is also more likely that these sequences will be of higher quality and therefore represent real variations. Relaxing clustering thresholds also decreases the number of MOTUs but there is the risk that multiple variants are being collapsed into a cluster. The clustering thresholds were tested on the five recognised variants and it was discovered that only the 99% threshold distinguished between all five whereas 98% and 97% thresholds collapsed them into four clusters. Using the 99% threshold with the most stringent parameters (no primer mismatches, removing singletons) on the samples from this study resulted in a total of 51 variants being identified (Table 2.7), which was substantially more than previously reported by other studies. A total of six different ITS variants were identified in samples using the jMHC approach and five of these 100% matched a sequence in GenBank therefore

allowing the ITS 'type' to be identified. The number of variants was more in line with what was expected, based on the results of other studies. The results from the jMHC approach were used for ITS strain frequency analysis. The sixth variant was detected in one Turtle Dove from Senegal (reads=295) and is a new strain that is 99% similar to GEO (confirmed by also being detected in Laughing Doves *Streptopelia senegalensis*,n=2, 5,000- 7,000 reads), hereafter named GEO-TD. This method was also applied to Fe-hyd subtype identification. Thirteen MOTU's based on all four fragments of the Fe-hyd region were identified. Four of these 100% matched sequences on GenBank allowing the 'subtypes' to be identified. The remaining nine sequences represent new Fe-hyd subtypes. Those discovered in species other than Turtle Doves are described in Chapter 4 and Chapter 5. Two new subtypes within Type C were detected: one from Turtle Doves sampled in 2014 (France, Senegal and UK), hereby labelled C8-TD and the other isolated from one Turtle Dove in France, 2014 hereby labelled C11-TD. Two subtypes of the Tcl-1 strain were also detected: one from Turtle Doves in France and Senegal 2014, hereby labelled T1-TD and the other isolated from France and Senegal, 2014 and UK 2015, hereby labelled T2-TD.

Comparing Sanger and NGS ITS sequences of the same sample revealed that the majority of samples (N = 28, see Table 2.8) reported the presence of the same strain. Two samples sequenced by Sanger were identified as a different strain when sequenced by NGS. The Fe-hyd sequences were consulted in these cases for firmer identification. One sample only had a sequence for the first Fe-hyd fragment but it was sufficient to distinguish between Type A and Type C and it supported the NGS result of Type A. The second sample had fragments 1 -3 of the Fe-hyd sequence and although it did not group with any recognised strains, it was a sister taxa to T1-TD and T2-TD, which are variations within the Tcl-1 strain. This was congruent with the NGS result as well. Some further samples (n=6) only sequenced by Sanger had unexpected identifications (four as 'new' as they did not match a sequence on GenBank and two as T. tenax –a parasite of humans) and were excluded from analysis as they could not be confirmed with re-sequencing. NGS detected a case of coinfection (between GEO and Type III in a bird from France) whereas the Sanger sequence failed. Overall, Sanger sequencing did not detect any cases of coinfection.

The phylogenetic tree based on the ITS region (see Figure 2.4) revealed three well-supported clades, one of which was more closely related to *T. vaginalis* and another which was more closely related to *T. tenax*. The new strain identified in this study (GEO-TD) grouped with the GEO strain in addition to *T. tenax*. The phylogenetic tree based on the Fe-hyd region revealed two well-supported clades. The relationships between Fe-hyd sub-types within the larger of the two clades were less resolved, perhaps due to the increased sequence variation in the sub-types. The phylogenetic trees based on neighbour joining and minimum evolution were congruent on credible nodes i.e those with high support (>60), however variation in topology is seen with nodes of low support (<50), which is to be expected. Weakly supported nodes (<50) are considered unresolved.

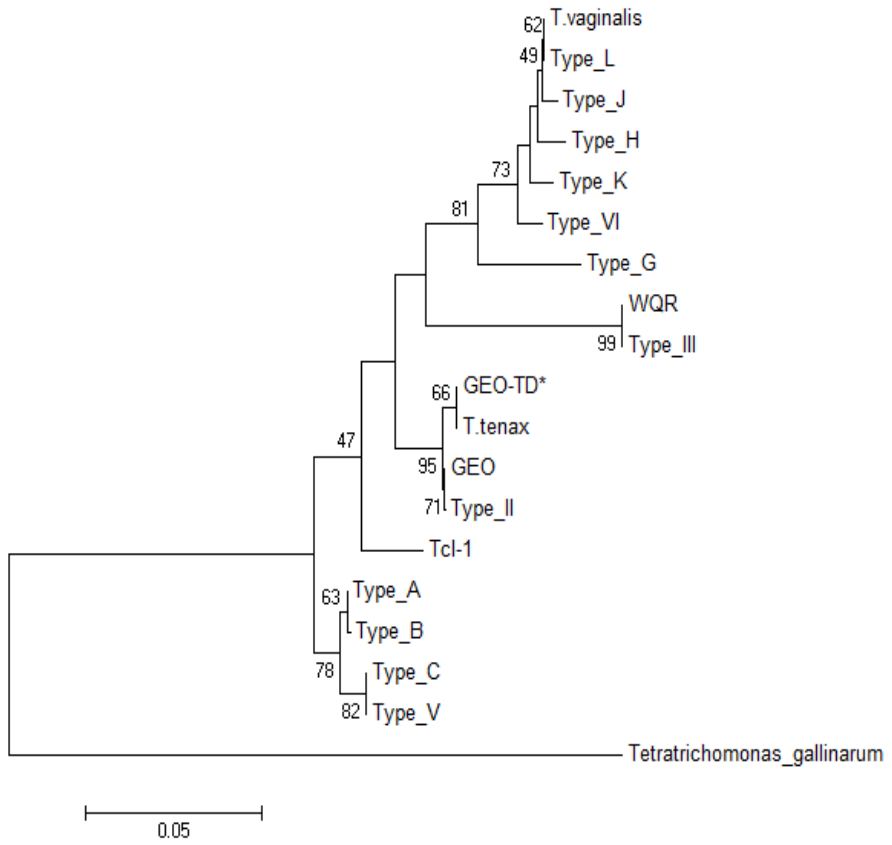


Figure 2.4: Phylogenetic tree based on an alignment of the ITS region (214bp), constructed using the neighbour- joining method with genetic distance measured by maximum composite likelihood. Branch reliability given as a percentage. Outgroup is *Tetratrichomonas gallinarum*, accession number AY244648 (Kutisova et al., 2005). Those marked with an * are new from this study.

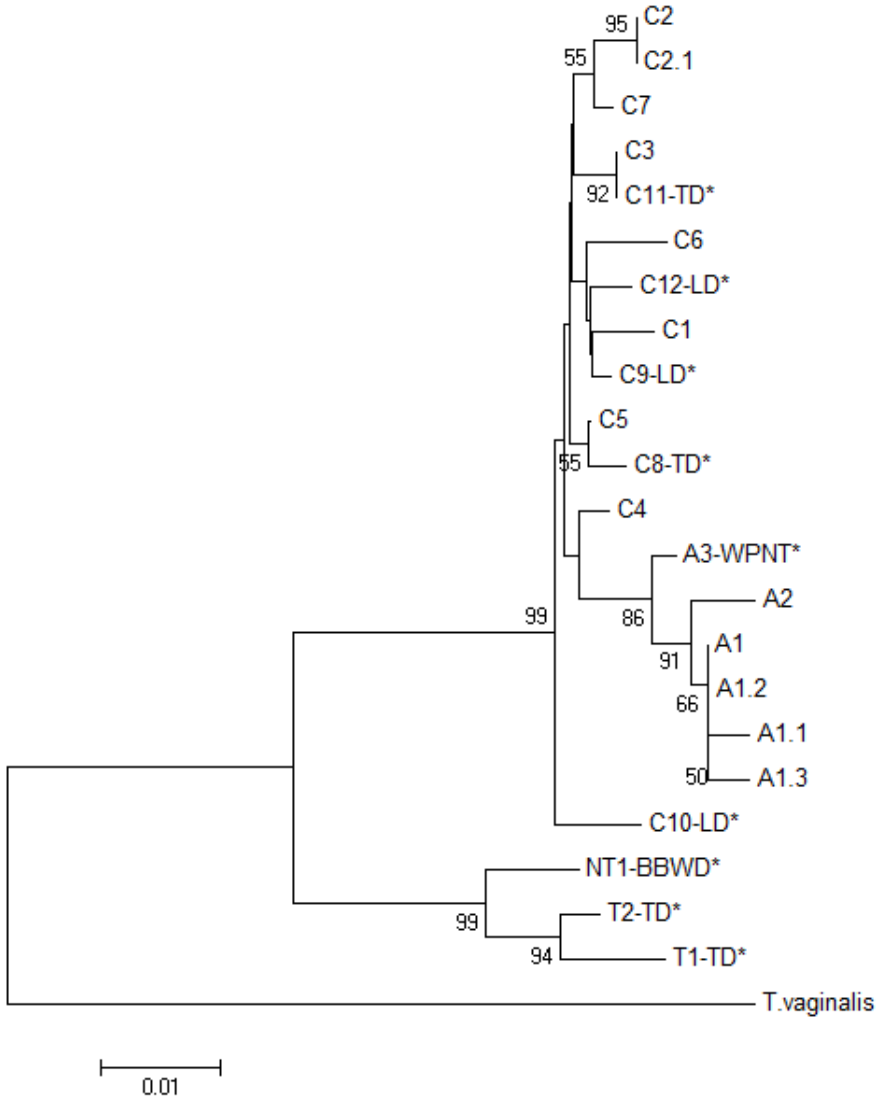


Figure 2.5: Phylogenetic tree based on an alignment of the Fe-hyd region (591bp), constructed using the neighbour- joining method with genetic distance measured by maximum composite likelihood. Branch reliability given as a percentage. Node values below 40 are not shown. Outgroup is *T. vaginalis*, accession number XM001310179 (Carlton et al., 2007). Fe-hyd sequence not available for *Tetratrichomonas gallinarum*. NB: this includes Fe-hyd sequences identified from all datasets, which was required for subsequent identification of partial Fe-hyd sequences. Those marked with an * are new from this study.

2.3.2 Strain frequency

The geographical and temporal variation in strain composition of *T. gallinae* (based on the ITS type) detected in Turtle Dove populations is portrayed in figure 2.6. Logistic general linear models supported the trends portrayed by figure 2.6 and revealed the degree to which the explanatory variables 'Year' and 'Country' can account for the variance observed in strain composition (Table 2.9). For two strains (Type A and Tcl-1), prevalence differed significantly between years (Type A, p=0.026; Tcl, p=0.002) (Table 2.9). Type A prevalence was higher in 2011 (57%) and 2012 (67%) compared to 2013 (0.06%) and 2014 (11%). Tcl-1 prevalence was higher in 2014 (26%) and 2015 (28%) than 2013 (0.06%). Type C was present in similar proportions across years (2011-2014: 27% - 36%), as was the GEO strain (2011, 2013-2015: 14%, 21%-68%) and Type III strain (2012, 2014, 2015: 0.07%, 0.09%, 0.05%). For all the strains except one (Type III), country is also a significant factor (Table 2.9). . The Type A strain is marginally more prevalent in the UK (38%) than France (0.03%). It has not yet been detected in Turtle Doves in Senegal. Type C is found at a higher prevalence in both the UK (28%) and France (40%) when compared to Senegal (0.04%) whereas the GEO strain is more prevalent in Senegal (71%) than the UK (24%) and France (18%). Tcl- 1 is more likely to be found in France (30%) than Senegal (21%). Type III is found at a similarly low prevalence in all three countries (0.02%-13%).

Different Fe-hyd sub-types were identified in the UK population in 2014 (A1: 60%, C8-TD: 40%, n=5) than 2015 (T2: 100%, n=2) however sample sizes were very small. Six Fe-hyd sub-types were detected in the population of France in 2014 with C8-TD found to infect the majority of Turtle Doves sampled (C8-TD: 67%, T1: 11%, A1: 5.6%, T2: 5.6%,C7: 5.6%, C11-TD: 5.6%, n=18). Three Fe-hyd sub-types were detected in the three Turtle Doves sampled in Senegal in 2014 (C8-TD, T1 and T2).

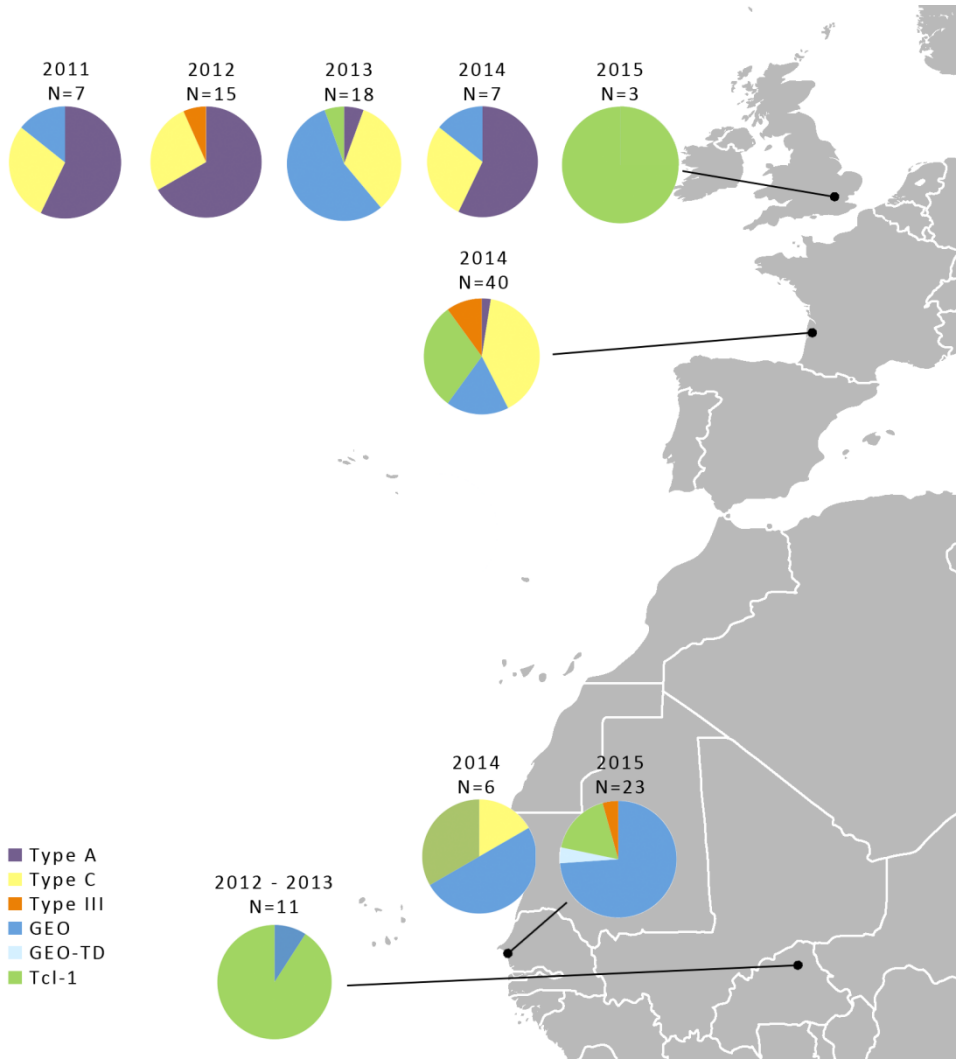


Figure 2.6: Pie charts reflecting the strain frequency composition of *T. gallinae* (based on the ITS type) in the sampled populations of Turtle Doves.

Table 2.9: Results of LRTs determining whether 'Year' and 'Country' are significant predictor variables for the variation observed in strain frequency shown in figure 2.6. Both terms included in full model. Significant results for the removal of a term from the full model are in bold and these terms were retained for the final model. Dev. = Deviance. Sample sizes: Type A (N=90); Type C – Type III (N=118).

	Type A			Type C			GEO			Tcl-1			Type III		
	Dev	Df	P value	Dev	Df	P value	Dev	Df	P value	Dev	Df	P value	Dev	Df	P value
Year	4.9592	1	**0.02595**	0.46265	1	0.4964	1.4923	1	0.2219	9.2046	1	**0.002414**	0.17447	1	0.6762
Country	4.917	1	**0.02659**	12.213	2	**0.002229**	13.923	2	**0.0009478**	6.0888	2	**0.04762**	4.3956	2	0.111

An AMOVA analysis was conducted on DNA haplotypes in Arlequin (Excoffier et al., 2005) to assess genetic differentiation of samples among years and countries which took into account variation across the DNA sequence instead of categorically defining the strains. Populations defined by different parameters were compared. The variance is highly significant (Fst value > 0.25) (Hartl and Clark, 1997) among populations under various partitions: year, country and year+country (Tables 2.9-2.11). All significance tests were calculated from 1023 permutations. Most of the variation was observed among populations which were temporally and geographically segregated i.e between populations grouped based on year+country (Table 2.10) Figure 2.7 displays a population comparison matrix based on these partitions, revealing the degree of genetic differentiation between the *T. gallinae* isolates infecting different Turtle Dove populations. The largest amounts of genetic differentiation were observed between *T. gallinae* isolates infecting Turtle Dove populations in the UK and Senegal, and in *T. gallinae* isolates infecting Turtle Doves between different years within the UK.

Table 2.10: AMOVA results for variance partitioned by year + country. Number of groups = 1. Significant Fst value highlighted in bold.

Source of variation	d.f	Variance components	Percentage of variation	Fixation index Fst
Among populations	7	23.44	30.56	**0.30557**
Within populations	139	53.26	69.44	

Table 2.11: AMOVA results for variance partitioned by year. Number of groups = 5. Significant Fst value highlighted in bold.

Source of variation	d.f	Variance components	Percentage of variation	Fixation index Fst
Among populations	4	16.04	20.19	**0.32968**
Among populations within groups	3	10.15355	12.78	
Within populations	139	53.26174	67.03	

Table 2.12: AMOVA results for variance partitioned by country. Number of groups = 3. Significant Fst value highlighted in bold.

Source of variation	d.f	Variance components	Percentage of variation	Fixation index Fst
Among populations	2	5.86973	7.54	**0.31605**
Among populations within groups	5	18.74227	24.07	
Within populations	139	53.26	68.39	

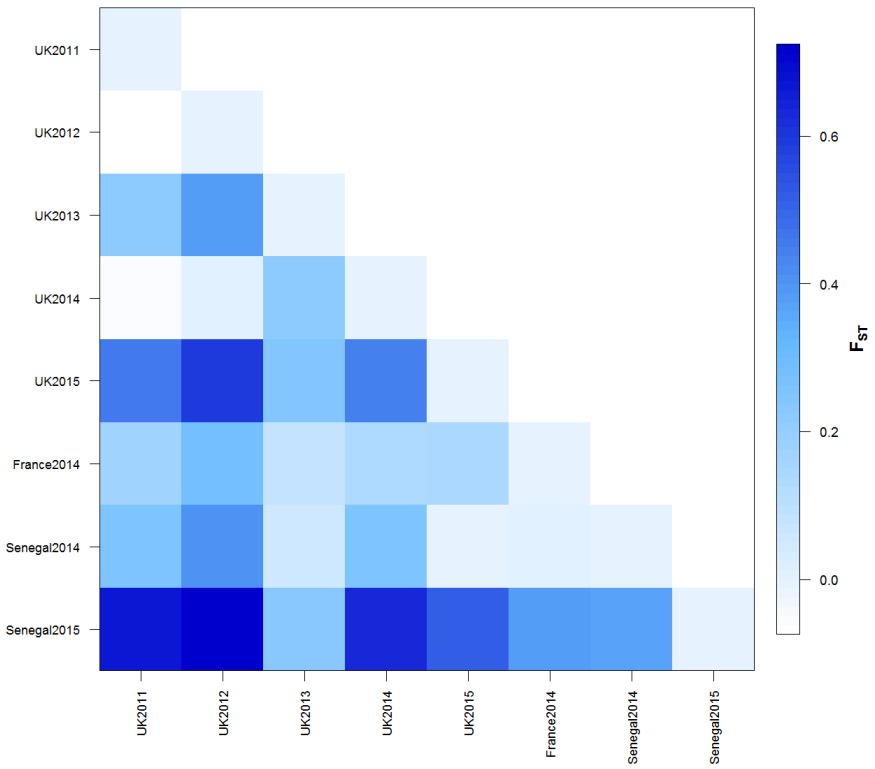


Figure 2.7: Population comparisons based on pairwise (FST) differences. Darker shades of blue indicate higher FST values and therefore increased genetic differentiation between the *T. gallinae* isolates infecting different Turtle Dove populations.

2.3.3 *T. gallinae* sample storage

Each Turtle Dove sample from Senegal in 2015 (n=45), except one, was stored on both Whatman FTA card and in ethanol. The storage methods were compared based on PCR and Sanger sequencing results (Table 2.13). When comparing the results from the same sample, the ethanol storage method detected 9 positive samples during PCR for which the FTA card method gave inconclusive results. The ethanol storage method also resulted in better sequencing reads and managed to obtain sequences from 15 samples that the FTA card storage method did not. The FTA card method only obtained one sequencing read from a sample for which the ethanol storage method failed. Where there were sequence reads for samples from both storage techniques, they were compared on sequence length (taking the longer of the reads if the sample was run twice) and strain identification. There were no conflicts on strain identification and overall, the samples stored in ethanol gave longer sequence reads than the samples stored on FTA cards (n=18, total n= 23) (ethanol stored: N=46, mean sequence length = 250 ±3.2; FTA card stored: N=32, mean sequence length = 227 ±5.3).

Table 2.13: Comparing the quality of data between samples stored in ethanol and samples stored on Whatman FTA card. PCR and DNA sequencing was repeated twice on each sample. Good quality result from PCR is a strong band visualised on gel, bad quality is either a faint band or non- distinct band. Good quality result from sequencing is both directions of sequencing run being assembled and DNA matched to *Trichomonas* sp., bad quality is sequence failure or poor quality sequence (small/ non- existent peaks on chromatogram, double peaks).

	Ethanol stored samples			FTA card stored samples		
Stage	Good quality	Poor quality	Total N	Good quality	Poor quality	Total N
PCR	86%	14%	43	70%	30%	44
Repeat PCR	100%	0%	18	97%	3%	29
Sequence	79%	21%	38	52%	48%	31
Repeat sequence	44%	56%	18	31%	69%	29

2.4 Discussion

Prevalence of *T. gallinae* infection was very high in all the Turtle Dove populations sampled but only one case of coinfection was detected. This overall high prevalence was surprising given the low to moderate prevalence (0- 50%) generally reported in Mourning Doves *Zenaida macroura* in the USA, the varied prevalence reported in Rock Pigeons *Columba livia* worldwide where approximately half of the populations had less than 50% prevalence and the other half over 50% prevalence and in endangered Mauritian Pink Pigeon populations, the average prevalence was 50.3% (Bunbury et al., 2008, Forrester and Foster, 2009). Recent screenings of UK Columbid populations revealed a generally high prevalence of *T. gallinae* infection in two of the four species of Columbiform sampled (Turtle Doves: 86% and Eurasian Collared Doves: 86%) whereas Woodpigeons and Stock Doves *Columba oenas* had moderate levels of infection (47% and 40% respectively) (Lennon et al., 2013). The earlier reports of *T. gallinae* prevalence in Columbids are likely to be underestimates as they would have relied on wet mount microscopy for detection which has been shown to be considerably less sensitive than the current molecular techniques (Bunbury et al., 2005). However, Mauritian Pink Pigeons and UK species of Columbiform were screened using the same techniques here (Bunbury et al., 2008). It is possible that some sampling bias has been introduced into this screening survey due to the capture methods employed. Turtle Doves were caught in the UK and France at bait sites which became a shared resource for the local bird population and possibly a

source of infection as a result (Villanua et al., 2006, Bunbury et al., 2007, Robinson et al., 2010). It is also possible that the birds sampled are more likely to be infected due to pathogen- induced changes in activity levels resulting in a dependence on these easily accessible food sources (Senar and Conroy, 2004). Turtle Doves sampled in Senegal in 2014 were not caught at bait sites however, and all were infected with *T. gallinae*. Furthermore, birds sampled in Senegal 2015 were caught using a mixture of capture methods (with and without bait) yet no difference in prevalence of infection was observed between here and the UK or France. This lends support to results from the UK and France being representative of the wider Turtle Dove population. It is likely that the use of bait will continue to be the only way to capture Turtle Doves in areas where they occur in low numbers (i.e breeding grounds as opposed to wintering grounds where birds roost together in high densities). This prevalence data has provided a baseline against which to test the impact of future strategies aiming to reduce *T. gallinae* infection. There are no baseline records of *T. gallinae* infection in Turtle Dove populations therefore it is unknown whether the high prevalence is a relatively recent occurrence or the result of a long term endemic infection. The level of genetic heterogeneity detected in Turtle Dove populations in this study suggests that *T. gallinae* infection is endemic, which is also presumed for Columbids and raptors in the USA, Spain and Austria (Gerhold et al., 2008, Sansano-Maestre et al., 2009, Grabensteiner et al., 2010). Molecular techniques have been used to establish the historic prevalence of emerging pathogen *Batrachochytrium dendrobatidis* (Bd) in amphibians using a qPCR assay (Talley et al., 2015). Museum samples collected between 1892 and 1989 were screened but the data was not directly comparable to the current prevalence of Bd as the technique is known to underestimate prevalence (Talley et al., 2015). It did indicate that the pathogen was present in populations over 120 years ago and that the prevalence remained constant between decades up until the 1940s, whereby it increased before returning to 1930s levels (Talley et al., 2015). Museum collections provide an opportunity to explore the history of pathogen infections and with the increased sensitivity of ancient DNA methods and NGS technology, this technique is worth exploring for the detection of *T. gallinae* (Sarkissian et al., 2015). It may be possible to evaluate clinical infection by *T. gallinae* in historic samples based on physical evidence in the host. Evidence of *T. gallinae* infection was discovered in tyrannosaurid specimens in the form of characteristic lesions on the mandible that are also seen on the mandible of modern birds, particularly raptors (Wolff et al., 2009). If large enough sample sizes of museum specimens representing different decades are possible, then this data may be linked to population trends in order to shed light on the potential role *T. gallinae* infection in driving declines.

Prevalence of infection by *T. gallinae* may always be high, considering the effective transmission route between parent and offspring and the difficulties in controlling that from a wildlife management perspective. The detection of a strain (Type A) associated with virulence (known as the finch epidemic strain) in the UK population and the evidence that it can cause mortality in both adult and nestling Turtle Doves necessitates monitoring of the pathogen to the level of strain

identification (Lennon et al., 2013, Stockdale et al., 2015). Strains represent variations within a parasitic species that are associated with different epidemiological traits such as pathogenicity or virulence. Avian blood parasite strains that differ in their host range and areas of transmission may only differ by 1bp in the cytochrome b gene (Waldenstrom et al., 2002, Beadell et al., 2004, Fallon et al., 2005, Perez-Tris and Bensch, 2005). In the case of *T. gallinae*, a 1bp difference in the ITS region results in a different ITS 'type' being identified with the term 'strain' being consistently applied to the virulent type responsible for the trichomonosis epidemic in Greenfinches (Lawson et al., 2011a, Chi et al., 2013, Ganas et al., 2014, McBurney et al., 2015). Different authors give these 'types' different names within the context of their paper which complicates efforts in identifying 'types' and determining host range and distribution. This issue was experienced within the research area of avian blood parasites therefore to standardize nomenclature and unify all the available information regarding each parasite, a public database was created (MalAvi) (Bensch et al., 2009). A similar resource would be highly valued by researchers within the *Trichomonas* community.

This study is the first to assess the prevalence and genetic strain composition of *T. gallinae* in Africa, which is also the over- wintering area of the migratory Turtle Dove. There appears to be differences in strain composition between the breeding grounds and the wintering grounds, with Type C being associated with the UK and France and the GEO strain being associated with Senegal. The individuals sampled whilst over- wintering are unlikely to belong to the breeding population of France, based on what tracking individuals from French breeding populations has revealed (Eraud et al., 2013). It is likely they belong to another European breeding population though, due to being part of the Western Palaearctic flyway (Marx et al., 2016). Discovering the European breeding grounds of the African birds sampled in this study or the wintering grounds of the European populations would allow the screening of these populations during both of these stages of their annual cycle and determine whether external factors over the migratory flyway have any effect and can change the strain composition present in one migratory population. There also appears to be a difference between breeding grounds in strain composition with the virulent Type A strain being associated with the UK when compared to France. There has been limited research on the infection of Turtle Doves by *T. gallinae* in other European countries. Thus far three Turtle Doves have been sampled in Spain and all three were infected with the Tcl – 1 strain (Martinez-Herrero et al., 2014). Another 42 Turtle Dove samples from Spain, 20 from Italy and 1 from Germany were screened for *T. gallinae* and despite a 92% infection rate in the combined sample, the Type A strain was not detected in these individuals however the Type C, Type V, Type II, Type III and another two previously undetected strains were (Marx et al., 2017). The Type A strain associated with the UK finch epidemics appeared to have emerged in the west of England and spread east into Europe (Lawson et al., 2011b). It is possible that infection is short-lived in Turtle Doves, as they either

clear it or it causes death (Stockdale et al., 2015), therefore it has not reached populations further south or on wintering grounds.

Type C is widely prevalent in the UK and French populations of Turtle Doves. It is one of the most widely reported types in the literature, commonly detected in Columbids and birds of prey and appears to be mostly non- pathogenic (Felleisen, 1997, Gerhold et al., 2008, Anderson et al., 2009, Sansano-Maestre et al., 2009, Grabensteiner et al., 2010, Chi et al., 2013, Lennon et al., 2013). Its wide geographical distribution and host range suggests it is likely to be an ancestral strain (Smith et al., 2003). The types 'GEO' and 'Tcl-1' are comparatively new in the literature with the GEO type only being described in Columbids from Australasia until this study and Tcl – 1 only detected in Turtle Doves from Spain (Martinez- Herrero et al., 2014, Peters & Raidal, unpub). It would be interesting to see whether Tcl – 1 becomes more prevalent in Europe over time and whether the GEO strain widens its distribution as these patterns would be consistent with these strains being recent additions to the western Palearctic populations and gradually dispersing. On the other hand, these current patterns could be due to under-sampling resulting in a lower likelihood of detection. This seems likely with the GEO strain, which presumably must be relatively widespread if it has reached the western Palearctic from Australasia.

This study was also the first to assess temporal variation in *T. gallinae* strain prevalence which was particularly evident over the five year span in the UK. Significant variation in strain frequency among years was observed suggesting strain turnover in the population. In particular, Type A occurred in much lower frequencies in 2013 in the UK which warrants further investigation into the factors driving this trend. Both biotic and abiotic factors may be influencing disease dynamics. All the Turtle Dove samples are independent therefore turnover of strains within hosts cannot be examined. It may be possible with environmental transmission of *T. gallinae* which would allow exposure to different strains over a lifetime and possibly result in coinfection or competition where one strain outcompetes and replaces the other (de Roode et al., 2005). It is known that adult Turtle Doves show a degree of site fidelity but first year birds disperse from their natal site (Marx et al., 2016). The variation in strain composition between years may reflect an influx of new first year birds into the local population. Unfortunately first year birds and older adults cannot be differentiated in order to compare strain composition between these two age classes. The difference in prevalence of the Type A strain between years in the UK is particularly interesting. It is high in 2012 but the following year, the prevalence of this strain drops. An alternative theory behind this change in strain composition is that the individuals infected with Type A in 2012 did not survive until 2013, neither did their offspring (if they had any) who are likely to have carried the same strain (Stockdale et al., 2015). Individuals infected with a different strain may have survived until the breeding season of 2013. The following year of 2014 sees a re-emergence of the Type A strain, perhaps because of spillover/ spillback from the Passerine population via shared resources (Robinson et al., 2010). If this pattern is

accurately reflecting the temporal variation in Type A prevalence, it suggests there could be fluctuating patterns, similar to other host- parasite systems (Lass and Ebert, 2006). Larger sample sizes and datasets spanning multiple years for all countries will allow the proper assessment of what ecological drivers may be influencing the *T. gallinae* strain composition.

Variation within the ITS 'types' was evaluated by examining the Fe-hyd region and identifying sub-types. Turtle Doves infected with the Type A strain are infected with the subtype A1, which is the prevalent subtype in infected free-ranging European bird populations and responsible for the finch epidemics in north-west Europe (Robinson et al., 2010, Neimanis et al., 2010, Lawson et al., 2011a, Ganas et al., 2014). This is in contrast to the sub-type A2 being involved in more mortality events in North America (Girard et al., 2014b). There is no further support for the variations of A1 detected in the study of Chi et al., (2013). Two new subtypes within Type C have been discovered, with one (C8-TD) present in the UK, France and Senegal indicating it is widespread and the other (C11-TD) only detected in France thus far. Subtypes of the Tcl-1 strain are also identified for the first time and detected in both Africa and Europe. This suggests that although the Tcl-1 strain has only recently been detected in European countries, it may be a result of under-sampling rather than being recently introduced to the population.

The strengths of the sampling and molecular analysis techniques used here to investigate prevalence of *T. gallinae* infection are that they are easily reproducible and strains can be identified accurately and reliably, as long as appropriate measures to prevent contamination between samples are in place. Contamination can happen at sample collection, DNA extraction, PCR and sequencing but including negative controls at each of the stages of molecular analysis would at least allow for the detection of contamination. During this protocol, negative controls were only included at the PCR stage therefore it could be improved by including negative controls during DNA extraction and sequencing to increase the validity of the results. Replicates of each NGS run (10%) gave the same result for each sample, increasing the confidence in the results obtained however; Sanger sequencing replicates were not performed. When the InPouches were first inoculated with the parasite, they were sealed carefully to ensure no loss of culture media however they were subsequently stored and incubated together. Although the InPouches were monitored for signs of leakage, this methodology could be improved by individually sealing the InPouches in plastic bags before being stored together, to further limit chances of cross-contamination. When the parasites were isolated from the culture media, scissors used to open the InPouches were cleaned with disinfectant wipes between each sample. The process was carried out within a fume cupboard and any spillages of the culture media resulted in a full wipe down of all surface areas with disinfectant wipes and a change of laboratory gloves. An extra pre-caution against contamination between samples recommended for future work would be to use filtered pipette tips, to prevent the risk of contaminating the pipette used. Overall, precautions

against contamination were taken during this protocol however there is room for improvement.

The application of NGS to *T. gallinae* samples has improved the accuracy and reliability of identifying strains. Reads occurring in high frequencies increase the confidence that the sequence represents a true strain which is particularly important when analysing intra-specific variation and expecting differences as little as 1 bp between sequences. If a potentially new strain was identified with Sanger sequencing, confirmation by re-sequencing is an extra costly and inconvenient step. Utilizing NGS also allowed the extent of coinfection between different strains to be assessed in Turtle Dove populations. Previously, only clonal cultures allowed the detection of multiple infections with one study revealing two birds out of 17 sampled to have coinfection between two strains of *T. gallinae* (Grabensteiner et al., 2010). Cloning and culturing is labour- intensive and an expensive method for effectively monitoring parasitic infection (Fuhrman et al., 2008, Supabandhu et al., 2008, Liu et al., 2010). Despite coinfection being rare in Turtle Dove populations, the continued application of NGS in the monitoring of this pathogen will facilitate early detection if multiple infections become more common. This will allow a thorough assessment of the factors which facilitate multiple infections and contribute to the under-studied research area of parasite diversity within a host (Bordes and Morand, 2011). Monitoring of this pathogen using sequencing technologies determines whether an infection is endemic or emerging. Rapid and accurate differentiation of strains is important for establishing appropriate management actions which will differ depending on whether the strain is endemic or not (Rachowicz et al., 2005). The application of NGS also has practical benefits in terms of reducing cost and sequencing multiple individuals simultaneously through pooling samples (Long et al., 2011, Schlotterer et al., 2014, Cao and Sun, 2015). It could be further developed so that PCR is no longer a required step which would have a further benefit of removing PCR-related errors in the output sequences (Zhou et al., 2013). Applying NGS to whole genome analysis of Bd revealed that the most rapidly evolving gene regions may encode putative virulence factors, which is concerning for the likelihood and severity of epizootics in the future (Farrer et al., 2013). The near ubiquity of *T. gallinae* infection in Turtle Doves and genetic variation of strains infecting populations suggests that infection is endemic. The strain that is associated with virulence (Type A) varies geographically, temporally and in whether it causes disease in the host. Comparative genome analysis for *T. gallinae* will allow research on what genetic changes underlie virulence which will contribute to the investigation of factors driving the emergence of trichomonosis.

In conclusion, the application of sequencing technologies to pathogen surveillance has detected temporal and spatial variation in the strain composition of a parasite. This is the first step to identifying which ecological factors may be driving these patterns which is important considering the potential threat of this parasite in a species with a Vulnerable conservation status. Furthermore, it also begins to address host-parasite relationships in a very complex system. Turtle Doves are

migratory, spending the summer months breeding in pairs in Europe, and the winter months roosting in high densities in Africa. They are exposed to a variety of nutritional and climatic stresses, all of which should be taken into account when assessing host-parasite interactions in this system.

Chapter 3

Presence of *T. gallinae* in shared environmental resources

3.1 Introduction

3.1.1 Supplementary Feeding

Establishing how a pathogen is transmitted is imperative to an epidemiological study. It is the mechanism behind infection spreading throughout a population and new transmission routes may lead to the emergence of a disease. Disease emergence occurs when a pathogen is transmitted to a novel and therefore naïve host population and establishes a transmission cycle, or if the incidence of disease in a natural host population increases to a rate that is higher than expected (Daszak, 2000). Anthropogenic changes to the environment in the form of urbanization, agricultural intensification and globalization can lead to changes in pathogen and host ecology by significantly altering transmission and exposure patterns (Schrag and Wiener, 1995, Daszak, 2000). This can result in increasing the proximity of domestic animals to wildlife which provides opportunities for pathogen spillover or introduce nonindigenous species along with their pathogens to naïve populations (Roelke-Parker et al., 1996, Berger et al., 1998, Edgerton et al., 2004, Plowright et al., 2011). Increasing host density or facilitating contact between a natural host and a novel host are common underlying mechanisms to disease emergences. Farming systems which allow contact between high density domestic bird flocks and wild birds have been highlighted as facilitating transmission, adaption and amplification of disease (Jones et al., 2013). The provision of supplementary feeding as a conservation measure to support game and wildlife populations also has the potential to increase this risk by increasing local densities and therefore transmission possibilities. Systematic provision of supplementary feed for wild birds is available in some agri-environment schemes. For example, in England, the 'Supplementary winter feeding for farmland birds' is an English Country Stewardship option (Natural England, 2015) being used to address the 'hungry gap' experienced by British farmland birds, which relates to the shortage of natural food between January and April (Siriwardena et al., 2008). This strategy is meant to be a short-term solution to feed birds whilst work to provide longer-term plots containing natural sources of food is ongoing. Compared to other agri-environment options, there is little research on the effects of supplementary feeding on farmland birds but it has been shown that declines in some target species were less severe where more food was provided and that deploying supplementary food sources at an appropriate spatial scale is critical to maximise their effectiveness (Siriwardena et al., 2007). A recent BTO study of Supplementary Winter Feeding, under the now closed Environmental Stewardship scheme, found that evidence for a temporal response by priority granivorous bird species (e.g. Grey Partridge *Perdix perdix*, Skylark *Alauda arvensis*, Linnet *Linaria cannabina*, Tree Sparrow *Passer montanus*, House

Sparrow *Passer domesticus*, Reed Bunting *Emberiza schoeniclus*, Yellowhammer *Emberiza citrinella* and Corn Bunting *Emberiza calandra*) and seed delivery at the fed patch was inconsistent and improving the efficacy of the delivery of supplementary feeding would improve its value as a viable option (Henderson et al., 2014). Studies on other avian systems have reported variable results regarding the impact of winter feeding on birds. Extra food was observed to increase the over-winter survival of Willow Tit *Poecile montanus* and Crested Tit *Lophophanes cristatus* populations which then led to doubled breeding populations the following spring (Jansson et al., 1981). The provision of peanuts has been shown to have a positive effect on the subsequent breeding season of Blue Tits *Cyanistes caeruleus* in Northern Ireland, shown by advanced egg laying dates and increased fledgling success; however, another study, also focusing on Blue Tits, found that winter-fed birds produced offspring that weighed less, were smaller and were less likely to survive (Robb et al., 2008b, Plummer et al., 2013b). The differences may be explained by the quality of the winter food, as the provision of fat was shown to impair egg production although this could be mitigated by the addition of vitamin E (Plummer et al., 2013a). Supplementary feeding timed for the pre-breeding season benefitted the Florida Scrub-Jay *Aphelocoma coerulescens* with a marked effect on reproductive output, including earlier laying attempts leading to larger clutch sizes and more re-nesting attempts (Schoech et al., 2008). This strategy could therefore increase the local bird population and support translocated individuals in newly established populations to promote rapid population growth (Schoech et al., 2008). A replicated supplementary feeding experiment tested whether the availability of invertebrate prey limits the breeding success and adult abundance of House Sparrows in suburban London. Daily mealworm provision over two successive breeding seasons of House Sparrow pairs nesting within 50m of feeders, had a large positive impact on the abundance of recently fledged birds (+62%), but only a small positive impact, limited mainly to small colonies, on the overall abundance of territorial males (Peach et al., 2015). Conservation interventions that enhance invertebrate availability for suburban House Sparrows may increase reproductive success but are unlikely, on their own, to lead to population growth or recovery (Peach et al., 2015). Supplementary feeding is a regular feature in re- introduction programmes, particularly with food specialists such as vultures, which has led to the term 'Vulture Restaurants' being coined. It has proven successful with Bearded Vultures *Gypaetus barbatus* in the Alps and Eurasian Griffons *Gyps fulvus* in the south of France, the latter resulting in a local population increase and attracting individuals from elsewhere in Europe (Frey et al., 2004, Houston and Piper, 2006). A feeding programme was used to address brood reduction in a trophic specialist, the Spanish Imperial Eagle *Aquila adalberti*, which was thought to be a result of sibling aggression that was subsequently reduced by supplementary feeding. Not only did this increase the number of fledglings per brood but facilitated the recovery of breeding success when their natural prey fell in numbers after succumbing to disease (Gonzalez et al., 2006). Supplementary feeding is also used to manage game wildlife, particularly in Europe and North America. This method maintains high densities of animals for hunting by increasing survival rates and reproduction

in addition to attracting and holding the animals in shooting areas (Putman and Staines, 2004, Selva et al., 2014). Pheasants *Phasianus colchicus* are captive-reared and fed wheat grain in preparation for the shooting season to maintain body condition (Draycott et al., 2005). Furthermore, they are continually fed throughout the shooting season, from summer to the end of winter, to hold them in the shooting estates (Draycott et al., 2005). Feeding used to end when the shooting season ended at the start of February, but research showed that continued feeding improved body condition of females in preparation for breeding, increased densities of territorial males and females during the breeding season and increased densities of young during the following autumn (Draycott et al., 1998, Draycott et al., 2005). Therefore supplementary feeding has become a continual, year- round practice.

All the examples above are targeted feeding programmes either for conservation or management of game wildlife. A non-targeted approach to food supplementation is the provision of bird feeders in back gardens, which has become so popular that 48% of households in the US, 46% in New Zealand and ~75% in the UK regularly feed birds (Cowie and Hinsley, 1988, Martinson and Flaspohler, 2003, Galbraith et al., 2014). Local positive impacts on breeding productivity and fledgling survival have been documented (Robb et al., 2008a), but the effects on bird populations nationwide is largely unknown. This highlights the limited understanding of the wider- scale impacts of supplementary feeding. Numerous concerns with supplementary feeding have been outlined, such as the formation of an ecological trap whereby populations or species become dependent on supplementary food and no longer seek out natural sources (Putman and Staines, 2004). A study on the use of supplementary food by Australian Magpies *Gymnorhina tibicen* found that despite extensively using suburban feeding stations, the majority of the food that they fed their young was natural (O'Leary and Jones, 2006). On the other hand, an increased tendency for birds to over- winter on breeding grounds instead of migrating to wintering grounds has been reported (Jokimaki et al., 1996, Plummer et al., 2015). Another main concern is the increase of predation in areas surrounding supplementary feeding stations. Predators that are attracted to the bait site also forage for prey nearby, which has been shown with increased predation of ground nesting birds in the vicinity of a vulture restaurant and modelling simulations show an increased risk of predation on arthropods local to winter bird feeding sites (Martinson and Flaspohler, 2003, Cortes-Avizanda et al., 2009). Furthermore, the provision of food may increase the population of predatory species such as Corvids and Brown Rat *Rattus norvegicus*, which are known egg predators (Marzluff and Neatherlin, 2006). An additional potential hazard of supplementary feeding is the increased risk of disease, transmitted either via direct contact between high densities of individuals at the feeding stations or via the food itself. One study concluded that the prevalence of bird mortality at winter feeders was mostly due to disease after finding an association with the type of feeder and the species composition at the feeder site (Brittingham and Temple, 1986). These relationships would not be expected if mortality was the result of starvation and hypothermia (Brittingham

and Temple, 1986). Not all the diseases were diagnosed but salmonellosis was highlighted as being the principal disease reported as a cause of mortality at bird feeders (Brittingham and Temple, 1986). A direct study of Mycoplasma conjunctivitis, a bacterial infection caused by *Mycoplasma gallisepticum* (MG) which has spread throughout the eastern population of House Finches *Haemorhous mexicanus* in North America, has been linked to transmission at bird feeders (Fischer et al., 1997). The disease is transmitted by direct contact, airborne droplets or dust and House Finches are a highly gregarious and mobile species, likely to assemble at bird feeders which are thought to enhance contact with infected birds or with contaminated surfaces (Fischer et al., 1997). It is also hypothesized that the bird feeders may prolong the life of an infected bird that otherwise would struggle to feed, enabling them to be a longer source of infection (Fischer et al., 1997). Despite being a source of transmission, bird feeders provide a predictable food source that could prevent starvation (Robb et al., 2008a). A more recent study found that house finch declines were greatest following an epidemic where the density of people providing food for the birds also fell dramatically, suggesting that the supplementary food had a positive indirect effect on survival (Fischer and Miller, 2015). When the prevalence and severity of infection with intestinal coccidians *Isospora* sp., and canarypox virus *Avipoxvirus*, was found to increase in House Finches along the rural-to-urban gradient, bird feeders were again implicated in being the driving factor behind the results (Giraudeau et al., 2014). Advice is available on how to minimize disease risk of bird feeders, by cleaning them regularly, but this type of advice is rarely based on evidence and a survey in New Zealand revealed that poor hygienic practices were widely reported (Galbraith et al., 2014).

3.1.2 Transmission routes of *T. gallinae*

Columbiforms are widely believed to be the natural host of *T. gallinae*, and the parasite is transmitted from an infected parent to their offspring via regurgitated food or crop milk (Stabler, 1947). Direct contact between individuals during courtship or cross-feeding has also been highlighted as a transmission route (Forrester and Foster, 2009). *T. gallinae* is able to persist in Columbid carcasses for at least 8 hours, possibly up to 24 hours, after host death whereby it is able to infect Falconiformes that prey upon these birds (Boal et al., 1998, Erwin et al., 2000). Recently, *T. gallinae* has been responsible for an emerging infectious disease in finches in NW Europe and has caused significant declines in the UK breeding population (Robinson et al., 2010). There is limited evidence of how the parasite is transmitted to these novel avian hosts although infection through contact with contaminated shared food and water resources is suspected (Anderson et al., 2009, Lawson et al., 2012, Stockdale et al., 2015). *T. gallinae* is sensitive to desiccation, and is considered more likely to contaminate water, rather than food. However, the presence of a pseudocyst form has been suggested (Stabler, 1947, Tasca and De Carli, 2003, Forrester and Foster, 2009). During this reversible life- stage, *T. gallinae* becomes spherical, having internalized the flagella, but no true cyst wall is present (Tasca and De Carli, 2003). This is

thought to behave as a resistant form under stressful environmental conditions, although the role it plays in transmission is yet to be elucidated (Forrester and Foster, 2009). A trichomonosis epidemic in Spanish Woodpigeons *Columba palumbus* was linked to the provision of supplementary food and UK Columbids screened at farms providing supplementary food were more likely to be infected with *T. gallinae* than those at farms without supplementary food (Hofle et al., 2004, Villanua et al., 2006, Lennon et al., 2013). It is important to identify the role of food supplementation in disease transmission as it is significant for all bird populations that rely on such resources.

3.1.3 Detecting *T. gallinae* in environmental sources

To date detection of *T. gallinae* in food or water sources has been sporadic. *T. gallinae* has been recovered from water containers, and the isolates successfully produced lesions in inoculated birds (Stabler, 1947) . Under laboratory conditions, *T. gallinae* survived in water for at least 120 minutes and on moist grain for up to five days in one study, and for up to 16 hours in water with organic material in another study (Kocan, 1969, Purple and Gerhold, 2015). More recent attempts involved sampling water sources that were seen to be used by Columbids, and screening grain dropped by an infected bird. However, only two out of fifteen water samples were positive for trichomonads, and no parasites were detected in the grain samples (Bunbury et al., 2007). In the UK, a farmyard grain pile and three artificial water sources tested at one site were positive for *T. gallinae* (Stockdale et al., 2015). Following the emergence of trichomonosis in the Canadian Maritime provinces in Canada, the bird seed at sites of mortality was screened but *T. gallinae* was only successfully isolated from one sample (McBurney et al., 2015).

Systematic confirmation of widespread *T. gallinae* presence in shared resources would contribute to establishing the extent of the issue and understanding of epidemiological pathways. It could also allow monitoring of the spread of the parasite without the invasive testing of animals. This chapter examines whether *T. gallinae* can be consistently detected in shared environmental resources and the extent to which it can persist in the environment. Variation in the presence of the parasite will be examined with reference to environmental variables and the species composition and density of birds using the shared resources. Detection trials within a laboratory setting, mimicking the temperatures and degree of dampness often experienced during a British summer, were undertaken to confirm the limitations of *T. gallinae* survival on wheat grain under such conditions.

3.2 Methods

3.2.1 Sample Collection

Shared food and water resources at the UK farmland sites described in Chapter 2 were sampled between May-August 2013-2015 to test for the presence of *T.*

gallinae. A further site was included in the fieldwork season of 2013, a golf course in Cambridgeshire (52°52'N, 0°08'E) due to local sightings of Turtle Doves. The food sources sampled included the bait sites previously mentioned in Chapter 2, which were laid to catch Turtle Doves along with a variety of other birds. These bait sites were 1.5m long and approximately 15cm wide and consisted of Wheat *Triticum* spp., Oil Seed Rape *Brassica napus*, or a standard wild bird seed mix (Maize *Zea mays* , Sunflower *Helianthus annuus*, Pinhead Oatmeal *Avena sativa*, Wheat, Red Dari Sorghum , Red and Yellow Millet *Panicum miliaceum*, Hempseed *Cannabis sativa* and Canary seed *Phalaris canariensis*). The bait sites were monitored for bird activity by camera traps (Bushnell Trophy Cam), which confirmed they were being used by a range of Columbiforms, Passerines and Galliformes. In 2013, a pilot study into the detection of *T. gallinae* in shared resources took place whereby a subset of bait sites were sampled (n=11) with the majority being sampled on multiple occasions over the season (n=10). Gamebird feeders (n=4), the ground below them (n=3), a poultry spoil heap (n=1), cereal farm plots (n=2) and bespoke seed-sown trial plots (n=3) as described by (Dunn et al., 2015) were additionally sampled. The types of water sources sampled in 2013 varied from upturned containers at ground level filled with rain water (n= 1), standing water at 4ft above ground level (n=1) water- logged areas of farmland that persisted over the season (n=2) to water troughs for livestock (n=1) (Figure 3.2). In 2014, a more comprehensive sampling strategy took place, whereby 11 bait sites were sampled every 7-10 days over a period of 10 weeks, with some exceptions due to logistical constraints. This sampling frequency was chosen on the basis that *T. gallinae* is able to persist on moist grain for up to 5 days in laboratory conditions therefore sampling every 7-10 days should allow re-infection rates of shared resources to be established (Kocan, 1969). A similar area of nearby arable field edge/ trial plots was sampled at the same time to provide a control for the bait sites. These plots were chosen to represent a low density food source with less seed provided in a given area, encouraging the birds to forage over a greater area. A standardised water source in the form of a 38cm (L) x 24cm (W) x 8cm (D) plastic tray was placed by 10 of the bait sites and regularly topped up with tap water to maintain a certain water level. A control water source was also created, using an identical plastic tray with a plastic lid fixed securely to the top and sections cut out to allow environmental effects but prevent access by animals (Figure 3.3). This tray was placed next to the experimental water tray with the water level also maintained and they were both sampled at the same time as the bait sites and farm/ seed mix trial plots. The location of the water trays next to the bait sites allowed usage by birds and other animals to also be recorded by the camera traps. In 2015, only a subset of bait sites in Essex (n=4) were sampled weekly over a period of 7 weeks due to logistical constraints. Three bait sites at farmland sites in Hampshire (previously described in Chapter 2) were also sampled on three separate occasions over the course of the season. Additionally, single samples from bait sites across Cambridgeshire, Norfolk, Lincolnshire and Suffolk were taken (n=8) opportunistically as part of a wider auto ecological study of Turtle Doves, which included two farms with bait sites previously sampled in 2013 and 2014 (Ouse Bridge and Hobbs Lot, locations given in Chapter 2) (new

sites: Abbey Farm: 52°58'N, 0°44'E; Feltwell: 52°49'N, 0°50'E; Frampton: 52°93'N, 0°01'E; Kelsale: 52°22'N, 01°47'E; Sizewell: 52°20'N, 01°61'E; Stuston: 52°36'N, 01°12'E) (Figure 3.1). All of the above sampling locations are restricted to the south and south- east of England to coincide with the current distribution of Turtle Dove. To provide an insight into whether *T. gallinae* can be detected at wider geographical scales, two different styles of garden bird feeder (tube feeder and platform) at a site in West Yorkshire (54°12'N, 01°58'E) were sampled weekly over the course of 3 weeks in 2015.

Figure 3.1: Map of additional sites for environmental sampling.

A

B

C

D

E

F

Figure 3.2: The range of water sources sampled at sites. A-B: Water logged areas of farmland. C: evidence of birds using water- logged area featured in B. D: water trough for livestock. E: standing water that has collected on top of the tank, 4ft above ground level. F: upturned container at ground level.

Sampling of the shared resources involved moistening a sterile viscose swab with saline solution or sterile water and running the swab through the entire length of the middle of the bait site. Pre-moistening the swab was thought to increase the chance of the parasite adhering to it. The condition of the bait pile was recorded as dry, damp or wet with the amount of seed left at the time of sampling as either none, some (less than half the original laid pile) or full (more than half of the original laid pile). Gamebird feeders were sampled by running the swab around the area where food is taken from the feeder. Farm plots, seed mix trial plots and a poultry spoil heap were sampled by running the swab along the same area of ground each week, covering a distance of 1.5m. The swab was inoculated into an individual InPouch TF culture kit (Biomed Diagnostics, Oregon), sealed and incubated at 37°C for seven days in order to culture the parasite. In order to sample natural water sources, a disposable pipette was used to collect 0.2ml of water that was then dispelled into an InPouch TF culture kit and treated as described above. One difference made with taking samples from the standardized water trays during 2014 was that the water was stirred briefly with the end of the disposable pipette before a water sample was taken. The condition of the water trays in terms of the amount of water present at the time of sampling was recorded as either dry, moist (reduced to moist algae/ sedimentary remains) or full (enough water to form a level). The users of a subset of bait sites (n=9) were recorded with camera traps. The proportion of Columbids, Galliformes and Passerines visiting the bait site during the 24 hours preceding sample collection was calculated as the proportion of photographs containing each species group. Climatic data were obtained from websites which held data from weather stations local to the sampling sites (http://www.tijou.co.uk/weather/mon201407.html, Essex and http://www.elyweather.org.uk/Data.html, Cambridge/ Norfolk sites).

A

B

C

Figure 3.3: Example of the standardised environmental sampling conducted in 2014. A: water tray and control water tray. B: Placement of water trays next to bait site. C: Example of low intensity feeding site e.g. crop margin.

3.2.2 Parasite presence in environmental samples

Parasite isolation, DNA extraction, PCR of the ITS 1/ 5.8S/ ITS 2 ribosomal region (hereafter referred to as the ITS region) and the Fe-hydrogenase region (hereafter referred to as the Fe-hyd region), Sanger sequencing and Illumina sequencing were performed using the same methods as described in Chapter 2. Each sample (n=448) was screened twice with PCR. If the result was inconclusive, the sample was run a third time and if the infection status could not be reliably determined (i.e due to the quality of the band), the sample was removed from analysis (discarded samples, 2014: n= 33; 2015: 26). The differences in sequencing techniques used between years also applies to these environmental samples i.e samples collected in 2013 were analysed using PCR amplification of the ITS region according to Robinson et al., (2010) and sent off for Sanger sequencing whereas samples collected in 2014 and 2015 were prepared for Illumina sequencing of both the ITS and Fe-hyd regions.

3.2.3 Statistical analysis

The package lme4 (Bates et al., 2015) in R (R Core Team, 2016) was used to perform generalised linear mixed effects analysis of the relationship between *T. gallinae* presence and environmental variables. The type of resource sampled was nested within farm as the random term in order to account for the repeated samples taken over a season. The response variable was the presence or absence of *T. gallinae* and therefore binary. The generalized linear mixed model was fitted by maximum likelihood with Laplace approximation and the default logit link function. The explanatory variables were chosen to represent characteristics considered likely to be important determinants of the presence of *T. gallinae* (Table 3.1). These include the types of shared resources present in the environment and their condition. Climatic data on a landscape scale is used to characterise environmental conditions that may affect the survival and persistence of *T. gallinae* in the shared resources. Separate analyses were conducted for water and food sources as different variables were recorded for them. The overall analysis of food resources involved three separate analyses as not every variable was recorded for every sample (Table 3.1). The first analysis omitted condition, seed and visitor variables but was conducted on a dataset that included all samples (n=226). A second analysis was then conducted on a partial dataset that included the condition and seed variables (n=86). A third analysis was finally conducted on another partial dataset that included the visitor variables (n=28). This took into account the proportion of Columbids, Galliformes and Passerines visiting the bait site during the 24 hours preceding sample collection. Only three samples from 2015 were positive for *T. gallinae* infection and were collected from two new sites therefore in the interest of maintaining a simple structure to the GLMM, 2015 samples were excluded from analysis.

Due to the number of potential explanatory variables to include in the GLMMs for all datasets (Table 3.1), univariate screening was performed to assess which variables had more explanatory power before combining them into one model

and then testing the adequacy of this model using Likelihood Ratio Tests (LRT). Firstly, a null model was compared to a set of models, each containing a single variable, using LRT to determine whether they were likely to influence the response variable ($p < 0.1$). If so, the variable was included in the full model. LRT was performed again to confirm the contribution of the variable to the full model and removed is statistical support was low ($p>0.1$). The adequacy of the amended model was re-tested.

The same approach to identifying molecular operational taxonomic units (MOTUs) as Chapter 2 was also adopted here. If a sequence was present with less than 50 reads in a sample, it was discarded as a potential artefact. This carried the risk of discarding sequences which were present in low numbers due to DNA degradation, which is likely to occur rapidly in environmental samples (Kocan, 1969) however these stringent measures increase the confidence we are only dealing with 'true' strains in the ecological analysis.

Table 3.1: List of explanatory variables assessed in the generalised linear mixed modelling.

Variable name	Description	Detail description	Model
Temperature	Climate data available at landscape scale	Average daily temperature (°C) recorded by nearest weather station	Water
			Food (model 1)
			Food (model 2)
			Food (model 3)
Rainfall	Climate data available at landscape scale	Average daily precipitation (mm) recorded by nearest weather station	Water
			Food (model 1)
			Food (model 2)
			Food (model 3)
Type (water)	Type of water resource present at farm	Water: Pond, opportunistic (present after rainfall, for example), water tray, control water tray.	Water
Type (food)	Type of food resource present at farm	High intensity (i.e. bait pile, gamebird feeder, poultry spoil), low intensity (i.e. farm plot or seed mix trial plot)	Food (model 1)

Seed	Amount of seed present	Categorical factor with three levels – full, some, none.	Food (model 2) Food (model 3)
Condition (water)	Condition of water source in terms of level of water present	Categorical factor with three levels- full, some, none.	Water
Condition (food)	Condition of bait pile in terms of dryness	Categorical factor with three levels – dry, damp and wet.	Food (model 2) Food (model 3)
Visitors	Other users of the bait site (during the 24 hours prior to sampling) who may have the potential to re-infect the site with T. *gallinae*	Abundance of Columbids, Galliformes and Passerines (measured by number of photos each appear in) Proportion of Columbids, Galliformes or Passerines in total abundance Proportion of hours Columbids, Galliformes and Passerines are present in	Food (model 3)
Year	Year the sample was taken	Categorical factor: 2013 or 2014.	Water Food (model 1) Food (model 2)
Farm	Farm where the resource sample was collected	Categorical factor, 12 levels	Random effect in all models

3.2.4 Laboratory detection trials

The persistence of *T. gallinae* on grain was tested within a controlled environment under three treatment variables (dry, moist and saturated) and three different temperatures. A climate summary of May, June and July in 2014 was evaluated to assess the minimum and maximum temperatures experienced during these months (http://www.metoffice.gov.uk/climate/uk/summaries/2014). The temperatures chosen were 10°C and 20°C, to reflect the mean temperature at night and the frequent warmest temperature during the day, and 35°C which is the temperature at which *T. gallinae* is cultured. A control was included for each treatment combination involving glass beads (Smith Scientific Ltd, UK) used instead of grain. Each treatment combination was done in three replicates. Petri dishes were disinfected with Virkon (Day-Impex Ltd, UK) and allowed to air dry. For the dry treatment, paper towel lined the bottom of each petri dish and 2g of dry wheat grain (~20 seeds) was measured out. The weight of grain was chosen to be comparable to the trials of Kocan (1969).

The culture of *T. gallinae* (live trophozoites from the cultures were counted using a haemocytometerwith an average concentration of 9 x 10^5 trichomonads per ml) was washed with 1ml phosphate buffered saline (Fisher Scientific, UK) three times and suspended in 1ml PBS before being mixed with the grain. Moist and saturated treatments had pre- washed moist paper towels lining the petri dishes and 2g wheat grain that had been soaked in PBS for an hour placed on top. 1ml of *T. gallinae* suspended in PBS was mixed with the grain. The saturated treatment was topped up with PBS to fully immerse the wheat grain. This level was maintained throughout the experiment. The petri dishes were incubated at either 10°C, 20°C or 35°C (using Brinsea, UK for 20°C & 35°C treatments or Sanyo MIR-553 BOD incubator for 10°C treatment). The paper towel lining the moist treatments held at 20°C and 35°C were kept moist by adding drops of water with a disposable pipette daily. Sample collection involved taking ten seeds/ glass beads from each treatment, placing them in a tube of TYM culture media (Diamond, 1983) and incubating them at 35°C for three days. To assess whether *T. gallinae* was alive in these samples, three separate drops of culture media from each sample were observed microscopically every day for at least three days or until it became positive (i.e. motile trichomonads were viewed). A sample was collected within 10 minutes of introducing *T. gallinae* to the treatment and repeated every 24hrs thereafter for 6 days in total.

3.3 Results

3.3.1 Detection and persistence of *T. gallinae* in shared resources

The results from screening shared environmental resources for the presence of *T. gallinae* infection are summarised in Table 3.2 and Figure 3.4. In 2013, 20 different food sources were sampled over seven sites and six different water

sources were sampled over five sites. In 2014, 24 different food sources were sampled over 11 sites and 21 different water sources sampled over 10 sites. In 2015, 10 different food sources were sampled over 9 sites. Due to the inconclusive infection status of some samples in combination with logistical constraints experienced during sample collection, there is a reduced dataset from 2014 and 2015. Some resources from 2014 only have results from one week of sampling (n= 2) and other resources have results from multiple sampling over a season but not every week (n= 41). Only two resources have conclusive samples from the full 10 weeks of sampling. Just over half of the samples taken in 2015 yielded inconclusive results (51% n=51). None of the four sites that were repeatedly sampled over the season gave conclusive results for the full seven weeks of sampling but still provided results for between three and five weeks.

Table 3.2: Prevalence of *T. gallinae* infection detected in the food and water resources sampled during a season.

		Positive at least once during season		Positive on repeated samplings	
Year	Resource	Prevalence	N	Prevalence	N
2013	Food	50%	24	33%	18
	Water	67%	6	0	2
2014	Food	67%	24	41%	22
	Water	71%	21	48%	21
2015	Food	12%	25	0	24

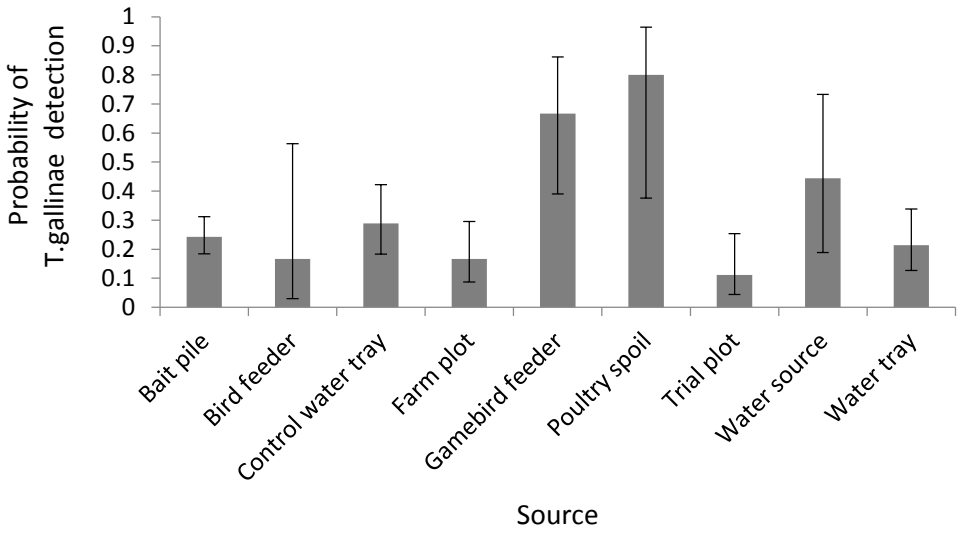


Figure 3.4: Summary of the prevalence of *T. gallinae* infection in the different types of shared resources. Total N sampled includes repeated testing of the same resource over a season. Does not include samples for which the infection status was inconclusive. Standard error bars represent Wilson score intervals. Sample sizes for bait pile (N=169), bird feeder (N=6), control water tray (N=52), farm plot (N=48), gamebird feeder (N=12), poultry spoil (N=5), trial plot (N=36), water source (N=9), water tray (N=56).

Taking into consideration the number of times a resource was detected to be positive out of total repeated sampling attempts, the range varied between 20% - 57% for food sources and 40% - 67% for water sources (Table 3.2). Gamebird feeders and poultry spoils, which attracted high densities of feeding birds, had the highest rates of *T. gallinae* detection (Figure 3.4), followed by natural water sources although the large error bars show that detection of *T. gallinae* was highly variable from all of these sources. Bait piles had moderate levels of *T. gallinae* infection and this probability was the least variable, as shown by the smallest error bars, whereas *T. gallinae* was detected the least in trial plots. In 2013, only two strains were detected from the shared resources: "Tcl-1" (n=9) and the "GEO" strain (n=5). Four food sources were infected with either one on separate occasions. Only Tcl-1 was detected in the water resources (n=3). In 2014, 10 food resources were infected with the Type A strain, 3 with Type C (Gerhold et al., 2008), one with Tcl-1 and one was co-infected with Type A and Type C. Eleven water resources were infected with Type A, four with Type C, one with Tcl-1, one with GEO and one was co-infected with Type A and Type C. All sequences bar one match a sequence 100% in GenBank. That one sequence is 99% similar to WQR strain and is represented by 8507 reads. Detection of this sequence, hereby labelled WQR-Env, occurred in Wood Pigeons (n=2, ~19,000-20,000 reads, see Chapter 4) providing confirmation that it is a new strain.

3.3.2 The effect of environmental variables on the detection of *T. gallinae* in shared resources

The following tables show that there are significant differences relating to environmental variables between the farms where shared resources were tested for the presence or absence of *T. gallinae*. There are also resource- specific differences in the variables that are associated with the detection of *T. gallinae*. The tables show the models fitted for each of the resources: water (Table 3.3) and food (Table 3.4). The fixed and random effect parameter estimates detailed in each table come from the glmer model fit. For the water source model, the terms 'Temperature' and 'State' were included in the full model and retained in the final model. For the food source model, the terms 'Temperature', 'Year' and 'Type' were included in the full model but 'Temperature' was subsequently dropped from the final model.

The detection of *T. gallinae* in water resources was most affected by temperature (Table 3.3). This positive relationship suggests that as the daily average temperature increases, so does the potential for detecting *T. gallinae* in a water resource. *T. gallinae* was also more likely to be detected in full water resources rather than dry water resources, although the confidence limits are large (Table 3.3). There was a similar likelihood of detecting *T. gallinae* in dry and moist water resources. Overall, this pattern suggests that the likelihood of *T. gallinae* detection increases when the resource is full of water as opposed to being dry or moist. The presence of *T. gallinae* in water resources was also shown to decrease from the year 2013 (44%, N= 9) to the year 2014 (25%, N=108) although this pattern was non-significant. The results from the GLMM were used to predict the probability of detecting *T. gallinae* in water sources of three varying conditions and across a range of temperatures (Figure 3.5).

Table 3.3: Results from a GLMM determining environmental factors associated with the likelihood of detecting *T. gallinae* in water resources. N is sample size for each category. P value for Likelihood Ratio Test where term is dropped from final model. Lower and Upper refer to 95% confidence limits. Reference 'State' category is 'Dry'.

Variable	N	P value for LRT	Odds Ratio	Lower	Upper
Intercept			0.00275	0.000119	0.0634
Temperature	117	0.0015	1.25082	1.082164	1.4458
State:		0.0357			
Dry	11				
Full	88		4.60394	0.544517	38.9267
Moist	18		1.02262	0.079195	13.2047

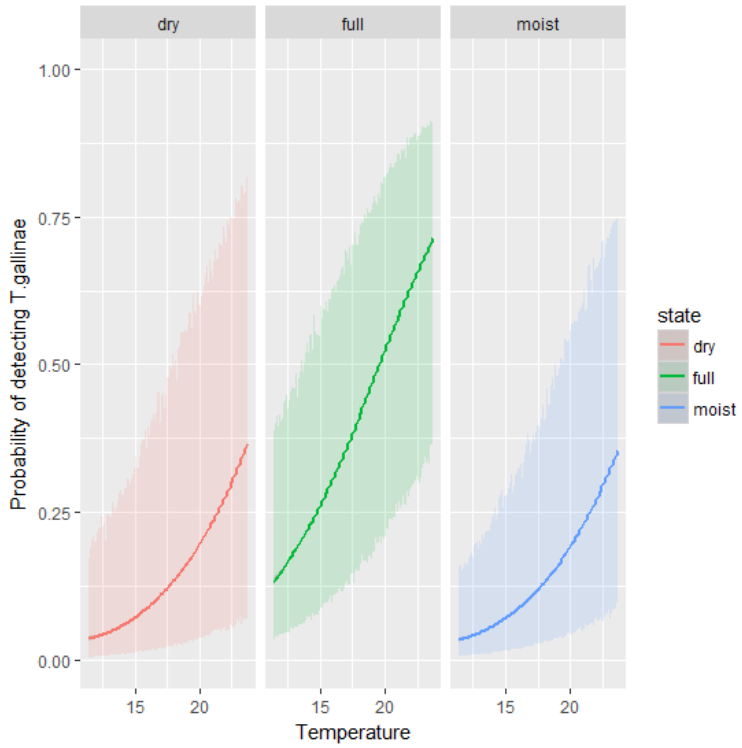


Figure 3.5: Predicting the probability of detecting *T. gallinae* in shared water resources based on the average daily temperature and the condition of the water source. Prediction estimates constructed from 1000 simulations. 80% prediction interval indicated by shaded area.

The detection of *T. gallinae* in food resources was most affected by the 'type' of food resource which was described as being 'high intensity' or 'low intensity' in terms of the amount of seed provided (Table 3.4). It was more likely to be detected in 'high intensity' food resources (Table 3.4). The year in which sampling took place also had a significant effect as *T. gallinae* was less likely to be detected in the year 2014 (22%, N=144) than the year 2013 (38%, N=82) (Table 3.4). The results from the GLMM were used to predict the probability of detecting *T. gallinae* in food sources depending on the type (e.g low intensity or high intensity) and year (Figure 3.6).

Table 3.4: Results from a GLMM determining environmental factors associated with the likelihood of detecting *T. gallinae* in food resources. Sample sizes: Year (2013, N=82; 2014, N=144), Type (high intensity, N=142; low intensity, N=84).

Variable	Estimate	Std. error	P - Value
Fixed effects			
Intercept	-0.1319	0.3942	0.73793
Year: 2013 - 2014	-0.8780	0.3740	0.01891
Type: High - Low intensity	-1.1743	0.3861	0.00236
	Std. dev.		
Random effects			
Grouping:			
Type: Farm	0		
Farm	0.9104		

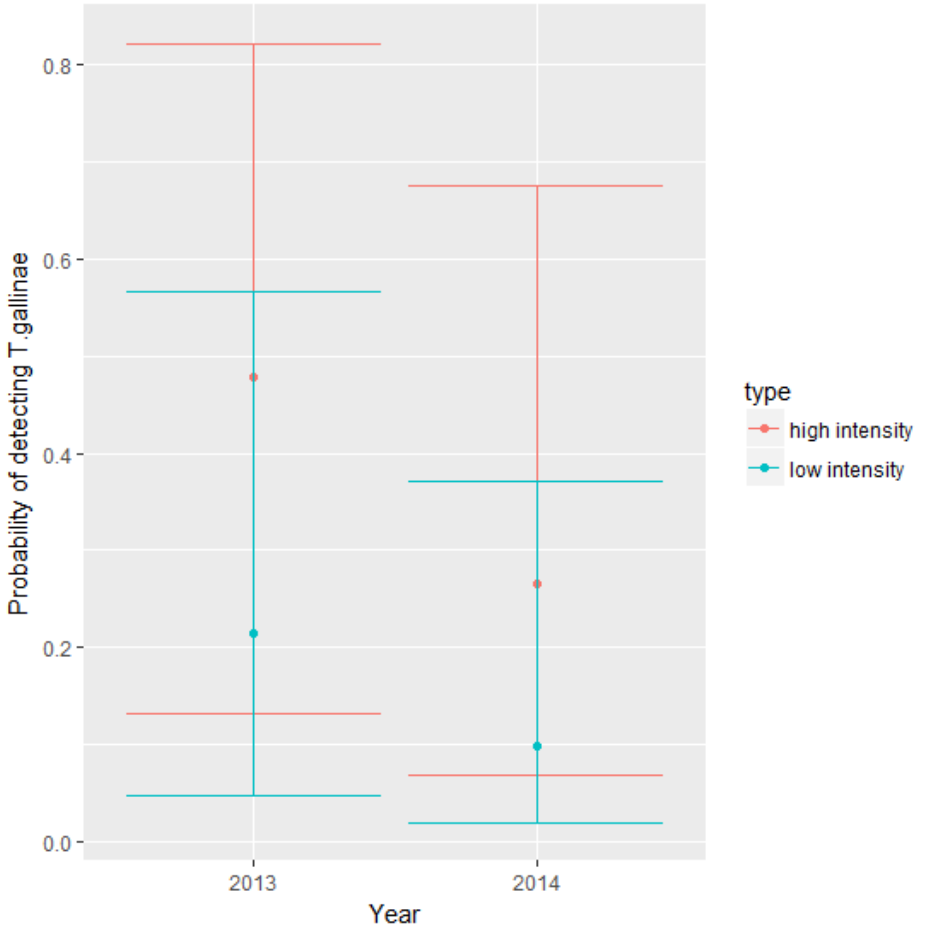


Figure 3.6: Predicting the probability of detecting *T. gallinae* in food resources depending on the 'Type' and 'Year'. Confidence intervals are based on fixed effect uncertainty and random effect variance.

Exploring the variables describing the condition of bait piles at the time of sampling with the levels defined within 'condition' and 'seed' revealed that neither had a significant relationship with the detection of *T. gallinae*. The analysis of these variables involved a reduced dataset on account of missing values therefore the full dataset was returned to with these variables omitted in order to more rigorously assess the effect of the other environmental variables. Three different approximations of 'visitors' were trialled within the model in order to examine slightly different approaches of capturing this information. There was no significant association between the presence of *T. gallinae* and any of the bird families. Models containing these terms in addition to condition and seed variables incurred convergence problems and it was concluded that the data were insufficient to adequately test all terms.

3.3.3 Detection trials of *T. gallinae* in the laboratory

Motile trichomonads were only observed in the moist and saturated treatments at 35°C after two and five days, respectively (Table 3.5). It suggests *T. gallinae*

cannot survive dry conditions regardless of temperature and can only survive in saturated conditions at high temperatures. High density clusters of non- motile trichomonads, which may be pseudocysts, were observed in the saturated treatment at 10°C and the dry treatment at 20°C after 1 day, the saturated treatment at 20°C after 2 days, the saturated treatment at 10°C again after 3 days and the moist treatment at both 10°C and 20°C after 4 days.

Table 3.5: Summary of *T. gallinae* survival under the different conditions tested.

Temperature	Medium	Treatment	Maximum length of survival (days)
10°C	Control	Dry	0
		Moist	0
		Saturated	0
	Wheat	Dry	0
		Moist	0
		Saturated	0
20°C	Control	Dry	0
		Moist	0
		Saturated	0
	Wheat	Dry	0
		Moist	0
		Saturated	0
35°C	Control	Dry	0
		Moist	0
		Saturated	0
	Wheat	Dry	0
		Moist	2
		Saturated	5

3.4 Discussion

There is evidence of *T. gallinae* being present regularly in all types of resource tested, except farm plots or sown seed plots. The higher prevalence in water sources compared to food suggests a greater risk for water. Furthermore, *T. gallinae* is more likely to be present in water sources when the daily average temperatures are warmer, suggesting warm summers may facilitate the transmission of *T. gallinae*. Full water resources as opposed to those that are in the process of drying out or have dried out appear to be more likely to be infected, although results were highly variable. If the water levels are particularly low, it is no longer an effective water resource and therefore unlikely to be attracting visitors. Full water resources, on the other hand, will be attracting visitors, particularly in dry weather, which may lead to high reinfection rates and therefore sustain *T. gallinae* presence. Furthermore, the detection of *T. gallinae* in water sources that contain high volumes of water, such as ponds, is surprising and could be the result of high re-infection rates. Turtle Doves, and other Columbids and Passerines, are known to regularly drink such water sources (Newton, 2008). There was no difference in the detection of *T. gallinae* between the artificial and naturally occurring resources, which varied in terms of the volume of water they held. The artificial water resources, once set up, only had their water levels topped up weekly but were not cleaned, therefore allowing an accumulation of organic material. Organic material appears to prolong the persistence of *T. gallinae* in water (Purple and Gerhold, 2015). Its presence in both artificial and naturally occurring water resources could explain the lack of variation in *T. gallinae* detection between the two types. The lack of difference between the intended control water treatment and other water resources is likely to be the result of pheasants managing to gain access to the control water treatment, which was either observed or there was evidence suggestive of this on a number of occasions. Treating shared water resources for *T. gallinae* may be possible but likely to be ineffective unless a wide number of water resources in a local area can be treated simultaneously. In a laboratory trial, *T. gallinae* can survive in chlorinated water with organic material but not in chlorinated water without organic material (Purple et al., 2015). It would not be feasible to replicate the latter treatment in the field or encourage an uptake of this practice among bird bath owners. The addition of aqueous water extract of garlic (AGE) to drinking water was found to increase the body weight and reduce mortality in infected Domestic Pigeon *Columba livia* nestlings, when compared to the control treatment and metronidazole treatment (Seddiek et al., 2014). Although metronidazole was effective in eliminating infection, AGE showed significant inhibition on *T. gallinae* growth which was dose dependent (Seddiek et al., 2014). The administration of garlic could therefore be a prophylactic agent for the prevention and treatment of *T. gallinae* however further trials are warranted (Seddiek et al., 2014).

The presence and persistence of *T. gallinae* in shared food resources is sufficient to be of conservation concern. *T. gallinae* was more likely to be detected in high density food sources such as seed piles which attract a high concentration of bird

species foraging in a small area, although it should be highlighted that results were again highly variable. It was less likely to be detected in low density food sources such as sown seed plots or uncropped cultivated margins that promote natural regeneration of vegetation from the seed bank, which encourage birds to forage at lower densities over a larger area. This suggests that if supplementary food is provided, it should be scattered over a wide area to encourage similar behaviour. The amount of seed left in bait piles or the degree of dampness at the time of sampling was not associated with *T. gallinae* presence. It is possible that an accumulation of *T. gallinae* existed regardless of the immediate conditions and the adoption of a pseudocyst form could play a role here. This morphological transformation is hypothesized to be triggered by adverse environmental conditions such as desiccation, increased oxygen tension or lower temperatures based on similar research with T. foetus (Granger et al., 2000, Tasca and De Carli, 2003). It may increase the survival time of T. foetus cells in the faeces which could be a trait that *T. gallinae* shares (Granger et al., 2000). PCR did not detect *T. gallinae* in non- cultured faceal samples from either clinically or sub- clinically infected birds although cultures from faecal samples were not tested (Dunn et al., 2016b). If *T. gallinae* can survive excretion by birds then this is another mode of infection of shared resources. Some lids of the control water trays were covered by bird faeces which may have dropped to the water and contaminated them. The proportion of other birds using the bait site was not associated with *T. gallinae* presence which was unexpected given Columbiforme populations are heavily infected therefore a high density of these species was expected to be related to positive infection (Lennon et al., 2013). It is possible that the degree to which other visitors influence the infection status of the bait site results from an interaction with other environmental variables which could not be tested here. Attempts to control a trichomonosis outbreak in Woodpigeons in Spain resulted in applying medication to shared food (Hofle et al., 2004). The amount of medication was estimated based on food intake of a normal bird but it is not guaranteed whether the bird takes its full food intake from one particular resource (Hofle et al., 2004). Subsequently, the bird is under-dosed which could lead to development of resistance by *T. gallinae* to the drug (Munoz et al., 1998). Furthermore, the medication could have a detrimental impact on non- target species. The number of Partridge chicks per female was reported to decrease during the subsequent breeding season of the trichomonosis-treated outbreak in Spain (Hofle et al., 2004). The drug, dimetridazole, can be toxic to birds (Reece et al., 1985). This increases the requirement for other methods, such as garlic, to be developed which have the potential to control local trichomonosis outbreaks without the risks of encouraging antibiotic resistance (Seddiek et al., 2014). Ultimately, prevention is better than cure. In addition to scattering the supplementary food, regularly changing the area where the seed is scattered to prevent an accumulation of *T. gallinae* in the environment is also encouraged. Shared environmental resources as a transmission route for *T. gallinae* has increased the parasite's exposure to a wider range of avian hosts with the added potential of being introduced to naïve populations where it could emerge as an infectious disease. Indeed, parasite spillover was suspected to be the cause of a

trichomonosis outbreak in British finches (Robinson et al., 2010). The prevalence of *T. gallinae* infection of bird feeders is currently unknown but a systematic nationwide survey could be possible with the advent of citizen science (Lawson et al., 2015). Households with bird feeders willing to take part in the survey could be sent a sample collection pack which is subsequently posted back to a laboratory for analysis. This would allow the mapping of positively infected feeders to trichomonosis outbreaks. Furthermore, this will increase the connectedness of the general public to this issue and likely encourage hygienic maintenance of bird feeders.

Strain diversity appeared to increase from the years 2013 to 2014 however a different method of DNA extraction, PCR and sequencing was used to analyse the samples from 2014 therefore this variation in strain composition being the result of adjustments in the methodology cannot be ruled out. There is limited research on whether different strains of *T. gallinae* respond differently to the culture media. A comparison of the effect of a virulent strain (named Jones' Barn) and a non- pathogenic (Lahore) strain on chick liver cells revealed that the virulent strain multiplied faster in the media than the non-pathogenic strain (Honigberg et al., 1964). One study looked at the growth and survival of two clonal cultures (one was identical to the virulent finch strain, the other does not match a sequence on GenBank) in six different types of culture media but the purpose was to optimize in vitro growth, not to compare strains (Amin et al., 2010). The graphs showing growth curves of the two *T. gallinae* isolates revealed very similar patterns (Amin et al., 2010). Notably, no cells from either clone were observed during the whole trial (264 hours) in the TYM culture media, the same culture media used in the detection trials here (Amin et al., 2010).

A parasite may be detected from the resource using PCR but whether it is metabolically viable, and therefore able to infect a bird, would ideally be confirmed by observing the culture media under a microscope for a number of days post-sample collection. Unfortunately, this was not logistically feasible during the fieldwork involved in this study. Evidence suggests culturing *T. gallinae* is required for detection and this involves inoculation with live trichomonads (Dunn et al., 2016b). This supports the assumption that positive environmental samples were likely to be infected with live trichomonads or possibly pseudocysts, that reversed their form once they encountered the more favourable conditions of the culture media (Pereira-Neves et al., 2003).

The levels of persistence of *T. gallinae* on grain were not as high as a previous study where trichomonads survived on moist wheat grain for 4 days at 30°C and 35°C and 2 days at 10°C and 25°C (Kocan 1969). In this experiment, *T. gallinae* only survived in moist and saturated treatments at 35°C for 2 and 5 days respectively. The high densities of non-motile trichomonads in a number of the colder/ drier treatments may have been pseudocysts which were difficult to identify using a light microscope. In order to clarify these results, the experiment ought to be repeated before any firm conclusions are drawn. Some modifications to the next experiment may improve the detection of motile trichomonads.

Firstly, the petri dishes should be filled with wheat grain as 2g only formed a small pile which, in the saturated treatments, the trichomonads could have slid off easily to circulate generally in the petri dish and not be sampled when seeds were collected. A second modification would be to use smaller culture vials for the seeds that are collected so a smaller volume of culture is sampled for microscopic observations. Finally, a different culture media should be used, such as HF medium, which was found to facilitate the best growth of *T. gallinae* in a comparison of six different media (Amin et al., 2010). Furthermore, if the non-motile trichomonads were indeed pseudocysts, this optimized media is more likely to encourage reversal of this life stage (Pereira-Neves et al., 2003).

Supplementary feeding is a regular conservation management tool with potential benefits to the reproductive output of a target species. The risks of disease transmission however, make this practice questionable. There is likely to be a trade-off between maintaining good body condition which may increase resources to fight infection or lowering exposure to transmission sources but increasing stress associated with limited natural food sources. Both outcomes have implications for reproductive productivity which affects the stability of the population and ultimately, the species. In other animal systems the body condition of red deer *Cervus elaphus*, was improved by supplementary feeding and deer in good condition carried lower nematode burdens, possibly related to the nutritional benefits of improved immune function (Vicente et al., 2007). Supplementary feeding however, also encouraged the aggregation of individuals and enhanced the potential risks of bovine tuberculosis (bTB) transmission (Vicente et al., 2007). Further effects of supplementary feeding were revealed by modelling which showed that the impact of provisioning depended on the anthropogenic food source, pathogen type and host immune system (Becker and Hall, 2014, Becker et al., 2015). Moderate levels of provisioning could lead to either pathogen extinction or maximise prevalence (Becker and Hall, 2014). The impact of supplementary feeding on parasite transmission and the overall health of the host is likely to be specific to each system and warrants independent investigation before the implementation of food provisioning as a conservation measure. In the case of providing supplementary feeding on farms in the UK, work to identify a practical and safe conservation solution is ongoing. The effects of climate change may exacerbate the problem, considering the average rise in global temperatures and predicted increase in precipitation in the UK (IPCC, 2013) which will provide environmental conditions that *T. gallinae* is more likely to survive in.

Chapter 4

Reservoir and spillover hosts of *T. gallinae*

4.1 Introduction

The ability of a pathogen to infect multiple host species has been identified as a risk factor to disease emergence in both humans and domestic animals (Cleaveland et al., 2001, Taylor et al., 2001). Furthermore, these generalist multi-host pathogens are common (Woolhouse et al., 2001). Understanding disease dynamics in such systems will help to enable protection of humans, livestock and species of conservation concern. Identifying reservoir hosts has important implications for parasite distribution and persistence. Reservoir hosts are host to the pathogen in the long-term, therefore maintaining it within a population or habitat, and have the ability to transmit the pathogen to a novel host. If contact between a reservoir host and a novel host results in transmission of infection, then this is known as a spillover event and the novel host becomes a spillover host (Daszak et al., 2001). If the spillover host is immunologically naïve to infection then it has the potential to escalate to an epidemic within this novel host species. Understanding the transmission dynamics within and between reservoir and spillover hosts is critical to controlling the disease and preventing onward spread which may result in an epidemic.

Transmission dynamics are varied, and specific pathways can be difficult to pin down if, for example, transmission from reservoir host to spillover host is sporadic. The zoonotic origin of the West African Ebola epidemic was proposed to be the result of a single event involving a two-year old playing near a colony of insectivorous Free-tailed Bats *Mops condylurus* (Saéz et al., 2014). Some practices may increase contact rates, such as the butchering of infected fruit bats in addition to non-human primates and Duikers *Sylvicapra grimmia*, which is common (Saéz et al., 2014, Kaner and Schaack, 2016). Human-to-human transmission, particularly nosocomial transmission, played a key role in the unprecedented spread of the epidemic (which affected Guinea, Liberia and Sierra Leone) (Raabe et al., 2010, Kaner and Schaack, 2016). An epidemic caused by isolated transmission events and subsequently maintained due to transmission within the susceptible spillover host also occurred with epidemics of severe acute respiratory syndrome (SARS) in Hong Kong, whereby large clusters of infection have been linked to single individuals or spatial locations (Riley et al., 2003). Conversely, repeated transmission between the reservoir host and spillover host may be responsible for the maintenance of an epidemic. Many human pathogens are not transmitted readily between humans but rely on contact with infected animals, such as rabies, caused by the RNA virus in the genus *Lyssavirus*, brucellosis *Brucella melitnis*, and bovine tuberculosis *Mycobacterium bovis* (Taylor et al., 2001). One of the best-documented cases is that of Lyme disease *Borrelia burgdorferi*, transmitted from animals to humans by deer ticks, after it

became the most common arthropod-borne disease in the United States (Barbour and Fish, 1993). Transmission between wild animals and closely related domestic counterparts can result in devastating infections of livestock. *Brucella* is easily established in wild populations of bison which can transmit brucellosis to cattle in the US (Dobson and Meagher, 1996); warthogs *Phacochoerus africanus* can transmit African swine fever to domestic pigs in sub- Saharan Africa (Gallardo et al., 2011) and, in a case where the species are not necessarily closely related, Badgers *Meles mele*, can act a reservoir of tuberculosis that may infect cattle in the UK (Donnelly et al., 2003). This transmission pathway can also occur in the opposite direction, with domestic animals being responsible for transmitting a pathogen to a wildlife population and threatening endangered species. Notable examples include domestic dogs transmitting CDV to African Lions *Panthera leo*, causing a severe population crash (Roelke-Parker et al., 1996); populations of both the endangered African Wild Dog *Lycaon pictus* and the endangered Ethiopian Wolf *Canis simensis*, being severely impacted by rabies spilling over from domestic dogs (Gascoyne et al., 1993, SilleroZubiri et al., 1996); and domestic sheep being responsible for transmitting scabies and pasteurellosis to Bighorn Sheep *Ovis canadensis* resulting in population crashes and local extinctions (Jessup et al., 1995).

Reservoir hosts may consist of multiple populations and even species, who contribute to the maintenance of the pathogen in varying degrees, although quantifying this is challenging (Haydon et al., 2002, Fenton et al., 2015). Some reservoir hosts may not be able to maintain pathogen persistence without the existence of a main reservoir host yet they are still able to transmit the pathogen (Fenton et al., 2015). This is demonstrated in the case of bovine tuberculosis (bTB; *Mycobacterium bovis*) in New Zealand, whereby the main reservoir host is considered to be the Brushtail Possum *Trichosurus vulpecula*, whereas the Red Deer and Ferrets *Mustela furo* are spillover hosts (Nugent, 2011). Red Deer and Ferrets can, however, become reservoir hosts if their densities become exceptionally high (Nugent, 2011). It is possible for spillover hosts to transmit infection back to the reservoir host, known as spillback, which plays an important role in the maintenance of the pathogen in the overall population (Nugent, 2011). Cane Toads *Bufo marinus*, syn. *Rhinella marina*, are suspected to have played a role in the emergence of an infectious disease caused by myxosporean parasites in two endangered amphibians (Green and Golden Bell Frog *Litoria aurea*, and the Southern Bell Frog *Litoria raniformis*) (Hartigan et al., 2011). The possibility that Cane Toads brought the parasites with them to Australia was ruled out yet they now carry the parasite and play a role in its dissemination (Hartigan et al., 2011). Spillback can result in amplifying the reservoir of infection. It can increase the spatial spread of the pathogen relative to the main reservoir host alone and allow greater persistence of a pathogen in the spillover host which, if they are long-lived, will also extend the risk of spillback (Nugent, 2011). This has important implications for any eradication plans for disease which will require management of infection in reservoir hosts and spillover hosts, to prevent any risk of spillback (Nugent, 2011).

There are three different strategies to controlling disease (Haydon et al., 2002). The first is to concentrate on controlling infection in the spillover host which, in the case of CDV in wild Indian Foxes *Vulpes bengalensis*, was identified as being the most effective intervention although it does not prevent future spillover from the reservoir of free-ranging domestic dogs (Belsare and Gompper, 2015). The second method is to target the transmission pathways between reservoir and spillover host. This requires knowledge of the main reservoir population acting as the source of transmission and how transmission is being achieved. Wildebeest-associated malignant catarrhal fever (WA- MCF) is a threat to cattle production in eastern and southern Africa whereby the only control method being exercised is the avoidance of grazing cattle in Wildebeest zones (Wambua et al., 2016). This unfortunately increases exposure to other vector- borne diseases and moving the cattle long distances to less nutritionally viable grazing grounds can reduce body condition (Bedelian et al., 2007, Lankester et al., 2015). A more effective means of blocking transmission would be to develop an effective vaccine (Wambua et al., 2016). The third strategy is to focus on controlling infection within the reservoir population. In the case of multi-host pathogens, an understanding of the host community is required as disease management will be more effective if it targets the reservoir host species that is responsible for maintaining the pathogen. In Spain, the endangered Iberian Lynx *Lynx pardinus*, is threatened with spillover of viral infections from feral cats *Felis catus*, and bTB from their wild ungulate prey, and these reservoir hosts are the subject of disease management actions (Delibes et al., 2000). This strategy is the only one which has the potential to eliminate disease but successful elimination requires the reservoir host and disease to be clearly identifiable. If the reservoir hosts are unknown, investigating the natural host of the pathogen is a good place to start (Haydon et al., 2002). These guided prevalence surveys helped identify the reservoir hosts of zoonotic cutaneous leishmaniasis in Iran and hantavirus reservoir hosts in Argentina (Yaghoobi-Ershadi and Javadian, 1996, Calderon et al., 1999). The persistence of infection in a reservoir population must also be demonstrated and can only be achieved with longitudinal studies (Haydon et al., 2002). The use of molecular techniques provides a minimally invasive and reproducible method to identifying pathogens. . The comparison of fine-scale variation between genetic strains of a pathogen has allowed the inference of inter-specific transmission of Giardia and Cryptosporidium in human and animal hosts (Xiao and Ryan, 2004, Feng and Xiao, 2011). Single-nucleotide polymorphisms (SNPs) in whole genome sequences revealed such fine-scale differences between pathogen isolates that it provided evidence for recent transmissions of bacterial lineages of *M. bovis* (responsible for bTB) between Badger and cattle hosts (Biek et al., 2012). The use of molecular techniques has provided valuable insights into multi-host systems by allowing the rapid assessment of the role of each reservoir host in the epidemiology of a pathogen.

The natural hosts of the etiological agent of trichomonosis *T. gallinae*, are members of the Columbiform order (Stabler, 1947). Although natural hosts can often be asymptomatic carriers, there has been a report of a trichomonosis

outbreak in Woodpigeons in southwestern Spain and Portugal, multiple sporadic outbreaks in the Pacific Coast Band- tailed Pigeon in California, and the disease is currently threatening the vulnerable Mauritian Pink Pigeon where infection is a major mortality factor in nestlings and fledglings (Hofle et al., 2004, Villanua et al., 2006, Bunbury et al., 2008, Girard et al., 2014b). The prevalence of infection by *T. gallinae* can vary hugely in populations (Bunbury et al., 2007, Forrester and Foster, 2009). The host range also includes birds of prey who become infected by feeding upon Columbid species and consequently infect their nestlings (Boal et al., 1998, Erwin et al., 2000). Furthermore, trichomonosis has recently become an emerging infectious disease in Passerines, mostly affecting finches, after an epidemic in Britain which subsequently spread to Fennoscandia then central Europe (Peters et al., 2009, Neimanis et al., 2010, Robinson et al., 2010, Lawson et al., 2011b, Zadravec et al., 2012, Ganas et al., 2014). Meanwhile, another outbreak has been reported in Canada (McBurney et al., 2015). *T. gallinae* is thought to have emerged in Passerines by transmission at bird feeding stations, following spillover from Columbids (Forzan et al., 2010, Neimanis et al., 2010, Robinson et al., 2010). Studies of *T. gallinae* infection in finches have thus far identified one clonal strain responsible for the epidemics (Neimanis et al., 2010, Lawson et al., 2011a, Chi et al., 2013, Ganas et al., 2014, McBurney et al., 2015). A subsequent survey revealed that this clonal strain was also preponderant in a sample of British Columbids who had all died between 2009 and 2012 (Chi et al., 2013). The investigations into the finch trichomonosis epidemic have thus far mostly focused on dead specimens. Included in some reports are mentions of non-finch Passerines found dead at the locality of the epidemic: five Blue Tits, one Coal Tit *Parus ater* and one Yellowhammer were found dead at the localities where outbreaks were observed in southern Fennoscandia and five Sparrows *Passer* spp. and three Great Tits *Parus major* were also found in places of disease outbreak in Slovenia (Neimanis et al., 2010, Zadravec et al., 2012). Of these Passerines, only one blue tit from southern Fennoscandia was examined post-mortem and had clinical signs consistent with trichomonosis (Neimanis et al., 2010). In the UK, gross necropsy carried out on a Reed Bunting, Blackbird *Turdus merula*, and House Sparrow, revealed they were infected with the finch epidemic strain (Chi et al., 2013). To my knowledge, no other strain of *T. gallinae* has been reported in European Passerines and there have been limited efforts in establishing the role Passerines play as a host in the epidemiology of *T. gallinae*. Screening live European Passerine populations has so far only been carried out in Spain and Slovenia whereby no birds, except one Magpie *Pica pica* in Spain, were found to carry the parasite (Martinez-Herrero et al., 2014, Zadravec et al., 2016). The genetic strain for the positively infected Magpie is unknown (Martinez-Herrero et al., 2014). In North America, several species of Passerine (House Finches, Scrub Jays *Aphelocoma californica*, American Crows *Corvus brachyrhynchos* and Common Ravens *Corvus corax*) housed at the wildlife rehabilitation hospital in California were screened for *T. gallinae* infection and found to carry the UK finch epidemic strain (Anderson et al., 2009). Live Passerines were also sampled during a trichomonosis epidemic in Canada, with most being infected by the UK finch epidemic strain although further genetic

variation was detected in some individuals, suggesting multiple spillover events were responsible for the emerging disease, rather than the single spillover event hypothesized to be responsible for the outbreak in the UK (Lawson et al., 2011a, McBurney et al., 2015).

In order to further understand the reservoir of *T. gallinae* in free-ranging avian populations, I use data collected from *T. gallinae* screening surveys of Columbid and Passerine populations, over a period of three (Passerines) to five (Columbids) years, to address the following questions:

To what extent are Columbid populations acting as a reservoir of the virulent *T. gallinae* strain?

How does *T. gallinae* infection and strain composition vary within the British bird population and what does this suggest about the role of Passerines in the epidemiology of *T. gallinae*?

4.2 Methods

4.2.1 Sample collection

In order to assess *T. gallinae* infection in potential reservoir hosts belonging to the same local community as the sampled Turtle Doves (Chapter 2), species of Columbiforms other than Turtle Doves were also caught and screened for *T. gallinae* infection (using the same methods detailed in Chapter 2) in the same locations (UK, France, Senegal and Burkina Faso). In the UK, Galliformes and Passerines were also caught and screened at the same locations as Turtle Doves, to assess *T. gallinae* infection and strain composition in potential spillover hosts. Galliformes were caught using whoosh nets whereas Passerines were caught using a mixture of whoosh netting and mist netting. For mist netting, a combination of 5- shelf mist nets (20ft (6m), 40ft (12m) and 60ft (18m) were set up at a subset of farms where habitat was suitable (2013 and 2014: Upp Hall and Ouse Bridge; 2015: Limesbrook and Ouse Bridge). Additional catching attempts using mist nets at a separate site took place during 2013-2015. This garden site (51°88'N, 0°59'E) in Essex was set within an arable landscape and provided supplementary feeding in the form of bird feeders. Another catching attempt using mist nets took place during the fieldwork season of 2015, at a site (near Salisbury, UK) representing semi- natural habitat with no local provision of supplementary feeding (50°98'N, 01°94'W).

4.2.2 Determining infection status

Sampling birds for the presence of *T. gallinae* followed the same protocol as described in Chapter 2. Parasite isolation, DNA extraction, PCR of the ITS 1/ 5.8S/ ITS 2 ribosomal region (hereafter referred to as the ITS region) and the Fe-hydrogenase region (hereafter referred to as the Fe-hyd region), Sanger sequencing, Illumina sequencing and DNA sequence analysis (including the neighbour-joining tree) were also performed using the same methods as

described in Chapter 2. The differences in techniques used between years also apply to these bird samples. Samples from 2011 were collected and analysed as part of the study conducted by Lennon et al. (2013) and samples from 2012 were collected and analysed as part of the study conducted by Stockdale et al. (2015).

4.2.3 Statistical analysis

Generalised linear modelling was performed in R (R Core Team, 2016) to determine associations between *T. gallinae* infection and variables relating to the host (Table 4.1). The response variable was the presence or absence of *T. gallinae* or a strain of *T. gallinae* and therefore binary. The first analysis examined geographical and temporal variation in the strain composition of *T. gallinae* infecting Columbid populations (Model 1).There was not a sufficient sample size for each independent variable within each year for the model to run with 'Year' as a categorical factor. Treating 'Year' as a continuous variable allowed the term to be tested in the model and represent temporal variation. Different Columbid species contributed to these different populations however species could not be included as a variable as the model would not converge, therefore diet was used to characterise some variation within the populations. The second analysis consisted of two parts which examined both *T. gallinae* prevalence (Model 2.1) and *T. gallinae* strain prevalence (Model 2.2a-2.2d) in the British bird population. Again, species could not be included as a variable as the model would not converge therefore the variables 'order' and 'diet' were used to capture some information relating to the type of host that was infected. The diet of the bird sampled was defined according to BTO fact sheets (BTO Bird Facts, n.d.) or, in the case of African species, Handbook of the birds of the world (Del Hoyo et al., 1992) (See Appendix, Tables 7.5 and 7.6 . The categories defined for purpose of this analysis are: granivorous (only grain), herbivorous (all plant matter, including grains), insectivorous (only invertebrates) and omnivorous (plant matter and invertebrates, regardless of frequency). It is recognized that the term 'omnivorous' usually applies to a bird that has a wide range of diet e.g. pheasant who will feed on seeds, berries, leaves, roots and small arthropods however in this study, it will also be used to describe a bird that may have a smaller range e.g. goldfinch *Carduelis carduelis* who will feed on small seeds and some invertebrates in summer. The data here are insufficient to address added levels of complexity associated with the extent of an omnivorous diet e.g. differences between pheasant and goldfinch.

Likelihood Ratio tests (LRTs) were used to compare null models to models containing one term each. The term was included in the full model if $p<0.1$. The adequacy of the model was then tested using LRT. The term was removed if statistical support was low ($p>0.1$) and the adequacy of the amended model was re-tested to give the final model. For some models, levels within a factor were combined if the sample size of a level was too small (Table 4.1). If the levels related to a geographical area, they were combined based on proximity e.g. Burkina Faso and Senegal combined to describe West Africa, or Cambridgeshire and Norfolk combined to describe north East Anglia. If the levels related to a

period of time, they were also combined based on proximity e.g. July or August and September combined to describe the 'late' stage of the breeding season, or May and June combined to describe the 'early' stage of the breeding season. For diet, insectivorous was removed as a category when n=1. Site type could not be included as a term in model 2.2d (Tcl) as the sample size for 'garden' was small (n=4) with no variation in presence. The variance in frequency of DNA haplotypes between Columbid populations and over different years was examined using AMOVA in Arlequin (Excoffier et al., 2005) so that the information in the full DNA sequence could be taken into account.

Table 4.1: List of explanatory variables assessed in the generalised linear modelling, including terms analysed for each model and whether levels had to be combined.

Variable	Description	Model	Combinations within a factor
Year	Year the bird was sampled. Continuous.	Model 1a (Type A)	
		Model 1b (Type C)	
		Model 1c (GEO)	
		Model 1d (Tcl-1)	
		Model 2.1	
		Model 2.2a (Type A)	
		Model 2.2b (Type C)	
		Model 2.2c (GEO)	
		Model 2.2d (Tcl-1)	
Country	Country the bird was sampled. Categorical factor with four levels: Burkina Faso, France, Senegal, UK.	Model 1a (Type A)	West Africa
		Model 1b (Type C)	West Africa
		Model 1c (GEO)	
		Model 1d (Tcl-1)	

Table 4.1 (continued): List of explanatory variables assessed in the generalised linear modelling, including terms analysed for each model and whether levels had to be combined.

Variable	Description	Model	Combinations within a factor
Diet	Categorical factor with four levels: granivorous, herbivorous, insectivorous, omnivorous.	Model 1a (Type A)	
		Model 1b (Type C)	
		Model 1c (GEO)	
		Model 1d (Tcl-1)	
		Model 2.1	
		Model 2.2a (Type A)	
		Model 2.2b (Type C)	Insectivorous removed
		Model 2.2c (GEO)	Insectivorous removed
		Model 2.2d (Tcl-1)	Insectivorous removed
Month	Month of the British breeding season that a bird was sampled. Categorical factor with five levels: May, June, July, August, September.	Model 2.1	Late season (August & September)
		Model 2.2a (Type A)	
		Model 2.2b (Type C)	Late season (July & September)
		Model 2.2c (GEO)	
		Model 2.2d (Tcl-1)	Early (May & June) & Late season (July & September

Table 4.1 (continued): List of explanatory variables assessed in the generalised linear modelling, including terms analysed for each model and whether levels had to be combined.

Variable	Description	Model	Combinations within a factor
County	Area of the UK where the bird was sampled. Categorical factor with three levels: Cambridgeshire, Norfolk, Essex.	Model 2.1	North East Anglia
		Model 2.2a (Type A)	North East Anglia
		Model 2.2b (Type C)	
		Model 2.2c (GEO)	
		Model 2.2d (Tcl-1)	
Order	Taxonomic order of the bird that was sampled. Categorical factor with three levels: Columbid, Galliform, Passerine.	Model 2.1	
		Model 2.2a (Type A)	
		Model 2.2b (Type C)	
		Model 2.2c (GEO)	
		Model 2.2d (Tcl-1)	
Site type	A description of the site where the bird was caught and sampled. Categorical factor with three levels: farm, garden, nature reserve (NB nature reserve only applies to dataset for Model 1).	Model 2.1	
		Model 2.2a (Type A)	
		Model 2.2b (Type C)	
		Model 2.2c (GEO)	

4.3 Results

4.3.1 Prevalence and strain summary

Over a period of five years (2011-2015), 166 Columbids, 90 Passerines and 13 Galliformes were caught in the UK. During 2014, 81 Columbids were caught in France. During 2012/2013, 74 Columbids were caught in Burkina Faso. Over a period of two years (2014-2015), 151 Columbids were caught in Senegal. Prevalence of *T. gallinae* infection in British Columbids remained high over the years sampled, although there was a noticeable decline in 2015 (Table 4.2). The highest prevalence of infection in UK Columbids (100%, n=5) was during 2012 and although this year was the smallest sample size, 100% infection in this bird order is not unusual, as it was also seen in Columbids in France (2013, n=3) and Columbids in Senegal (2014, n=78 and 2015, n=16). Columbids sampled in Burkina Faso revealed a similar proportion of *T. gallinae* infection (69%, n=55) to most years of UK Columbid screening (2011-2014: 60%-100%, see table for respective sample sizes) although sub-optimal sample storage means this is likely to be an underestimate of true prevalence. British Columbids had the highest prevalence of *T. gallinae* infection (66%, n=109) over all the years sampled in comparison to Galliformes (33%, n=12) and Passerines (38%, n=90). The UK breeding season of 2013 however, revealed a particularly high proportion of Passerines being infected (70%, n=23), although similar levels of infection were also observed in the Columbid population (79%, n=29). The sample size for gruiformes was too small (n=1) to draw conclusions from. The decline of *T. gallinae* infection in Columbids during the final year of sampling was mirrored in Galliformes and Passerines also.

Strain information was not obtained for every positively infected sample, hence the difference in sample sizes between Table 4.2 and Figure 4.3. Samples collected from the UK in 2013 and analysed by Sanger sequencing were identified as one of four ITS types: Type A, Type C, GEO and the Tcl-1 strain. Samples collected from Senegal in 2015 that were also Sanger sequenced were identified as one of four *T. gallinae* ITS types: Type C, GEO, Tcl-1, Type III or *T.tenax*. The majority of ITS sequences (88%, n=93) sampled from hosts that were analysed by NGS were identified as the Type A, Type C or the GEO strain. A new ITS strain was discovered that is 99% similar to the Tcl-1 strain, hereafter named Tcl_BBWD (n=9, ~55-5,000 reads). A further four new sequences were also detected but they each occurred in one sample therefore detection in further samples are ideally required before they are considered a new strain. Names are given here for reference: Sen-NQD is 96% similar to the Tcl-1 strain, detected from a Namaqua Dove *Oena capensis* (Senegal 2014, 642 reads), GEO-NQD is 99% similar to GEO strain, detected from a Namaqua Dove (Senegal 2014, 1540 reads), GEO-LD is 98% similar to GEO strain, detected from a Laughing Dove (Senegal 2014, 544 reads) and Tcl-LD is 98% similar to Tcl-1 strain, detected from the same Laughing Dove (Senegal 2014, 498 reads). No cases of coinfection between different ITS types were detected. The relationship of the new sequences and potentially new

sequences to known ITS types can be visualised in Figure 4.1. One of the new strains isolated from a Laughing Dove (GEO-LD) along with one of the new strains isolated from a Namaqua Dove (GEO-NQD) group with the GEO-TD strain identified during this study and previously recognised GEO strain and Type II strain, in addition to *T. tenax*. The new strain isolated from the Black-billed Wood Dove *Turtur abyssinicus* (Tcl-BBWD) and the strain isolated from another Laughing Dove (Tcl-LD) form a clade with previously recognised Tcl-1 strain. The other strain isolated from a Namaqua Dove (Sen-NQD) is in a separate clade to the *T. gallinae* – like strains (Type A, Type B, Type C and Type V). It should be noted that the WQR-strain is identical to the Type III strain in the region of overlap but it is 27bp longer.

Table 4.2: Prevalence of T. *gallinae* infection in adult birds sampled in the UK, France, Burkina Faso and Senegal between 2011- 2015. Results from 2011 from Lennon et al., (2013), samples from 2012 from Stockdale et al., (2015). Birds from Burkina Faso were caught during the winter of 2012-2013 but results are collated into one year (2012).

Country	Order	Species (Latin name)	2011	2012	2013	2014	2015	TOTAL
UK	Columbiforms	Collared Dove *Streptopelia decaocto*	86% (n=7)	100% (n=1)	100% (n=2)	80% (n=5)		87% (n=15)
		Feral Pigeon *Columba livia domestica*			75% (n=4)			75% (n=4)
		Stock Dove *Columba oenas*	50% (n=2)		86% (n=14)	65% (n=20)	50% (n=4)	70% (n=40)
		Wood Pigeon *Columba palumbus*	56% (n=18)	100% (n=4)	67% (n=9)	47% (n=15)	25% (n=4)	56% (n=50)
TOTAL (UK Columbids)			63% (n=27)	100% (n=5)	79% (n=29)	60% (n=40)	38% (n=8)	66% (n=109)
UK	Passeriformes	Blackbird *Turdus merula*			100% (n=3)	0% (n=2)	50% (n=2)	57% (n=7)
		Blue tit *Cyanistes caeruleus*			50% (n=2)		0% (n=1)	33% (n=3)
		Bullfinch *Pyrrhula pyrrhula*					0% (n=1)	0% (n=1)
		Chaffinch *Fringilla coelebs*			100% (n=2)	33% (n=3)	0% (n=2)	43% (n=7)
		Dunnock *Prunella modularis*			100% (n=1)	50% (n=2)	14% (n=7)	30% (n=10)

Table 4.2 continued: Prevalence of T. *gallinae* infection in adult birds sampled in the UK, France, Burkina Faso and Senegal between 2011- 2015. Samples from 2011 from Lennon et al., (2013), samples from 2012 from Stockdale et al., (2015). Birds from Burkina Faso were caught during the winter of 2012-2013 but results are collated into one year (2012).

Country	Order	Species (Latin name)	2011	2012	2013	2014	2015	TOTAL
UK	Passeriformes	Goldfinch *Carduelis carduelis*			100% (n=1)	50% (n=2)	0% (n=1)	50% (n=4)
		Greenfinch *Chloris chloris*			0% (n=1)	30% (n=10)		27% (n=11)
		Great tit *Parus major*			33% (n=6)		0% (n=2)	25% (n=8)
		House sparrow *Passer domesticus*				40% (n=5)	0% (n=3)	25% (n=8)
		Jackdaw *Corvus monedula*			50% (n=2)	0% (n=1)		33% (n=3)
		Jay *Garrulus glandarius*				25% (n=4)		25% (n=4)
		Long-tailed tit *Aegithalos caudatus*					50% (n=2)	50% (n=2)
		Magpie *Pica pica*				100% (n=2)		100% (n=2)
		Pied wagtail *Motacilla alba*			100% (n=1)			100% (n=1)
		Robin *Erithacus rubecula*			100% (n=3)			100% (n=3)
		Rook *Corvus frugilegus*				0% (n=1)		0% (n=1)
		Common Starling *Sturnus vulgaris*			100% (n=1)	0% (n=5)		17% (n=6)

Table 4.2 continued: Prevalence of T. *gallinae* infection in adult birds sampled in the UK, France, Burkina Faso and Senegal between 2011- 2015. Samples from 2011 from Lennon et al., (2013), samples from 2012 from Stockdale et al., (2015). Birds from Burkina Faso were caught during the winter of 2012-2013 but results are collated into one year (2012).

Country	Order	Species (Latin name)	2011	2012	2013	2014	2015	TOTAL
		Yellowhammer *Emberiza citrinella*				0% (n=4)	75% (n=4)	38% (n=8)
TOTAL (UK Passeriformess)					70% (n=23)	29% (n=42)	24% (n=25)	38% (n=90)
UK	Galliformes	Grey partridge *Perdix perdix*				0% (n=2)		0% (n=2)
		Common pheasant *Phasianus colchicus*				33% (n=3)		33% (n=3)
		Red-legged partridge *Alectoris rufa*			100% (n=1)	50% (n=4)	0% (n=2)	43% (n=7)
TOTAL (UK Galliformes)					100% (n=1)	33% (n=9)	0% (n=2)	33% (n=12)
UK	Gruiformes	Common moorhen *Gallinula chloropus*				0% (n=1)		0% (n=1)
TOTAL (UK gruiformes)						0% (n=1)		0% (n=1)
France	Columbiforms	Woodpigeon *Columba palumbus*			100% (n=2)			100% (n=2)
		Collared Dove *Streptopelia decaocto*			100% (n=1)			100% (n=1)
TOTAL (France Columbids)					100% (n=3)			100% (n=3)

Table 4.2 continued: Prevalence of T. *gallinae* infection in adult birds sampled in the UK, France, Burkina Faso and Senegal between 2011- 2015. Samples from 2011 from Lennon et al., (2013), samples from 2012 from Stockdale et al., (2015). Birds from Burkina Faso were caught during the winter of 2012-2013 but results are collated into one year (2012).

Country	Order	Species (Latin name)	2011	2012	2013	2014	2015	TOTAL
Burkina Faso	Columbiforms	African Mourning Doves *Streptopelia decipiens*		75% (n=4)				75% (n=4)
		Laughing Dove *Streptopelia senegalensis*		69% (n=49)				69% (n=49)
		Vinaceous Dove *Streptopelia vinacea*		50% (n=2)				50% (n=2)
TOTAL (Burkina Faso Columbids)				69% (n=55)				69% (n=55)
Senegal	Columbiforms	Black-billed Wood Dove *Turtur abyssinicus*				100% (n=14)	100% (n=1)	100% (n=15)
		Laughing Dove *Streptopelia senegalensis*				100% (n=30)	100% (n=4)	100% (n=34)
		Namaqua Dove *Oena capensis*				100% (n=33)	100% (n=10)	100% (n=43)
		Vinaceous Dove *Streptopelia vinacea*				100% (n=1)	100% (n=1)	100% (n=2)
TOTAL (Senegal Columbids)						100% (n=78)	100% (n=16)	100% (n=94)

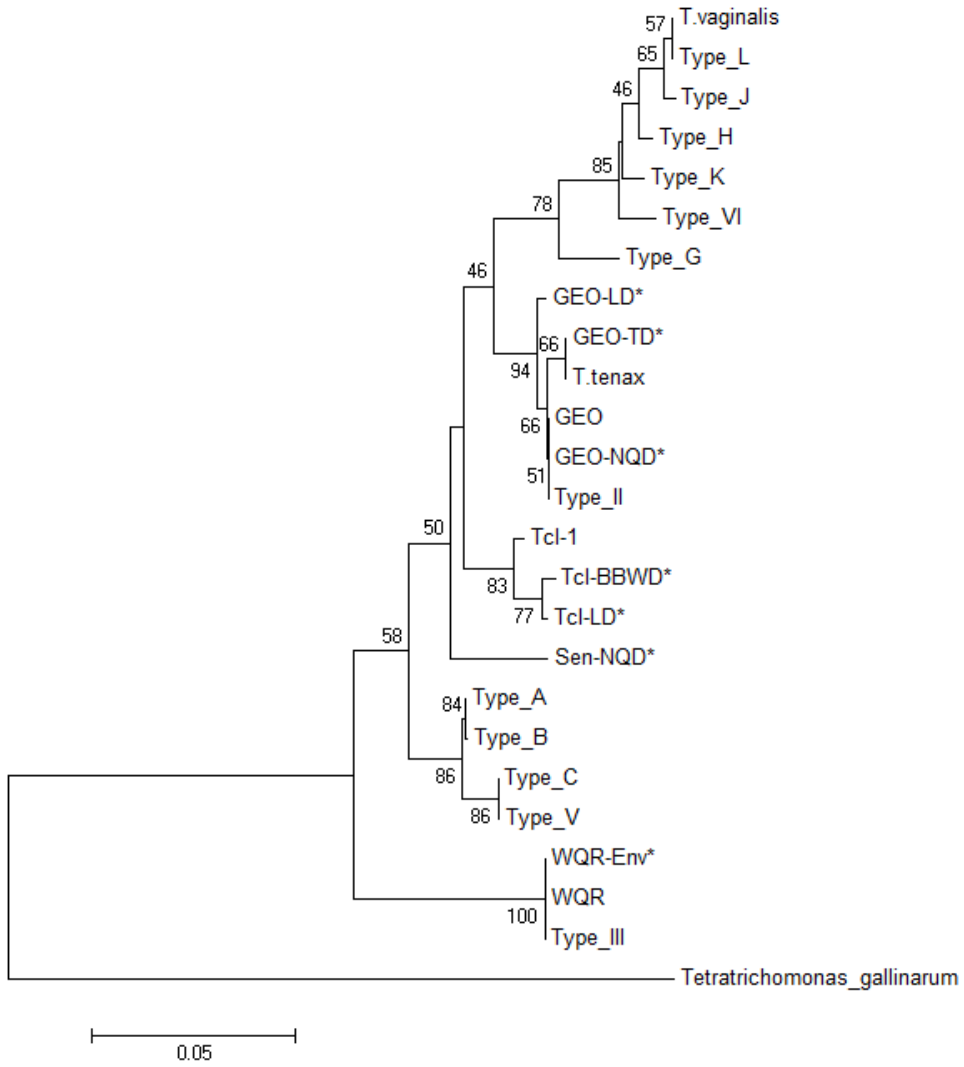


Figure 4.1: Phylogenetic tree including potential new *T. gallinae* strains from this study based on an alignment of the ITS region (214bp), constructed using the neighbour-joining method with genetic distance measured by maximum composite likelihood. Representatives of other strains detailed in Table 3 of Chapter 2. Branch reliability given as a percentage. Node values below 40 are not shown. Outgroup is *Tetratrichomonas gallinarum*, accession number AY244648 (Kutisova et al., 2005). Sequences marked with * are new from this study.

Strain information from amplifying the Fe-hyd region in host samples has allowed a further five samples to be identified, which did not previously give a result for the ITS region. Nevertheless, only 43% (n=93) of samples which had the ITS region successfully amplified also had the Fe-hyd region amplified. This is a similar result to the Turtle Dove dataset, whereby 44% (n=78) of samples identified by the ITS region, also had Fe-hyd sub-type information. Furthermore, the majority of Fe-hyd sequences in both datasets (Turtle Doves: 68%, n=34, other hosts: 52%, n=43) were identified based on partial fragments and not the full length sequence. Figure 4.2 depicts the variation in Fe-hyd sub types between bird families according to year and country. The subtype A1 of the virulent Type A strain is the only subtype to be detected in hosts from the UK and France. The subtype A2 was detected in a Columbid (Black-billed Wood Dove) from Senegal. A total of six Type C subtypes were detected in all the hosts sampled with some evidence of geographical separation. The subtype C4 occurred in both the British and French populations. Subtype C6 was found in the French and Senegalese population. The C8-TD variant was only discovered in the French population and the C11-TD variant was only found in the British population. Another three new variants were isolated from Laughing Doves in the Senegalese population (C9-LD, C10-LD, C12-LD). Two sub-types within the Tcl-1 strain described in Chapter 2 have also been found in other French Columbids (T1-TD) and Senegalese Columbids (T2-TD). The sub-type of one of the potentially new ITS strains, which is 99% similar to the Tcl-1 strain, hereafter named NT1-BBWD has only been detected in Senegalese Columbids thus far. Unresolved sub-types are partial sequences which did not group with reference strains or new MOTUs identified from this study based on full length sequences (see Chapter 2 for methods).

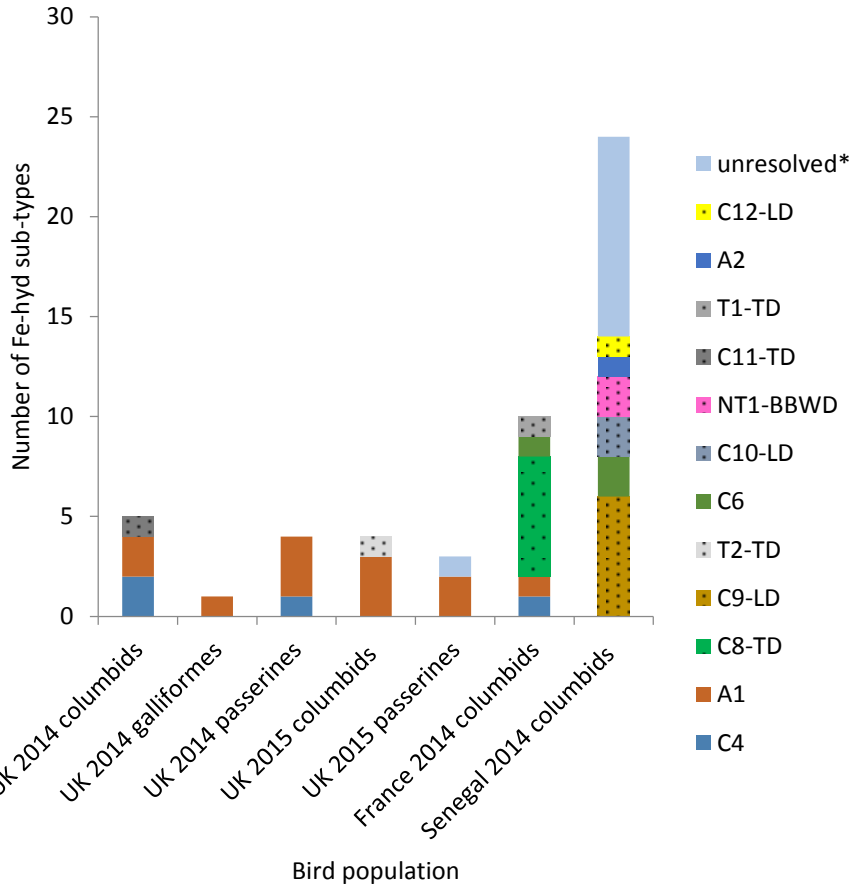


Figure 4.2: Number of Fe-hyd sub-types detected in bird populations defined by order, country and year. Additional strains detected where amplification of the ITS region for a sample failed. Black dots represent new Fe-hyd sequences discovered during this study. *Unresolved sub-types (n=11) consist of different sequences that are combined into one group in this graph.

4.3.2 Columbid populations as a reservoir of the virulent Type A strain

Moderate to high proportions of the virulent Type A strain were detected in British Columbid populations in most years (2012, 2014, 2015: 40%- 87%, see Figure 4.3 for sample sizes). The proportion of Type A was however, relatively low (13%, n=31) during 2013. Comparing the strain composition in Columbids, with and without Turtle Doves, allows inference on whether Turtle Doves are contributing to the presence of Type A (Figure 4.3). The strain composition remains similar for years 2012-2014 with the presence of Type A not varying by more than 10% when Turtle Doves are excluded from the dataset. Turtle Doves appear to decrease the proportion of Type A in the UK Columbid population in 2015 by introducing Tcl-1 into the strain composition but the sample size is small. Turtle Doves also contribute the GEO strain to overall strain composition in Columbids in 2013. The finch epidemic strain was only detected in one sample in France (Turtle Dove) and two samples in Senegal in 2014 (Black-billed Wood Dove and Namaqua Dove), reflecting very small proportions of the overall strain

composition in these populations. Overall, there appears to be variation in *T. gallinae* strains infecting Columbids from different geographical populations (Figure 4.3). The highest diversity of *T. gallinae* strains was detected from Columbids in France where six strains were detected in 2014. The strain composition is also relatively diverse in Senegal, with five strains being detected each year but three of these strains being different between years. The strain diversity is lowest in Burkina Faso with only two being detected, along with the year 2015 in the UK, although these are also the smallest sample sizes. The sample size for the UK in 2013 was almost double the sample size of 2014, yet the number of strains detected remained the same (n=4) with some variation between years.

The results of an AMOVA reveal an almost strong differentiation (FST value =0.25 where > 0.25 is considered strong) (Table 4.3) (Hartl and Clark, 1997) in the ITS strains infecting different Columbid populations (defined according to year and country sampled), supporting the observed temporal and geographical variation displayed in Figure 4.3.

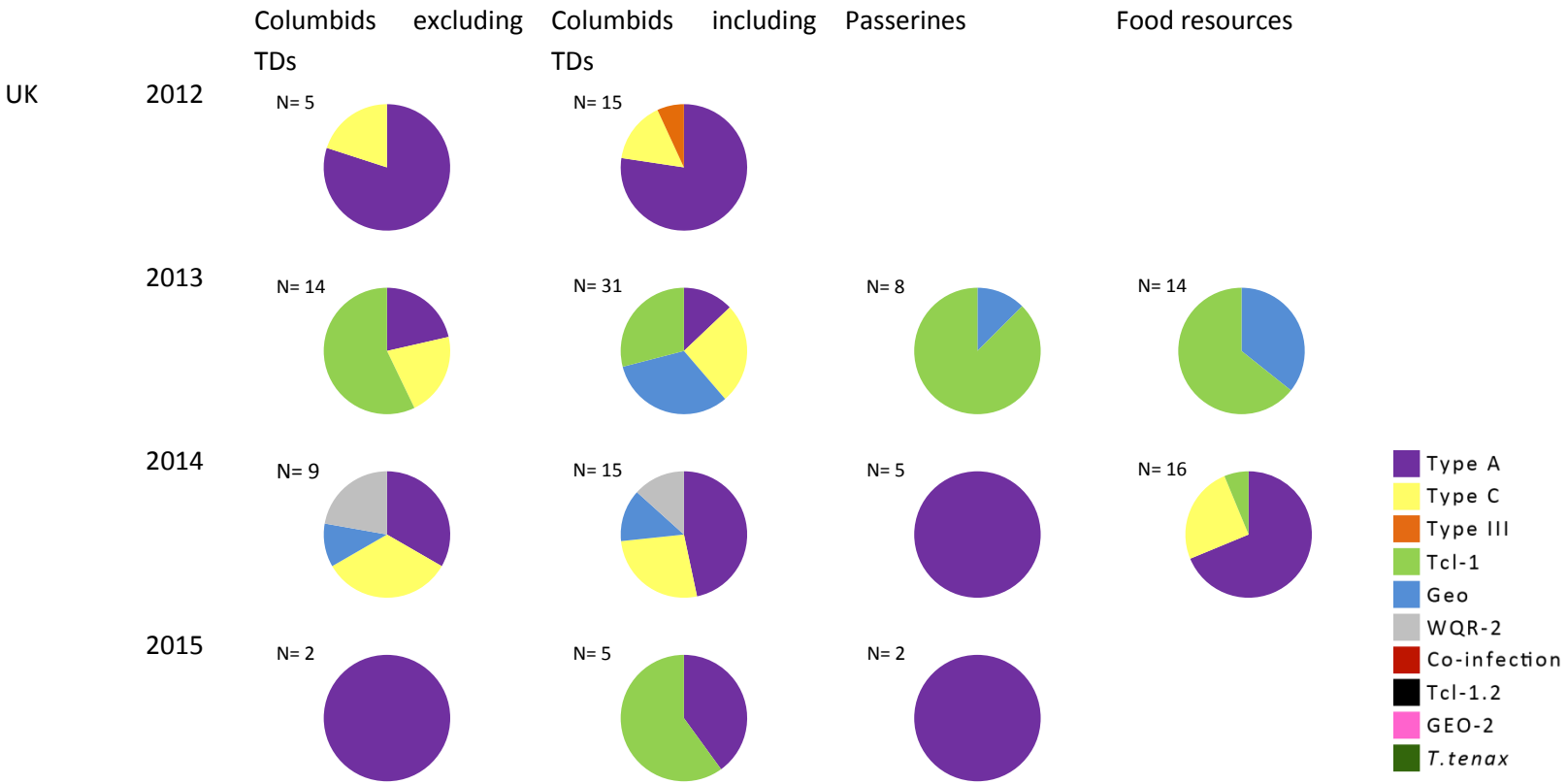


Figure 4.3: Pie charts reflecting the strain composition (based on the ITS region) of *T. gallinae* infecting Columbid populations, allowing the comparison of resident Columbid species (i.e excluding Turtle Doves) with all Columbid species (i.e. including Turtle Doves) in different countries and between different years. The strain composition infecting Passerines and shared food resources is also shown for the UK during different years. Potential new sequences only detected in one sample are not included.NB: additional strains detected based on Fe-hyd region only not included.

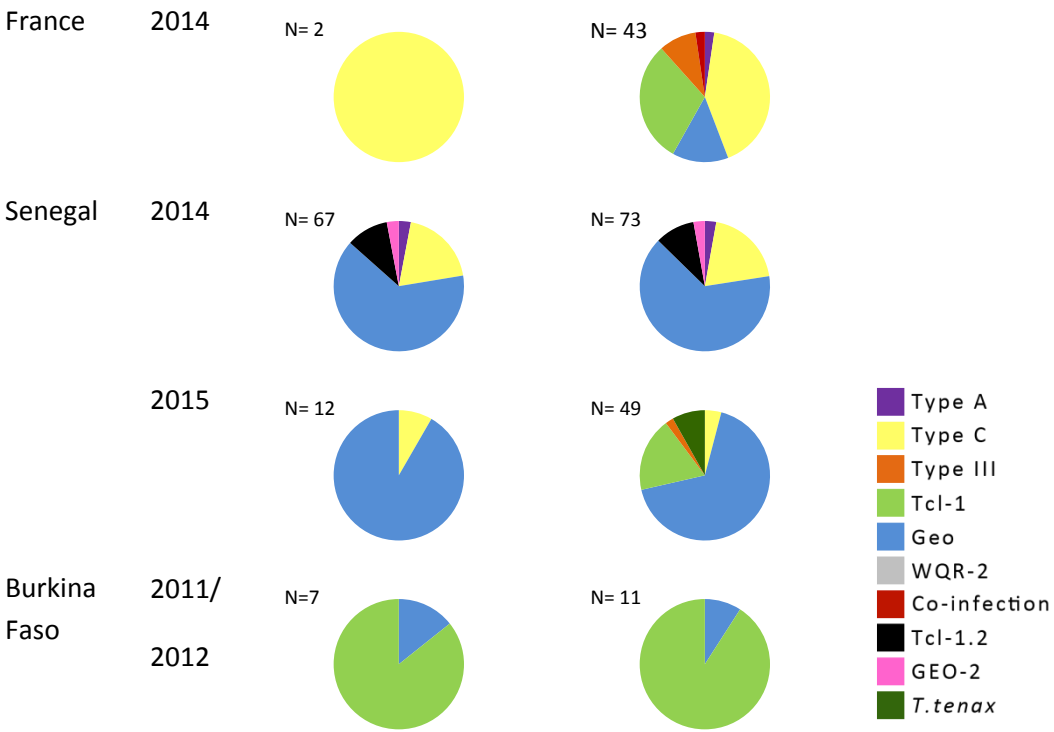


Figure 4.3 continued: Pie charts reflecting the strain composition (based on the ITS region) of *T. gallinae* infecting Columbid populations, allowing the comparison of resident Columbid species (i.e excluding Turtle Doves) with all Columbid species (i.e. including Turtle Doves) in different countries and between different years. The strain composition infecting Passerines and shared food resources is also shown for the UK during different years. Potential new sequences only detected in one sample are not included. NB: additional strains detected based on Fe-hyd region only not included.

Table 4.4 shows results from generalised linear modelling reveal associations between strains and countries. Diet is also an important term for the prevalence of Type C and GEO, although it is confounded with species which could not be included as a term here. Year is an important term for the Tcl-1 strain (Table 4.4). The virulent Type A strain is more prevalent in the UK (42%, n=67) than France (2.6%, n=38) and West Africa (0.02%, n=119) (Figure 4.4). The Type C strain is more prevalent in France (47%, n=38) than in the UK (22%, n=67) and West Africa (13%, n=119) (Figure 4.4). Furthermore, omnivorous Columbids are more likely to be infected with Type C than granivorous Columbids and marginally more likely to be infected than herbivorous Columbids (Figure 4.4). Herbivorous Columbids are also marginally more likely to be infected with Type C than granivorous Columbids (Figure 4.4). The GEO strain was more prevalent in Senegal (73%, n=108) than Burkina Faso (0.09%, n=11), France (0.16%, n=38) and the UK (0.18%, n=67) (Figure 4.5). Columbids that have granivorous or herbivorous diets are more likely to be infected with the GEO strain than Columbids with an omnivorous diet (Figure 4.5). The Tcl-1 strain is more prevalent in Burkina Faso (91%, n=11) than all the other countries (Senegal (n=108), France (n=38), UK (n=67): 10% - 34%) (Figure 4.5). It is also more likely to be found in France (34%, n=38) and the UK (18%, n=67) than Senegal (10%, n=108) (Figure 4.5). Unlike the other strains, prevalence of the Tcl-1 strain varied significantly with year, showing an increase from 2014 to 2015 (Figure 4.5).

Table 4.3: AMOVA results for variance partitioned by year + country. Number of groups = 1. Significant Fst value highlighted in bold

Source of variation	d.f	Variance components	Percentage of variation	Fixation index FST
Among populations	7	0.1	24.88	**0.25**
Within populations	237	0.29	75.12	

Table 4.4: Results of LRT tests determining factors associated with T. *gallinae* strain prevalence in Columbid populations (Model 1). All terms included in full model. Significant results for the removal of a term from the full model are in bold and these terms were retained for the final model. Dev. = Deviance. N=224.

	Type A			Type C			GEO			Tcl-1		
	Dev.	df	P value	Dev.	df	P value	Dev.	df	P value	Dev.	df	P value
Year	1.01	1	0.32	0.05	1	0.82	3.47	1	0.63	4.76	1	**0.03**
Country	37.87	2	**5.98e-09**	27.51	2	**1.05e-06**	46.15	2	**9.55e-11**	7.54	2	**0.023**
Diet	0.31	2	0.86	21.22	2	**2.46e-05**	25.03	2	**3.68e-06**	0.58	2	0.75

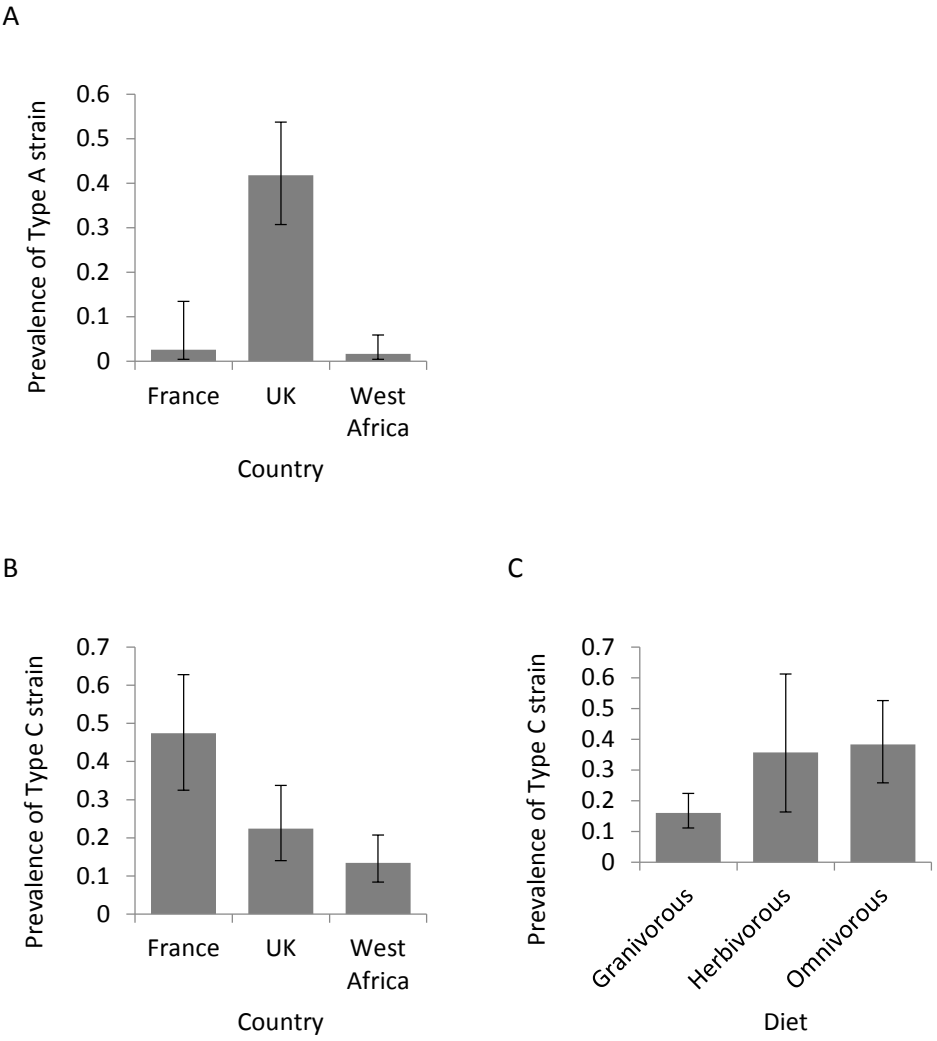


Figure 4.4: Significant terms in final model for prevalence of *T. gallinae* strains in Columbid populations. Mean prevalence ± SE of: Type A strain depending on (A) country; Type C strain depending on (B) country, (C) diet. Standard error bars represent Wilson score intervals. Sample sizes: France (N=38), UK (N=67), West Africa (N=119), granivorous (N=163), herbivorous (N=14), omnivorous (N=47).

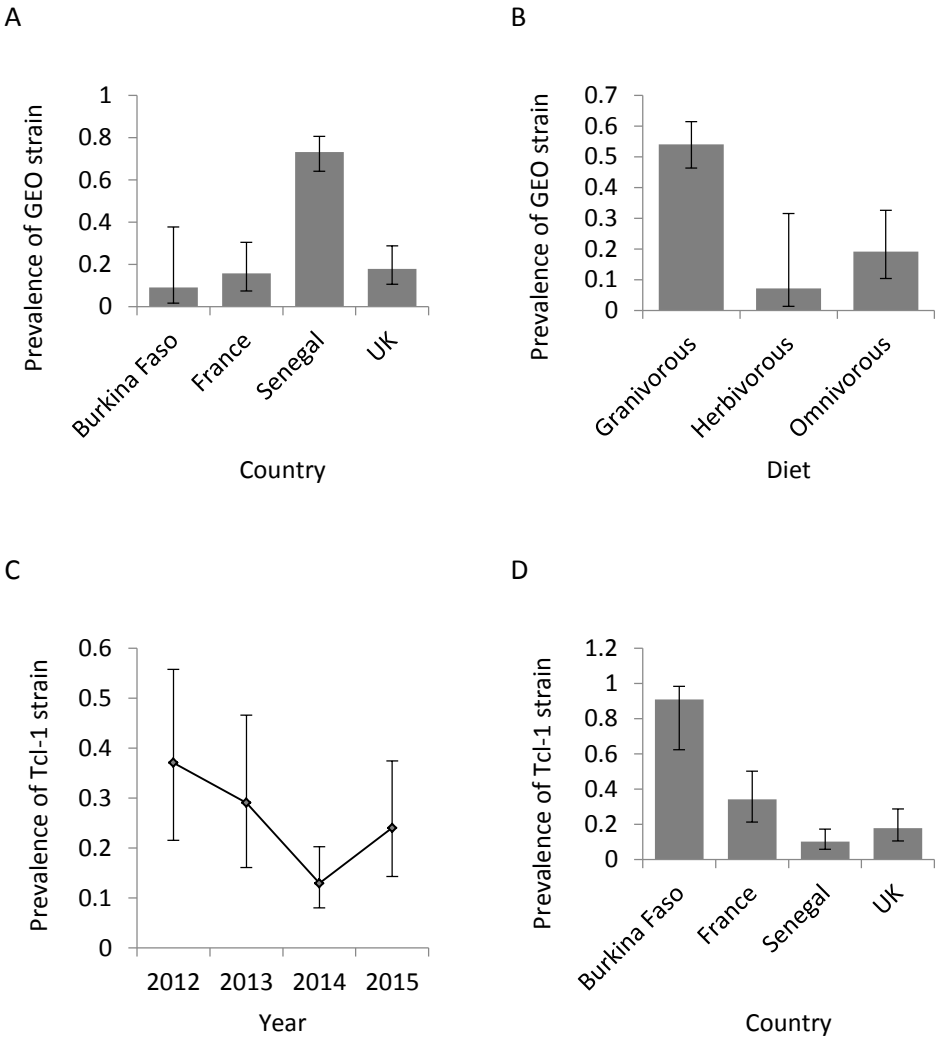


Figure 4.5: Significant terms in final model for prevalence of *T. gallinae* strains in Columbid populations. Mean prevalence ± SE of: GEO strain depending on (A) country and (B) diet; Tcl-1 strain depending on (C) Year and (D) Country. Standard error bars represent Wilson score intervals. Sample sizes: France (N=38), UK (N=67), Senegal (N=108), Burkina Faso (N=11), granivorous (N=163), herbivorous (N=14), omnivorous (N=47), 2012 (N=27), 2013 (N=31), 2014 (N=116), 2015 (N=50).

4.3.3 The role of Passerines in the epidemiology of *T. gallinae*

More strains were detected in British Columbids than Passerines although this could be an artefact of sample size (Figure 4.3). Comparing the strain composition between British Columbids, Passerines and food resources within the same year reveals similarities (Figure 4.3). In 2013, the two highest occurring strains in the Columbid population were GEO and Tcl-1 which were the only two strains detected in Passerines and shared food resources. There is only strain information for one Galliform sample, a Red-legged Partridge which was infected with Tcl-1 in 2013. In 2014, Type A is dominating Columbid populations, with Type C being the second most common. Only Type A was detected in Passerine populations and Type A was also the most detected strain in food resources, with Type C being the second, similar to the Columbid population.

Terms capturing temporal variation and characteristics of the host were important for the prevalence of *T. gallinae* infection in the British bird population (Table 4.5). *T. gallinae* prevalence has significantly decreased over the years sampled (Figure 4.6). Birds that were caught in June were more likely to be infected (74%, n=133) than birds caught in May (43%, n=70) (Figure 4.6). Columbids were more likely to be infected (79%, n=152) than Passerines (38%, n=90) (Figure 4.6). Birds with a granivorous diet were more likely to be infected (96%, n=54) than birds with an herbivorous (58%, n=48) or omnivorous diet (51%, n=150) (Figure 4.6). In this dataset, only Turtle Doves were categorized as having a granivorous diet therefore Turtle Doves are more likely to be infected than the other birds sampled as part of this study.

Table 4.5: Results of LRT tests determining factors associated with *T. gallinae* infection in the British bird population (Model 2.1). Terms highlighted in grey were not included in the full model with statistics given for comparison of term to null model. Remaining terms included in full model. Significant results for the removal of a term from the full model are in bold and these terms were retained for the final model. Dev. = Deviance. N=255.

	T. gallinae infection		
	Dev.	df	P value
Year	15.967	1	**6.446e-05**
Month	15.825	3	**0.001231**
County	0.87101	1	0.3507
Site type	1.8333	2	0.3999
Fed	2.6214	1	0.1054
Order	8.2323	2	**0.01631**
Diet	21.767	3	**7.293e-05**

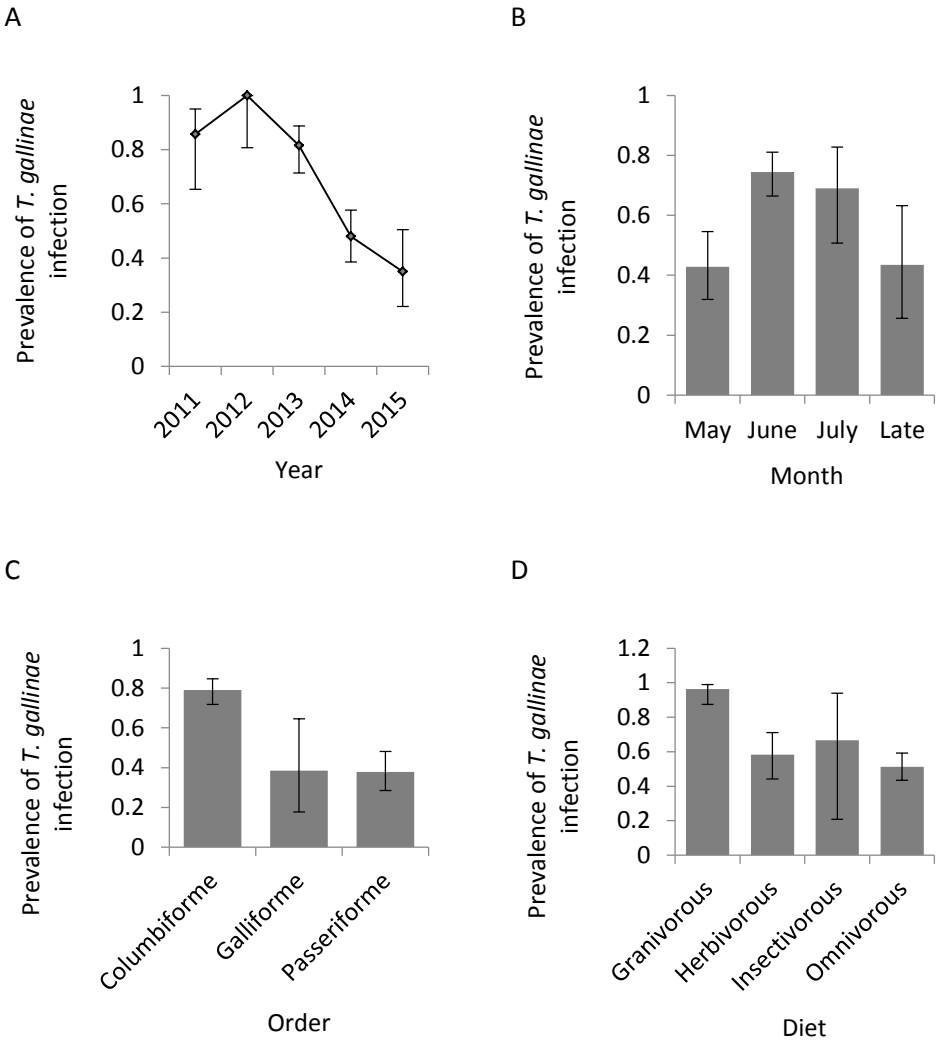


Figure 4.6: Significant terms in final model for prevalence of *T. gallinae* infection in British bird population. Mean prevalence ± SE of infection depending on (A) year, (B) month ("Late" = August and September combined), (C) order and (D) diet. Standard error bars represent Wilson score intervals. Sample sizes: 2011 (N=21), 2012 (N=16), 2013 (N=76), 2014 (N=102), 2015 (N=40), May (N=70), June (N=133), July (N=29), Late (N=23), Columbiform (N=152), Galliform (N=13), Passeriform (N=90), granivorous (N=54), herbivorous (N=48), insectivorous (N=3), omnivorous (N=150).

Generalised linear modelling analysing factors associated with strain presence in British bird populations has shown that different strains are associated with different factors (Table 4.6). The presence of a strain was not found to be associated with a particular taxonomic order and did not significantly vary over years within the British bird population (Table 4.6). Birds caught and sampled on a site that provided supplementary food were more likely to be infected with the Type A strain (48%, n=61) than birds that were caught and sampled from a site that did not provide supplementary food (27%, n=22) (Figure 4.7). Birds that were sampled in May were more likely to be infected with the Type C strain (44%, n=16) than birds that were sampled in June (12%, n=56) or during the late season (July/September) (0.09%, n=11) (Figure 6). Birds with a granivorous diet were more likely to be infected with the GEO strain (30%, n=37) than birds with an omnivorous diet (0.03%, n=34) (Figure 4.7). Omnivorous birds were more likely to be infected with the Tcl-1 strain (35%, n=34) than granivorous birds (11%, n=37) (Figure 4.7).

Table 4.6: Results of LRT tests determining factors associated with *T. gallinae* strain prevalence in the British bird population (Model 2.2a-2.2d). Terms highlighted in grey were not included in the full model with statistics given for comparison of term to null model. Remaining terms included in full model. Significant results for the removal of a term from the full model are in bold and these terms were retained for the final model. Dev. = Deviance. N= sample size for each response variable, note that they are small hence low power.

	Type A (N=36)			Type C (N=14)			GEO (N=13)			Tcl-1 (N=19)		
	Dev.	df	P value	Dev.	Df	P value	Dev.	df	P value	Dev.	df	P value
Year	0.16	1	0.69	0.1	1	0.75	0.26	1	0.61	0.08	1	0.77
Month	2.62	3	0.45	6.58	2	**0.04**	2.3	3	0.51	1.62	1	0.2
County	1.88	1	0.17	2.15	2	0.34	4.07	2	0.13	3.99	2	0.14
Site type	1.79	1	0.18	0.13	1	0.72	1.4	1	0.24	NA	NA	NA
Fed	3.06	1	0.08	2.49	1	0.11	1.06	1	0.3	0.39	1	0.53
Order	0.05	2	0.97	2.87	2	0.24	2.12	2	0.35	3.75	2	0.15
Diet	2.2	3	0.53	1.67	2	0.43	9.14	2	**0.01**	6.31	2	**0.04**

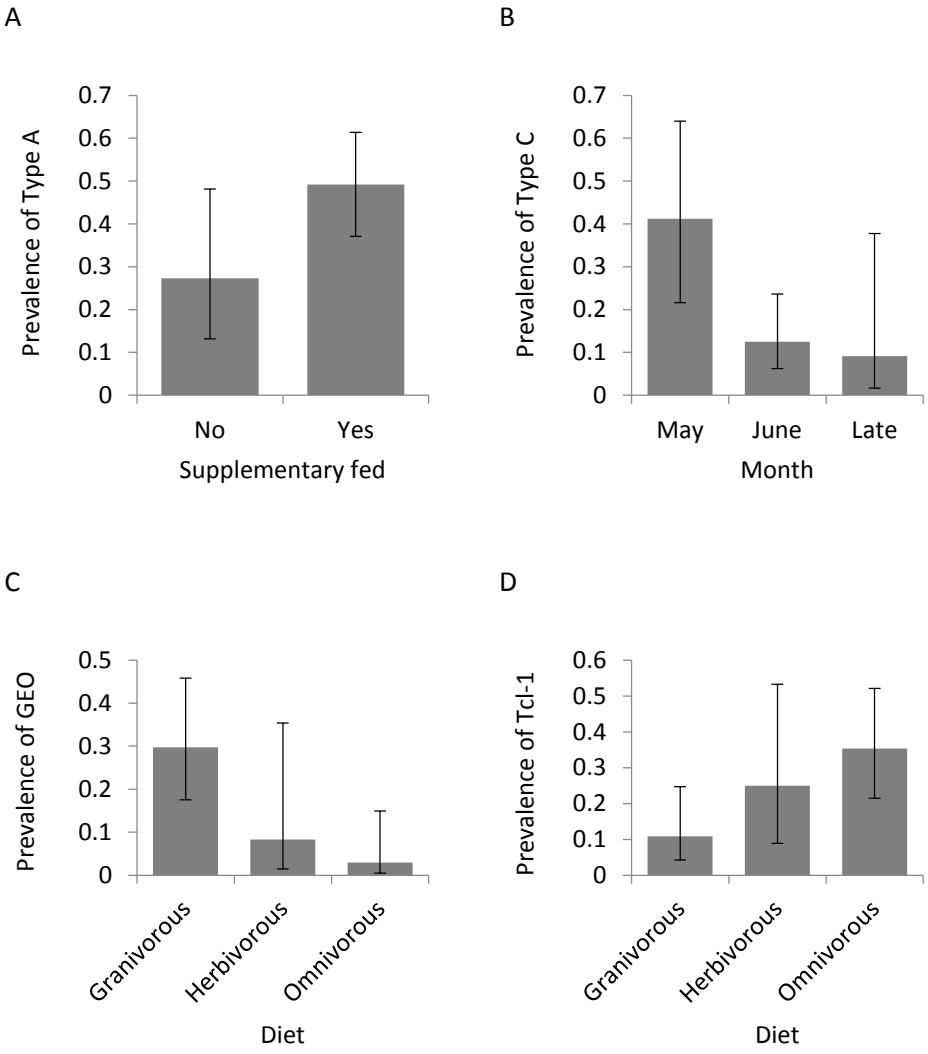


Figure 4.7: Significant terms in final model for prevalence of *T. gallinae* strains in British bird population. Mean prevalence ± SE of: Type A strain depending on (A) provision of supplementary food at site; Type C strain depending on (B) month; GEO strain depending on (C) diet; Tcl-1 strain depending on (D) diet. Standard error bars represent Wilson score intervals. Sample sizes: supplementary fed (N=61), not supplementary fed (N=22), May (N=16), June (N=56), Late (N=11), granivorous (N=37), herbivorous (N=12), omnivorous (N=34).

4.4 Discussion

This study has increased the knowledge of *T. gallinae* strain diversity circulating in Columbid populations from different countries and provided the first evidence of variation in the *T. gallinae* strains infecting UK Passerine populations. Based on analysis of the Fe-hyd region, further genetic variation within the Type C strain has been detected and an insight into the variation within the Tcl-1 strain has been acquired. The clonal strain (A1) remains the only Type A variant to be detected in free-ranging UK bird populations. Overall, this information is used to expand what is known about the role of Columbids as the reservoir of the finch epidemic strain and shed light on the role Passerines play in the epidemiology of *T. gallinae*.

A high prevalence of *T. gallinae* infection persisted in the UK Columbid population over a period of five years, although a decrease in prevalence was observed during the final year of the study but the sample size for 2015 was particularly small. *T. gallinae* infection was also more prevalent in Columbids than Passerines. This supports the theory that UK Columbids are a reservoir for *T. gallinae* infection which is based on the parasites ubiquitous distribution in Columbids worldwide and recently indicated by a survey conducted in the UK (Lennon et al., 2013, Amin et al., 2014). Further evidence, in the form of longitudinal studies demonstrating that parasite prevalence is maintained in the host, which this study provides here, increases the support (Haydon et al., 2002). Confirmation is ultimately achieved when infectiousness of the host is demonstrated. One of the main aims of this study was to determine the extent that Columbids are a reservoir of the finch epidemic strain. Temporal variation in the predominant *T. gallinae* strain infecting the UK Columbid population was revealed and strains carried by Turtle Doves have the potential to alter the overall strain composition. The finch epidemic strain was preponderant in the Columbid population during 2012 and 2014. In 2013, the GEO strain was dominant but it was only detected in Turtle Doves, otherwise the Tcl-1 strain would have been the most common strain. In 2015, the Tcl-1 strain was dominant in the Columbid species considered however this may not be an accurate representation of the population due to the small sample size. Interestingly, the strain composition of *T. gallinae* infecting Passerines reflects the dominant strains in the Columbid population within the same year. This suggests that the *T. gallinae* strains are spilling over from the Columbid reservoir to the Passerine population in proportion to their frequency. Continued monitoring of *T. gallinae* strain infection in UK bird populations will allow this idea to be investigated by increasing sample sizes. If a difference between strain composition in Columbid and Passerine populations becomes apparent then it suggests other factors, such as variation in transmission, virulence or susceptibility among host species are influencing host- parasite dynamics. The strains detected from shared food resources in 2013 are the only ones reported in Passerines that year. In 2014, the finch epidemic strain was recovered from the majority of food resources and it was the only strain reported in Passerines. The temporal variation in *T. gallinae* strain composition being mirrored in Columbids, Passerines and

shared food resources, in addition to the lack of an association between strains and bird families, provides further evidence that *T. gallinae* infection is being shared between taxonomic families via this transmission route.

Although Columbids appear to be a reservoir for *T. gallinae* on a wide geographical scale, as demonstrated by the high prevalence of infection in different Columbid populations here, the finch epidemic strain is associated with the UK population. Different strains dominate the Columbid populations in other countries however there are also shared strains between countries, which could be the result of migrating birds. The GEO strain is most prevalent in Senegal, which is an over-wintering site of Turtle Doves who migrate along the western Palaearctic flyway (Eraud et al., 2013). Turtle Doves are also the source of the GEO strain in British Columbid populations during 2013, which suggests that this strain may have spread between countries via this migration route. The spread of the finch epidemic strain from Britain to Fennoscandia and subsequently to central Europe is thought to be via migrating Chaffinches, although the ability of sub-clinically infected birds to migrate long distances is unknown and warrants further investigation (Lawson et al., 2011b, Lehikoinen et al., 2013, Ganas et al., 2014). It is a concern that Turtle Doves may carry the finch epidemic strain to over-wintering sites in Africa where they roost in high densities and have the potential to transmit this strain via watering holes to naïve Passerine populations (Gerhold et al., 2013, Purple and Gerhold, 2015). The fact that the Type A strain detected in two samples in Senegal (2014) were of a different Fe-hyd subtype (A2) than the one responsible for the European finch epidemic (A1), suggests that the A1 strain has not reached Africa yet. Perhaps Turtle Doves infected with the finch epidemic strain in the UK are unable to complete the migration to back to Africa therefore reducing the risk of spreading this particular strain. The A2 subtype however, is mostly responsible for trichomonosis outbreaks in USA therefore it has the potential for virulence but no trichomonosis outbreaks have yet been reported from Africa (Girard et al., 2014b).

Overall, there was a decrease in the prevalence of *T. gallinae* infection in the British bird population over the years studied. The breeding seasons of 2012 and 2013 were subject to particularly adverse weather conditions with unusually high amounts of rainfall during 2012 and cold temperatures during the spring of 2013 (Met Office, 2016a, Met Office, 2016b). Declines in many farmland bird species occurred following these two years which are believed to reflect the lack of food availability as a result of the poor weather (Walker et al., in prep). This may have elevated *T. gallinae* infection in bird populations due to an increased reliance on supplementary food and increased susceptibility to infection due to stress. The prevalence of infection in Passerines is much higher than reported from the studies in Spain and Slovenia (Martinez-Herrero et al., 2014, Zadravec et al., 2016). The need to investigate other factors, such as host density, feeding practices and climate in order to understand the differences in the impact of trichomonosis outbreaks in different countries has already been highlighted (Lehikoinen et al., 2013). The same also applies to the differences in infection

prevalence in Passerines between different countries. Seasonal variation in infection prevalence is also suggested by the results of this study, as birds sampled in June are more likely to be infected than those sampled in May. Birds are experiencing cumulative effects of breeding by June, in comparison to May, therefore this could translate into higher levels of stress (van de Crommenacker et al., 2011) and being less able to clear the parasite. Granivorous birds, represented only by Turtle Doves in this study, are also more likely to be infected. This is concerning, considering the Vulnerable conservation status of this bird (Birdlife International, 2015). They may be experiencing high levels of stress due to the factors that are driving the population decline, meaning they are more prone to infection (Appleby et al., 1999, Lochmiller and Deerenberg, 2000, Navarro, 2004). It also suggests this species is the biggest risk of infection to Passerines that share the same food resources, although this may be offset by the low densities of Turtle Doves occurring in a given area. Columbid species which carry a lower prevalence of infection but occur in higher densities could pose a similar risk. It is possible that increasing the diversity of *T. gallinae* strains by introducing new ones into the population could decrease the exposure of Passerines to the finch epidemic strain. Birds sampled on sites with supplementary food were not more likely to be infected, as suggested by a previous analysis (Lennon et al., 2013), however if the birds were infected then they were more likely to be carrying the finch epidemic strain. This suggests that shared food resources could be the main transmission route for this strain. All Passerines sampled were sub-clinically infected, including those found to be infected with the finch epidemic strain. This finding is not unprecedented as dead Passerines without macroscopic lesions were found to be infected with the finch epidemic strain (Ermgassen et al., 2016). It is possible that these birds were sampled at the incubation stage of infection before clinical signs developed, had the disease but only with microscopic lesions of necrotic ingluvitis or had resolved *T. gallinae* infection with no viable parasites present (Ermgassen et al., 2016). Evidence for the latter option comes from trials with Columbids showing that infection with a pathogenic strain provides immunity against subsequent infection with a virulent strain (Stabler, 1948). If Passerines are able to be asymptomatic carriers of the finch epidemic strain, this could result in further spread of the parasite and increased contact rates with other spillover hosts. Furthermore, it suggests Passerines are now contributing to the maintenance of *T. gallinae* in the population and could amplify the reservoir of *T. gallinae* if they transmit the pathogen back to Columbid hosts i.e spillback (Haydon et al., 2002, Nugent, 2011).

Future work ought to focus on what is driving the temporal trends in *T. gallinae* strain composition in an effort to manage the occurrence and spread of the finch epidemic strain. This can only be achieved by continuing to monitor *T. gallinae* infection in free-ranging populations as part of a longitudinal study so that variations in strain composition can be examined in relation to environmental and demographic factors. Forming a conservation strategy depends on whether the reservoir host or spillover host is being targeted. Option A would be to treat the

spillover host which is more likely to be effective if the epidemic is the result of sporadic transmission events. The frequency that *T. gallinae* is transmitted by shared resources is likely to be high given findings in Chapter 3 therefore targeting treatment in the spillover host is futile if reinfection rates are high. The dependency on supplementary food probably varies between individuals or local populations relative to the health status of the bird and the availability of natural food resources in the local environment. Option B would be to target the transmission route. This could reduce prevalence of infection in the spillover host and perhaps the prevalence of the finch epidemic strain in the reservoir host. In this case, the recommendations mentioned in Chapter 3 should be followed where only low densities of seed are provided and greater hygiene on sources of supplementary feed for garden and game birds is encouraged. Preventing access of Columbids to shared resources by building a cage around the food which still allows access by Passerines might prevent spillover of infection from Columbids but not control against further transmission between Passerines. Option C would be to target the reservoir host. Treating wild bird populations is logistically difficult and could potentially negatively impact non- target species (Hofle et al., 2004). Reducing the density of the reservoir host through culling has been implemented in the control of bovine tuberculosis in order to manage populations of Brushtail Possums *Trichosurus vulpecula* (Roberts, 1996). This strategy is controversial and support from simulation modelling is required to justify this approach (Caley, 2006). In the context of trichomonosis, it is complicated by the multi-host dynamics contributing to the reservoir of *T. gallinae* and the fact that one species, the Turtle Dove, has a deteriorating vulnerable conservation status (IUCN, 2015) and is currently the focus of a new conservation management plan (Fisher et al., 2016). The application of mathematical modelling would further inform conservation management. These models have a threshold parameter known as the basic reproduction number, R_o (van den Driessche and Watmough, 2002). If $R_o<1$ then a disease free equilibrium is locally stable indicating that a disease cannot invade the population whereas if $R_o>1$, then it is unstable and warns that disease invasion is possible (van den Driessche and Watmough, 2002). These models can be used to investigate which variables affect transmission and therefore R_o with the goal of identifying those to focus on in disease control programs. A conceptual framework has been developed, based on this key threshold, to quantify each host species' contribution to parasite persistence in a multihost system (Fenton et al., 2015). This approach would facilitate informed decisions regarding *T. gallinae* management and increase our understanding of this multi-host community. In conclusion, a conservation strategy which targets the transmission route has the potential to control infection in the spillover population in addition to the prevalence of the finch epidemic strain in all bird populations. Considering this particular strain is responsible for mortality in adult Turtle Doves and nestlings (Stockdale et al., 2015), this approach could have a beneficial impact on this declining species of bird.

Chapter 5

Impact of infection on reproductive output, body condition and post- fledging survival.

5.1 Introduction

Emerging infectious diseases are frequently highlighted, being responsible for local population crashes and in some susceptible ecosystems, such as endemic species on islands, contributing to extinctions (De Castro and Bolker, 2005, Heard et al., 2013). However, the effect of endemic infections (the constant presence of diseases or infections within a given geographic area or population) is not as apparent. Historically, infection by these parasites were not thought to be highly pathogenic, although experimental research has since revealed a cost of infection to host fitness (Merino et al., 2000, Knowles et al., 2010). Furthermore, hosts are more likely to have multiple infections than single infections, yet the effects of multiple infections are only recently being explored (Combes, 2001, Pedersen and Fenton, 2007, Poulin, 2007). For populations or species that are already experiencing stress, from other sources such as reduced food availability or increased predation pressure, there may be synergistic interactions with infection, further contributing to population declines. Species reduced to vulnerable levels may then be less resistant to environmental perturbations. The Paridae pox epidemic in wild tits (Paridae family) lowered average population growth rates in the UK (Lachish et al., 2012), which caused concern when the disease also emerged in Europe as European wild tit populations are experiencing a reproductive asynchrony with peak food availability as a result of climate change, possibly resulting in increased stress (Visser et al., 1998, Literak et al., 2010).

Determining the demographic impact of parasitic infections at the population level can be difficult, and is often only possible in the case of major changes in population numbers (Friend et al., 2001). Discreet shifts in abundance, due to long term population depression, reduced reproductive effort or indirect mortality are more difficult to detect (Friend et al., 2001). Sub-lethal effects can have as profound an effect on host dynamics as lethal effects and can act as a destabilizing force in host populations (Anderson and May, 1981, Sait et al., 1994, Boots et al., 2003). Parasites can impose energy demands, alter behaviour, affect morphology and appearance, and reduce growth and fecundity (Marcogliese, 2004). A well-studied system for the impact of parasitic infection on reproductive success has been endemic haemosporidian infection in wild Passerines. Correlations between blood parasite infection and the onset of breeding have been found in Great Tits, with parasitized females laying later and hatching their eggs later (Allander and Bennett, 1995). Field-based medication trials that experimentally manipulate parasite burdens provide better evidence of a causal relationship between parasitism and life history traits (Moller 1997). Medicated female Blue Tits (when compared to non-medicated females) had higher hatching

success, higher provisioning rates and increased fledging success (Merino et al., 2000, Tomas et al., 2007, Knowles et al., 2010). Furthermore, a reduced inequality in hatching probability and fledgling mass within broods was observed, with within-brood effects appearing to explain higher fledging success (Knowles et al., 2010). In another study, the nestlings of non-medicated females were found to experience a higher infestation rate by the ecto-parasitic blowfly *Protocalliphora azurea*, which was thought to be mediated by parental effort, causing differences in nestling susceptibility or time spent on nest sanitation activities (Tomas et al., 2007). Detrimental impacts of haemosporida infection were apparent with only a partial reduction in the intensity of parasitism (through lower doses of medication) therefore there may be far greater deleterious effects of infection that are not being detected (Merino et al., 2000). Overall, the sub-lethal effects of parasite exposure are often considered at the level of the individual but this may scale up to the level of the population. If prevalence of infection is high, then parasites can have a significant impact on the host population and regulate abundance (Anderson and May, 1979, May and Anderson, 1979). It is difficult to establish the population effects due to confounding factors that can obscure causal relationships but long-term studies on discrete populations have established a link between individual and population-level effects. For instance, Neogregarine *Mattesia* sp., infection in the Spruce Needleminer *Epinotia tedella* caused slight delays in adult emergence, a decreased adult life span and suppression of egg development (due to a negative impact on ovaries) which led to a reduction in fertility (Munster-Swendsen, 1991). A high frequency of infection in a generation resulted in a low mean fertility of the population which had a delayed density-dependent effect on host density (Munster-Swendsen, 1991).

Coinfection (the simultaneous infection of an individual host by multiple parasite species), also known as multiple infection or polyparasitism, is more likely to occur than a single infection and has a broader biological significance (Telfer et al., 2010, Thumbi et al., 2014), yet most research to date has focused on single host-parasite interactions (Petney and Andrews, 1998, Pedersen et al., 2007, Bordes and Morand, 2009, Rigaud et al., 2010). The outcome of coinfection is mediated by competition between the co-existing parasites, which may be at the intraspecific or interspecific level (Bordes and Morand, 2011). They may compete directly for resources (e.g. blood) or indirectly through their effects on the immune system, such as parasite-induced immune-depression (Cox, 2001, Graham, 2008). Ultimately, the interaction could be synergistic whereby infection with one parasite predisposes the host to infection by other types of parasite. For example, helminth species richness in the wild Wood Mouse *Apodemus sylvaticus* is enhanced by the presence of the intestinal nematode *Heligmosomoides polygyrus* (Behnke et al., 2009). On the other hand, the interaction could be antagonistic, whereby the presence of one parasite reduces the survival or fecundity of the co-existing parasite, resulting in a negative interaction between abundance of the two species (Fenton et al., 2010). Due to a conflict of interests between parasites occupying the same host, a virulent host exploitation strategy

could give a parasite a competitive advantage (Nowak and May, 1994, van Baalen and Sabelis, 1995). Theoretical models support this suggestion and have shown that coinfection leads to selection for higher levels of virulence and highly polymorphic parasite populations, resulting in very complex dynamics (Nowak and May, 1994). A potential feedback mechanism between population dynamics and evolution has been identified, whereby a high prevalence of co- infection favours increased virulence but when pathogens become more virulent, the transmission rate will decrease, favouring lower virulence levels again (van Baalen and Sabelis, 1995). Empirical studies have also provided support for the theory that coinfection drives the evolution of virulence. Mixed genotype infections of Schistosomes *Schistosoma mansoni*, within a species of snail *Biomphalaria glabrata*, were more virulent than single genotype infections in terms of reductions on host reproductive success and survival (Davies et al., 2002). Furthermore, the parasite's reproductive rate also increased in mixed strain groups (Davies et al., 2002). Mixed clone infections of *Plasmodium chaubaudi* in mice also resulted in higher virulence, in terms of a loss in body weight and lower blood counts resulting in anaemia (Taylor et al., 1998). Mounting an immune response against more than one genotype is likely to be more costly for hosts, which therefore suffer higher virulence (Taylor et al., 1998, Davies et al., 2002). Despite mixed infections leading to dominance by the more virulent strains of bacterium *Pasteuria ramose* in their waterflea host *Daphnia magna*, it also resulted in higher fecundity in the hosts, revealing an advantage of parasite competition for the host relative to single infections (Ben-Ami et al., 2008). Coinfection does not necessarily result in increased virulence, as revealed when Gower & Webster (2005) examined within-host competitiveness between genetic strains of *Schistosoma mansoni*. The reproductive success of a virulent strain was reduced in the presence of a faster-replicating parasite genotype with low virulence (Gower and Webster, 2005). Therefore, since the less virulent strain had a competitive advantage, its evolution was favoured in this particular system. The effect a parasite has on its host, in terms of being lethal or sub-lethal, may explain whether the outcome of multiple infection leads to increased or decreased virulence (Schjorring and Koella, 2003). Both outcomes, however, can be explained by host exploitation. Sub-lethal impacts may decrease the growth rate of the host and if the parasite's growth depends on the host size then competition will decrease virulence (Schjorring and Koella, 2003). Overall, increased virulence as a result of multiple infections is not as general as once previously thought.

Trichomonosis can have adverse effects on wild bird populations. It has become a conservation concern in the Mauritian Pink Pigeon where infection is a cause of mortality in nestlings and fledglings (Bunbury et al., 2007, Bunbury et al., 2008). Epidemics have resulted in a 35% and 21% decline in British Greenfinch, and Chaffinch breeding populations, respectively, although Chaffinch populations have since recovered (Robinson et al., 2010, Lawson et al., 2012). It is also responsible for high levels of nestling mortality in Cooper's Hawks *Accipiter cooperi*, and is the most frequent diagnosis for the cause of death in Mourning Doves *Zenaida*

macroura, from the south eastern United States (Boal et al., 1998, Gerhold et al., 2007). Sub-clinical infection can also have a negative impact on individuals in the form of reduced body mass and fat deposition (Rupiper and Harmon, 1988, Villanua et al., 2006). The resulting poor body condition may render the individual more susceptible to secondary disease and predation (Villanua et al., 2006). Considering the Vulnerable status of the Turtle Dove in the UK and throughout Europe (Birdlife International, 2015) and the high prevalence of *Trichomonas gallinae* infection that is consistent between years (reported in Chapter 2), investigating the impact of infection is of paramount importance. Trichomonosis is known to cause mortality in both adult and nestling Turtle Doves (Stockdale et al., 2015) but most infection detected during the breeding seasons of 2013- 2015 was sub-clinical. The assumption that sub- clinical infection is non-pathogenic has been disproved in the system of endemic haemosporidian infection in Passerines, and so the potential sub-lethal impacts of *T. gallinae* infection need to be investigated. Furthermore, a study in 2011 revealed coinfection of blood parasites (*Haemoproteus, Plasmodium* or *Leucocytozoon*) or of *T. gallinae* and a blood parasite in 71% of sampled Turtle Doves, although the sample size was small (Stockdale, 2012). The impact of coinfection in a closely related species, the Mauritian Pink Pigeon, revealed that the presence of *T. gallinae* and *Leucocytozoon* in at least one parent resulted in their chicks failing to fledge (Bunbury, 2006).

In this chapter I will investigate the impact of *T. gallinae* strain infection and coinfection with blood parasites, on reproductive output and body condition in adult Turtle Doves and the impact of *T. gallinae* strain infection on nestling body condition and post- fledging survival. A negative impact of the *T. gallinae* strain associated with virulence is expected, along with an increasing number of coinfections.

5.2 Methods

5.2.1 Adult Turtle Dove parasite sampling and monitoring reproductive output

Turtle Doves were caught and sampled for the presence of *T. gallinae* infection (as described in Chapter 2) during the breeding seasons of 2012-2014 on farms located in East Anglia. Blood samples were also collected from each individual, using superficial venepuncture of the brachial vein, to screen for the presence of haemosporidian parasites. Blood samples were stored on Whatman FTA cards (Sigma Aldrich) at room temperature or in a sterile Eppendorf tube and frozen within 1-8 hrs.

Each bird was fitted with a radio tag (PicoPip3, Biotrack, Dorset, UK) that was fixed to the base of the tail feathers and secured around the two middle tail feathers using dental floss and a small dab of cyanoacrylate glue. Most Turtle Doves are known to shed their tail feathers before migration (Browne and Aebischer, 2003a), and so these fittings were only temporary. The weight of the tag (1.7g)

was less than 1.5% of adult body weight (130-180g), which is well within the recommended weight limit, hence unlikely to have adverse effects. Turtle Doves were monitored for at least five days out of every seven throughout the season (May- September of 2013 and 2014), until their tag was dropped/ failed, they were recovered dead, or they left the area. If an individual began to display signs of nesting (being in the same location for at least 3 consecutive days, within a time period characteristic of when each sex is known to incubate), the area was searched for a nest. Nests were visited every 2-3 days and the contents recorded until the nest was empty due to predation or successful fledging (Dunn et al., 2016a). Care was taken to cause minimal disturbance to the surrounding vegetation so that it did not facilitate predation.

5.2.2 Medication trials

Both Turtle Dove nests (n=7) and Woodpigeon nests (n=19) containing a brood of two were used in medication trials during the breeding season of 2014. Including Woodpigeons in the medication trials increased the sample size for investigating the impact of *T. gallinae* infection on Columbid nestlings. The Turtle Dove nests were found as a result of radio-tracking the adult whereas the Woodpigeon nests were found by searching appropriate nesting habitat (e.g. hedgerows, dense bushes and trees). When the nestlings reached five days old post-hatching, they were weighed to the nearest 1g using a digital balance (Satrue, Taiwan), had standard morphometrics taken (tarsus length, head and beak length to the nearest 1mm using Vernier callipers (Redfern and Clark, 2001)) and were screened for the presence of *T. gallinae* using the same technique as described in Chapter 2. The first nestling to be processed was medicated with 2.5mg carnidazole (1/4 of a tablet) (Spartrix, Petlife Harkers, Suffolk, UK) under Home Office licence, which was washed down the oesophagus with sterile saline solution (~5ml). The choice of which nestling to medicate was considered to be random, as the first nestling brought out of the bag was not necessarily the larger of the two. Examining the raw data supports this approach, as half of the nestlings (n=26) processed first were the smaller of the pair (in terms of mass) on the first visit. The nestling was monitored for at least 10 minutes to ensure it did not act as an emetic. The control nestling was administered the same volume of sterile saline solution. When the Turtle Dove nestlings reached seven days old, they were re- screened for the presence of *T. gallinae* to establish re- infection rates from the parents and the same morphometrics were taken in order to assess the impact of *T. gallinae* infection on growth rate. Woodpigeon nestlings remain in the nest for a longer period of time than Turtle Dove nestlings (21 days vs 11 days) allowing the window between first and second measurements to be extended to a week, which was thought to increase the likelihood of detecting changes in growth rate.

5.2.3 Turtle Dove post- fledging survival

In 2014, Turtle Dove nestlings were radio- tagged in the nest at seven days old when they met the minimum recommended weight of 50g to carry the tag. The

tag was attached to a leather leg ring (total weight 1.2g) which was designed to degrade and detach from the bird before migration (Dunn et al., 2016a). The nestling was monitored until the battery of the radio-tag ran out (~4 weeks), the tag was shed, or the fledgling was recovered dead. This allowed the comparison of post- fledgling survival between nestlings medicated against *T. gallinae* infection and control nestlings. The location of the nestling was established every day, using triangulation, and if the bird was in the same location for more than two consecutive days, a visible sighting was required to confirm whether or not it was still alive. If the nestling was found dead but largely intact (i.e. no signs of predation) and the carcass relatively fresh (i.e no maggot infestation) it was sent to the Garden Wildlife Health initiative (http://www.gardenwildlifehealth.org/) for post- mortem examination.

5.2.4 Parasite strain identification

For *T. gallinae*, this was achieved using the same methods described in Chapter 2. For haemosporidia, the same DNA extraction technique was performed on 1/8 of a circle holding a blood sample on the Whatman FTA card. In order to detect the presence of parasites within the genera *Plasmodium, Haemoproteus* and *Leucocytozoon*, PCR amplification of the cytochrome b region of the mitochondrial genome took place. Primers HMRf [f] and H15730 [r] were used to target a 378bp length in *Haemoproteus* sp. and *Plasmodium* sp. (Table 5.1). The PCR reaction consisted of the following: 1X Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 0.4µM forward and reverse primer (Sigma- Aldrich, UK) and a volume of sterile double distilled water (ddH_20) to bring the total PCR reaction to 9µl whereby 1µl of DNA was then added. A negative control of sterile double- distilled water and a positive control of *Haemoproteus* sp. and *Plasmodium* sp. was included in every PCR run. PCR thermal cycling was performed as follows: 15 minutes denaturation at 95°C, then 35 cycles of 30 seconds at 94°C, 60 seconds at 52°C and 90 seconds at 72°C, followed by 10 minutes at 72°C for a final elongation. Primers Leu-new 1F [f] and - LDRd [r] were used to target a 302bp length in *Leucocytozoon* sp. (Table 5.1). The PCR reaction consisted of the following: 1x Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 0.2µM forward and reverse primer (Sigma- Aldrich, UK) and a volume of sterile double distilled water (ddH_20) to bring the total PCR reaction to 9µl whereby 1µl of DNA was then added. A negative control of sterile double- distilled water and a positive control of *Leucocytozoon* sp. was included in every PCR run. PCR thermal cycling was performed as follows: 15 minutes denaturation at 95°C, then 35 cycles of 30 seconds at 95°C, 60 seconds at 56°C and 60 seconds at 72°C, followed by 10 minutes at 72°C for a final elongation. A Gene Amp 9700 PCR system (Applied Biosystems, Foster City, CA) was used to run the PCR reactions. The PCR products were electrophoresed through a 1% agarose gel, which was stained with ethidium bromide, in 1x TBE buffer and visualised by UV light. Each sample was screened twice to check the consistency of the result. A subset of samples were Sanger sequenced to confirm the target regions were being amplified, according to the

methods described in Chapter 2. All samples positive for *Haemoproteus/Plasmodium* or *Leucocytozoon* were prepared for Illumina sequencing according to the methods set out in Chapter 2.

Table 5.1: List of primers used to amplify the cytochrome b region of the mitochondrial genome from haemosporidia.

Primer	Sequence	Reference
HMRf	GGTAGCWCTAATCCTTTAGG	Martinez et al., 2009
H15730	CATCCAATCCATAATAAAGCAT	Fallon et al., 2003
Leunew1F	GGWCAAATGAGTTTCTGGG	Quillfeldt et al., 2014
LDRd	CTGGATGWGATAATGGWGCA	Merino et al., 2008

5.2.5 DNA sequence analysis

DNA sequence analysis of the ITS region and Fe-hyd region followed the same protocol as described in Chapter 2. DNA sequence analysis of the HMRf-H15370 region and Leunew1F- LDRd region was also the same with the following amendment: paired end reads were again aligned using FLASH 1.2.11 (Magoc and Salzberg, 2011) but with a 122bp region of overlap for the HMRf-H15370 region and 198bp region of overlap for the Leunew1F- LDRd region. The MHC technique was followed as detailed in Chapter 2. The resulting blood parasite sequences were queried against the NCBI-BLAST database (Altschul et al., 1997) and the MalAvi database (Bensch et al., 2009) to determine the closest sequence matches and identify strains.

5.2.6 Impact of parasite infection on reproductive output and mass

All analyses were conducted in R version 3.3.2 (R Core Team, 2016). Modelling with the number of nesting attempts or fledglings as the response variable was not possible as the sample sizes were too small (see Table 5.2 for sample sizes, number of nesting attempts varied between 0-3; number of fledglings varied between 0-4). The response variable was simplified to a binary response i.e. whether the bird nested or not and whether the bird produced fledglings or not over a breeding season and data were combined across years (2012-2014). Fisher's exact test was applied to each dataset (Table 5.2). Power analysis was conducted using the software program G*Power 3.1.9.2 (Faul et al., 2007) to assess the power of the current study and what sample sizes would be required for a more rigorous analysis. The following parameters were specified: test family = exact, statistical test = Fisher's exact test, type of power analysis= a priori). The effect size was calculated based on a power of 0.8 and detecting a difference between the two groups being compared at a significance level of 0.05. The prevalence of infection used for the power analysis is reported in the results section of each test, along with sample sizes.

The distribution of adult mass (g) was assessed and found to be normal in the dataset on parasite presence or absence or overall number of infections (Shapiro-Wilk: W=0.97, p=0.19) therefore a linear model was conducted, which also included the term 'Year' to account for annual variation (Table 5.2). The distribution of adult mass (g) did not remain normal for the following dataset on *T. gallinae* strains (Shapiro-Wilk: W=0.86, p=0.0002) therefore a generalised linear model with gamma distribution and a log link function was fitted. The distribution of nestling mass (g) was normal (Shapiro-Wilk: W=0.96, p=0.33) therefore linear modelling was used to analyse the dataset (Table 5.2).

Table 5.2: Sample sizes and methods of statistical analysis

Response variable	Predictor variable	N	Analysis
Nesting	No. of infections	30	Fisher's exact test
	T. gallinae strain	19	Fisher's exact test
Fledglings	No. of infections	28	Fisher's exact test
	T. gallinae strain	18	Fisher's exact test
Adult mass	Presence/ absence of parasite + Year	49	Linear model
	No. of infections + Year	49	Linear model
	T. gallinae strain + Year	41	Generalised linear model
Nestling mass	*T. gallinae* strain + Year	34	Linear model
Growth rate	Group (Medicated/ Control)	52	T-test
Survival	Group (Medicated/ Control)	12	Fisher's Exact test

5.2.7 Medication trials

Head- bill length and tarsus length (indicative of structural size; Knowles et al., (2010) and body mass were used as indicators of offspring growth. The growth rate in each of these indicators was calculated by M_2-M_1/ hours (M_1=1st measurement, M_2 = 2nd measurement, hours= no. of hours between the measurements being taken). The growth rates were compared between medicated and control nestlings (Woodpigeons and Turtle Doves combined). The data for each of the growth indicators did not significantly differ from a normal distribution, which was tested using the Shapiro-Wilk test (Head- bill: W=0.98, p=0.36, Mass: W= 0.97, p=0.16, Tarsus: W=0.97, p=0.21). An F-test was used to check that none of the datasets violated the assumption of homogeneity of variance (Head- bill: F25= 1.02, p=0.96, Tarsus: F25=1.47, p=0.34, Mass: F25=1.45, p=0.36). Paired T-tests were used to analyse the data as the conditions of normality and homoscedasticity were fulfilled (Table 5.2).

5.2.8 Turtle Dove post fledging survival

One Turtle Dove nest was excluded from the analysis as the radio- tag detached and post- fledging survival could no longer be monitored. Due to the small sample size (n=12), a McNemar's test was conducted to establish whether or not there was a difference between the medicated and control group with regards to survival to the end of the 30 day monitoring period (Table 5.2). Power analysis was conducted to assess the power of the current study and what sample sizes would be required for a more rigorous analysis.

5.3 Results

5.3.1 Haemosporidia prevalence and lineages

Parasitic infection, in terms of *T. gallinae*, *Haemoproteus* sp. and *Leucocytozoon* sp., was determined for a total of 49 adult Turtle Doves. Only one of these birds was negative for all the parasites screened for during this study and four birds (8%) were infected only with *T. gallinae*. Most birds were infected with all three parasites (43%, n=21) or with *T. gallinae* and *Haemoproteus* sp. (35%, n=17). Six birds (12%) were infected with *T. gallinae* and *Leucocytozoon* sp. (See Table 5.3) Neither infections with a single haemosporidian nor coinfections between haemosporidia only were observed during this study. These data represent 2011-2014 combined as there was little variation in prevalence between years. Strain information for each parasite was gained from a subset of these samples (*T. gallinae*, n=41; *Haemoproteus*, n=27; *Leucytozoon*, n=15).

Table 5.3: Prevalence of types of infection screened for during this study (*T. gallinae*, *Haemoproteus* sp., and *Leucocytozoon* sp.). N=49

Type of infection	Prevalence
No infection	2%
T. gallinae only	8%
T. gallinae & *Haemoproteus* sp.	35%
T. gallinae & *Leucocytozoon* sp.	12%
T. gallinae, Haemoproteus sp., and *Leucocytozoon* sp.	43%

Overall, nine lineages were detected in this study, representing *Haemoproteus* sp. (n=2) and *Leucocytozoon* sp. (n=7) (Table 5.4). The two *Haemoproteus* sp. lineages differ by 2bp and a search on the MalAvi database did not return any results but both matched a sequence on GenBank (AB741490) with 100% cover and 100% and 99% identity respectively (Table 5.4). This sequence had previously been isolated from an Oriental Turtle Dove *Streptopelia orientalis* in Japan (Yoshimura et al., unpubl). The seven *Leucocytozoon* sp. lineages matched 5 strains on the MalAvi database. LA-TD matches AEMO02 on MalAvi and 3 sequences (KT779209, KJ488804 and HF543617) on GenBank, with 100% identity and 100% cover. KT779209 was isolated from a Red Turtle Dove *Streptopelia tranquebarica* in Taiwan (Huang et al., unpubl.), KJ88804 was isolated from a Woodpigeon in Northwest Iberia (Drovetski et al., 2014) and HF543617 was isolated from Milvus sp. in Spain. LB-TD matches STRORI02 on MalAvi and AB741508 on GenBank, with 100% identity and 100% cover. LC-TD matches STRORI01 on MalAvi with 100% identity and AB741491 with 99% identity and 100% cover. AB741508 and AB741491 were both isolated from Oriental Turtle Doves in Japan (Yoshimura et al., unpubl.) LD-TD matches AEMO02 on MalAvi with 99% identity and three sequences (KT779209, KJ88804 and HF543617) on GenBank with 99% identity and 100% cover. LE-TD matches COLIV04 on MalAvi and AB741506 on GenBank, with 100% identity and 100% match. AB741506 was isolated from a Woodpigeon *Columbia livia* in Japan (Yoshimura et al., unpubl). LG-TD matches CIAE02 on MalAvi and 8 sequences (KU761603, KJ488908, KJ577832, KC962152, KC962151, HF543631, JX418201, EF607287) on GenBank with 100% identity and 100% match. KU761603 (Little Bittern *Ixobrychus minutus*,Turkey (Yildirim et al., unpubl.)), KJ488908 (Lesser Spotted Woodpecker *Dendrocopos minor* (Drovetski et al., 2014)), KJ577832 (Mongolian Gull *Larus mongolicus*, Mongolia, (Neabore et

al., unpubl.)), KC962152 (Common Buzzard *Buteo buteo*, Turkey, (Ciloglu et al., 2016)), KC962151 (Long-legged Buzzard *Buteo rufinus*, Turkey, (Ciloglu et al., 2016)), HF543631 (*Milvus* spp, Spain, (Perez-Rodriguez et al., 2013)), JX418201 (Besra *Accipiter virgatus*, Phillippines, (Silva-Iturriza et al., 2012)) and EF607287 (Western Marsh Harrier *Circus aeruginosus* ,Germany, (Krone et al., 2008)). LJ-TD matches STRORI02 on MalAvi with 99% identity and AB741508 on GenBank with 100% identity and 100% cover. Some samples were coinfected with different lineages of haemosporidia (*Haemoproteus* sp: n=18, *Leucocytozoon* sp: n=8).

Table 5.4: Lineages detected as part of this study, closest matches on MalAvi and GenBank databases are listed. Note that lineage labels form part of a wider study therefore not necessarily consecutive.

Lineage (this study)	Parasite	MalAvi Match	% identity	GenBank Match	% overlap	% identity	No. of adults	Citation	Host species of GenBank match
HB-TD	*Haemoproteus*	NA		AB741490	100%	100%	12	(Yoshimura et al., unpubl.)	Oriental Turtle Dove
HC-TD	*Haemoproteus*	NA		AB741490	100%	99%	10	(Yoshimura et al., unpubl.)	Oriental Turtle Dove
LA-TD	*Leucocytozoon*	AEMO02	100%	KT779209	100%	100%	2	(Huang et al., unpubl.)	Red Turtle Dove
				KJ488804	100%	100%		(Drovetski et al., 2014)	Woodpigeon
				HF543617	100%	100%		(Perez-Rodriguez et al., 2013)	*Milvus* sp.
LB-TD	*Leucocytozoon*	STRORI02	100%	AB741508	100%	100%	7	(Yoshimura et al., unpubl.)	Oriental Turtle Dove
LC-TD	*Leucocytozoon*	STRORI01	100%	AB741491	100%	99%	1	(Yoshimura et al., unpubl.)	Oriental Turtle Dove
LD-TD	*Leucocytozoon*	AEMO02	99%	KT779209	100%	99%	1	(Huang et al., unpubl.)	Red Turtle Dove

Table 5.4 continued: Lineages detected as part of this study, closest matches on MalAvi and GenBank databases are listed. Note that lineage labels form part of a wider study therefore not necessarily consecutive.

Lineage (this study)	Parasite	MalAvi Match	% identity	GenBank Match	% overlap	% identity	No. of adults	Citation	Host species of GenBank match
LD-TD	*Leucocytozoon*	AEMO02	99%	KJ488804	100%	99%		(Drovetski et al., 2014)	Woodpigeon
				HF543617	100%	99%		(Perez-Rodriguez et al., 2013)	*Milvus* sp.
LE-TD	*Leucocytozoon*	COLIV04	100%	AB741506	100%	100%	2	(Yoshimura et al., unpubl.)	Woodpigeon
LG-TD	*Leucocytozoon*	CIAE02	100%	KU761603	100%	100%	1	(Yildirim et al., unpubl.)	Little Bittern
				KJ488908	100%	100%		(Drovetski et al., 2014)	Lesser Spotted Woodpecker
				KJ577832	100%	100%		Neabore et al., (unpubl.)	Mongolian Gull
				KC962152	100%	100%		(Ciloglu et al., 2016)	Common Buzzard
				KC962151	100%	100%		(Ciloglu et al., 2016)	Long-legged Buzzard
				HF543631	100%	100%		(Perez-Rodriguez et al., 2013)	*Milvus* sp.
				JX418201	100%	100%		(Silva-Iturriza et al., 2012)	Besra
LJ-TD	*Leucocytozoon*	STRORI02	99%	EF607287	100%	100%	1	(Krone et al., 2008)	Western Marsh Harrier
				AB741508	100%	99%		(Yoshimura et al., unpubl.)	Oriental Turtle Dove

5.3.2 Impact of infection on nesting attempts

Only one bird was not infected by either *T. gallinae*, *Haemoproteus* sp. or a *Leucocytozoon* sp. and it did nest during this study. Similar proportions of birds nested regardless of the number of parasite coinfections: single (67%, n=3), double (71%, n=14) and triple (83%, n=12) (Figure 5.1). These differences were not statistically significant (Fisher's test, p= 0.78). Comparing these different groups within this study revealed that in all cases the power was low and much larger sample sizes would be required to detect significant differences ($p<0.05$) and increase the power of the study to at least 0.8. The power for comparing birds infected with either one or two parasites was 0.01 and a future sample size of 2147 for each group would be required. The power for the comparison between one and three parasites was 0.05 and a future sample size of 125 for each group would be required. The power for the comparison between two and three parasites was 0.06 and a future sample size of 207 for each group would be required.

Birds that were infected with the Type A strain appeared less likely to have any nesting attempts (63%, n=8) than birds infected with the GEO strain (86%, n=7) or Type C strain (100%, n=5) (Figure 5.1) however, this difference was not statistically significant (Fisher's test, p=0.39). A power analysis revealed that the power of this study to detect significant differences between the strains was low for every pairwise comparison (Type A & GEO: 0.06, Type A & Type C: 0.03, GEO & Type C: 0.0008). An increase in sample size is required to detect a significant difference at the $p<0.05$ level where the power of the study is at least 0.8 (Type A & GEO: 62 each, Type A & Type C: 17 each, GEO & Type C: 55 each).

The odds ratio suggests that birds infected with a strain not typically associated with virulence (Type C or GEO) were almost 6 times more likely to have a nest attempt (odds ratio = 5.95) than birds infected with a strain associated with virulence (Type C or GEO, 92%, n=12; Type A, 63%, n=8) (Figure 5.1), however this difference was not statistically significant and the confidence interval was very large (Fisher's test, CI 0.37-376.09, p=0.26). A power analysis revealed that the power to detect a significant difference between these two groups was low (0.28). An increase in sample size to 36 in each group is required to detect a significant difference at the $p<0.05$ level where the power of the study is at least 0.8.

5.3.3 Impact of infection on fledglings

The one bird with no parasitic infections produced at least one fledgling during the breeding season. Of the birds infected by one parasite, 33% (n=3) produced fledglings which decreased to 15% if the bird was infected by two parasites (n=13). Of the birds infected by three parasites, 64% of birds (n=11) produced fledglings (Figure 5.2). Parasitic infection was found to be statistically significant (p=0.036) and figure 5.2 shows the significant difference lies between infection

with two and three parasites. Comparing the different groups within this study revealed that the power was either low or moderate and larger sample sizes would be required to detect significant differences ($p<0.05$) and increase the power of the study to at least 0.8. The power for the comparison between one and two parasites was 0.06 and a future sample size of 96 for each group would be required given the current estimations of effects size. The power for the comparison between one and three parasites was 0.06 and a future sample size of 47 for each group would be required. The power for the comparison between two and three parasites was 0.63 and a future sample size of 18 for each group would be required.

Birds that were infected with the Type A strain were less likely to produce fledglings (17%, n=6) than birds infected with either the Type C strain (80%, n=5) or GEO strain (43%, n=7) (Figure 5.2) however, this difference was not statistically significant ($p=0.14$). A power analysis revealed that the power of this study to detect significant differences between the strains was low to moderate for the comparison between Type A and Type C (0.37), but low for Type A and GEO (0.05) and Type C and GEO (0.13). An increase in sample size is required to detect a significant difference at the $p<0.05$ level where the power of the study is at least 0.8 (Type A & Type C: 12 each, Type A & GEO: 54 each, GEO & Type C: 31 each).

The odds ratio suggested that birds infected with a strain not typically associated with virulence (Type C or GEO) were approximately 6 times more likely to produce fledglings (odds ratio = 6.28) than birds infected with a strain associated with virulence (Type C or GEO, 58%, n=12; Type A, 17%, n=6) (Figure 5.2), however this difference was not statistically significant and the confidence interval was very large (Fisher's test, CI 0.48-376.31, $p=0.15$). A power analysis revealed that the power to detect a significant difference between these two groups was low (0.28) and that a future sample size of 25 in each group would be required to detect an effect at the $p<0.05$ level with a power of at least 0.8.

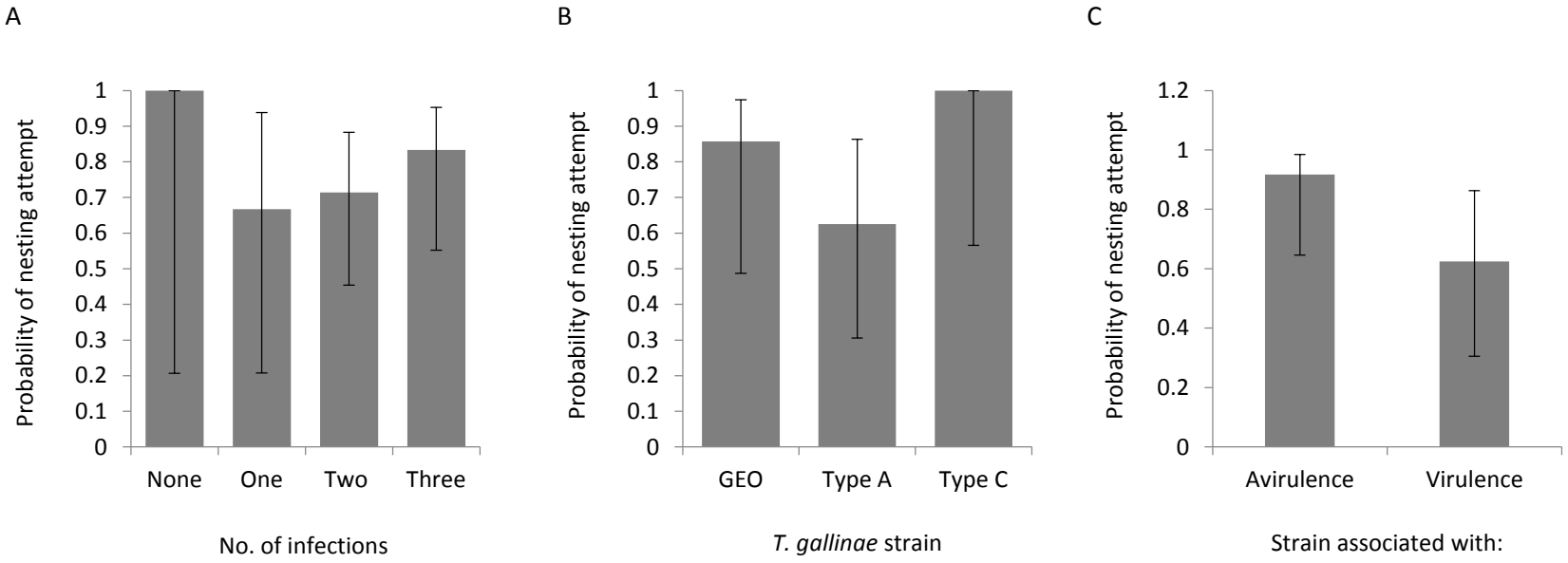


Figure 5.1: Bar graphs showing the associations between parasitic infection and mean reproductive output (nesting probability). Parasitic infection is represented by the number of infections considering *T. gallinae*, *Haemoproteus* sp. and *Leucocytozoon* sp. in graph A. Infection by *T. gallinae* strain is represented in B and C. Standard error bars represent Wilson score intervals. Sample sizes: No. of infections; none (N=1), one (N=3), two (N=14), three (N=12); GEO (N=7), Type A (N=8), Type C (N=5); avirulence (N=12), virulence (N=8).

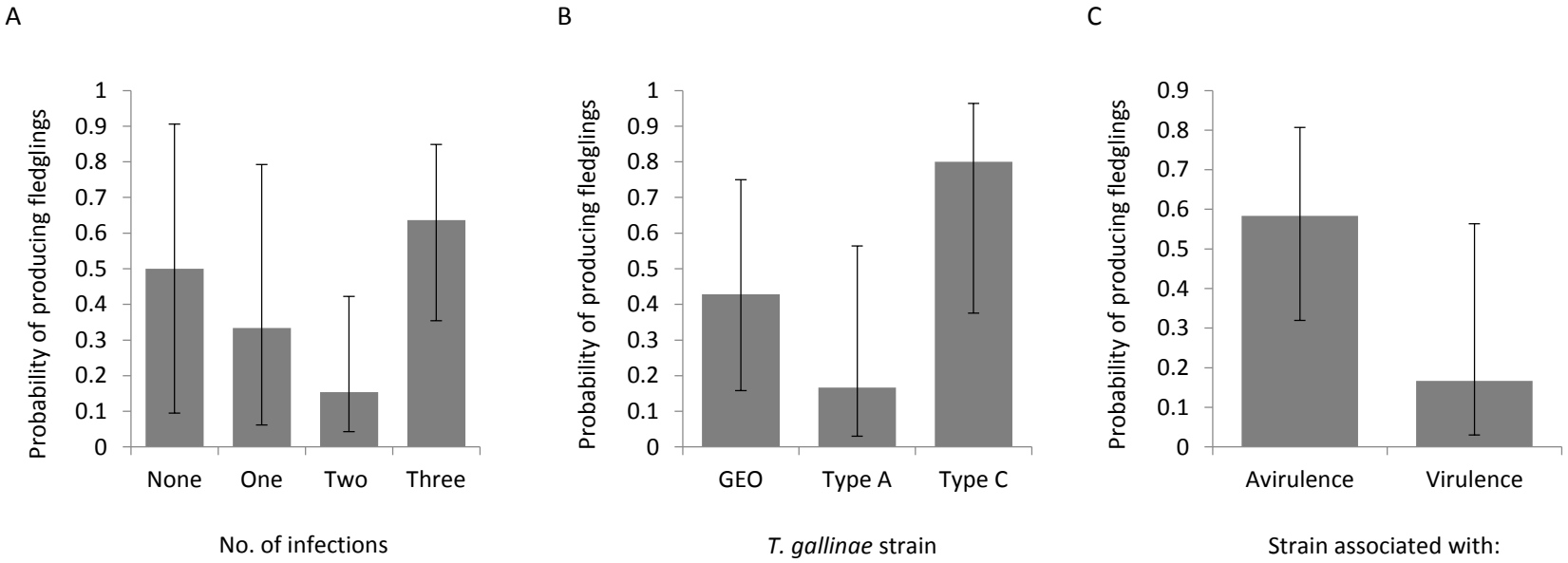


Figure 5.2: Bar graphs showing the associations between parasitic infection and mean reproductive output (fledgling probability). Parasitic infection is represented by the number of infections considering *T. gallinae*, *Haemoproteus* sp. and *Leucocytozoon* sp. in graph A. Infection by *T. gallinae* strain is represented in B and C. Standard error bars represent Wilson score intervals Sample sizes: No. of infections; none (N=1), one (N=3), two (N=13), three (N=11); GEO (N=7), Type A (N=6), Type C (N=5); avirulence (N=12), virulence (N=6).

5.3.4 Impact of infection on mass

Infection by *T. gallinae*, *Haemoproteus* sp. or *Leucocytozoon* sp. was not associated with variation in adult mass (g) (Table 5.5). The number of parasitic infections (between 0 and 3) was not associated with variation in adult mass (g) (Table 5.5, Figure 5.3). The year in which the bird was sampled was not a significant term in either model (Table 5.5).

Infection by either the Type A, Type C or GEO strain was not associated with variation in adult mass (g) (Table 5.6, Figure 5.3). The year in which the bird was sampled was not a significant term in the model (Table 5.6). Infection by either a strain associated with avirulence (Type C or GEO) or a strain associated with virulence (Type A) was not associated with variation in adult mass (g) (Table 5.6, Figure 5.3).

There was an indication of variation in nestling mass at 7 days old between those infected with the GEO strain and those infected with the Type C strain (Figure 5.4) however this was not significant (Table 5.7). Infection by either a strain associated with avirulence (Type C or GEO) or a strain associated with virulence (Type A) was not associated with variation in nestling mass (g) at 7 days old (Table 5.7, Figure 5.4).

Table 5.5: Results of F-test determining factors associated with adult mass (g).

Model		Sum of squares	Df	P value
A	Year	1191	3	0.15
	T. gallinae	274	1	0.26
	Haemoproteus sp.	19	1	0.77
	Leucocytozoon sp.	5	1	0.87
B	Year	1191	3	0.15
	No. of infections	309	3	0.69

Table 5.6: Results of LRT determining factors associated with adult mass (g).

Variables	Deviance	Df	P value
Year	0.052014	3	0.5419
T. gallinae strain	0.0092984	2	0.8252
Year	0.059108	3	0.4546
Strain type (Virulence)	0.00999	1	0.506

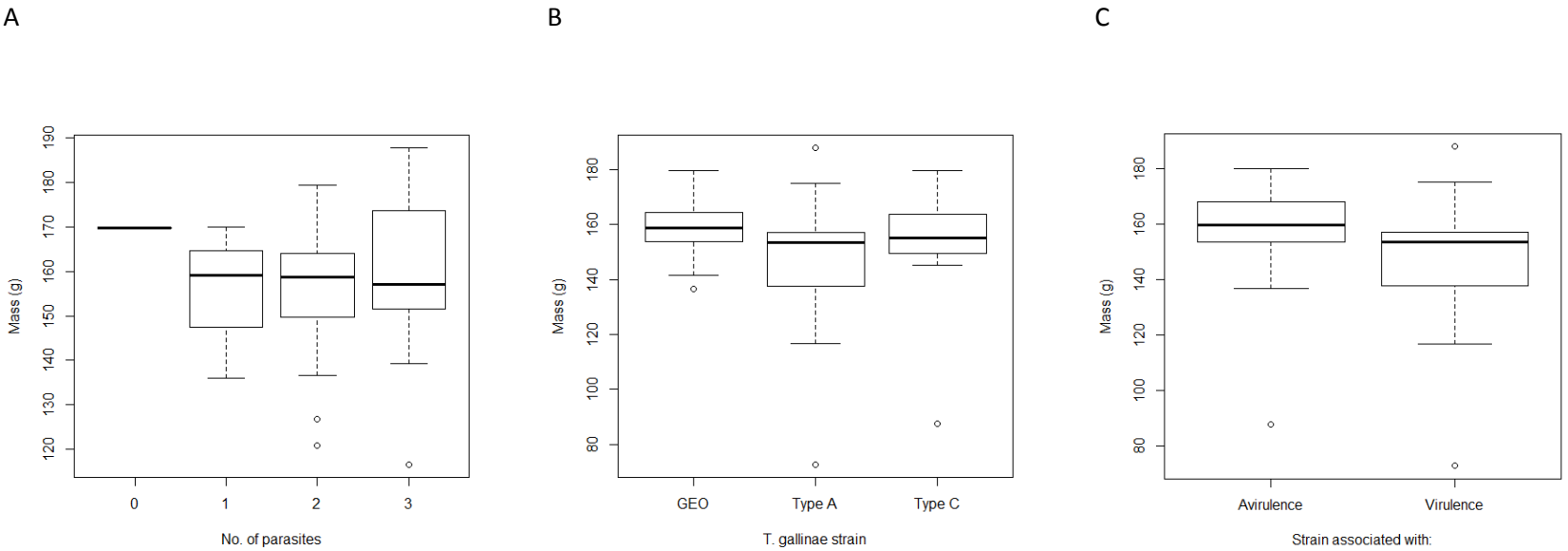


Figure 5.3: Boxplots revealing the variation in adult mass (g) when infected with A) a number of parasites and B-C) *T. gallinae* strains.

Table 5.7: Results of F-test determining associations with variation in nestling mass at 7 days old.

	Sum of squares	Df	P value
Year	476	3	0.42
T. *gallinae* strain	755	3	0.23
Year	476	3	0.45
Strain (Virulence)	132	1	0.39

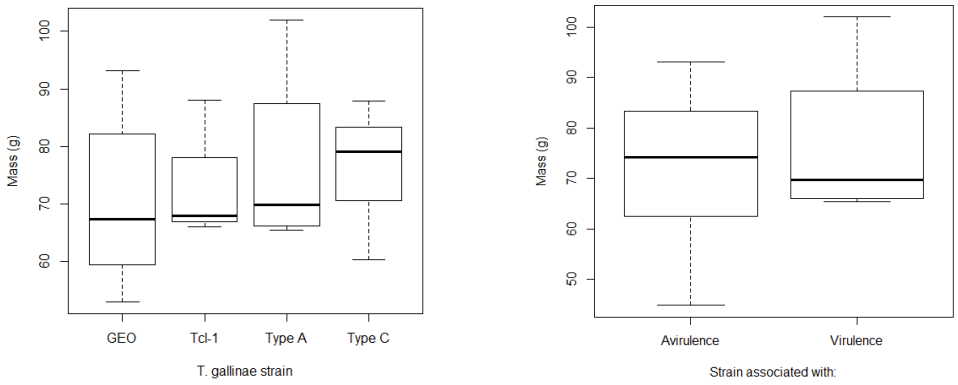


Figure 5.4: Boxplots showing the differences in nestling mass at 7 days old depending on type of *T. gallinae* infection.

5.3.5 Impact of medication on nestling growth

Control group and medicated group consisted of both Turtle Dove and Woodpigeon nestlings. Growth rate in head-bill was not significantly different between the control group (mean= 0.066mm/hour) and the treated group (mean = 0.07mm/hour, paired t_{25}=0.64, p=0.53) (Figure 5.5). Growth rate in tarsus was not significantly different between the control group (mean=0.057mm/hour) and the treated group (mean= 0.056mm/hour, paired t_{25}=0.02, p=0.99) (Figure 5.5). Some individuals were recorded as having negative tarsus growth although this is likely to be due to inconsistencies in measuring this particular indicator of growth, despite the same person being used to record measurements on first and second visits. Growth rate in mass was not significantly different between the control group (mean= 0.758mm/hour) and the treated group (mean= 0.76mm/hour, paired t_{25}= 0.02, p=0.99) (Figure 5.5). Figure 5.6 reveals that *T. gallinae* infection prevalence in control nestlings decreased slightly between the first and second visit, revealing that a smaller proportion were infected by the second visit. Infection prevalence in medicated nestlings was the same on the first and second visit.

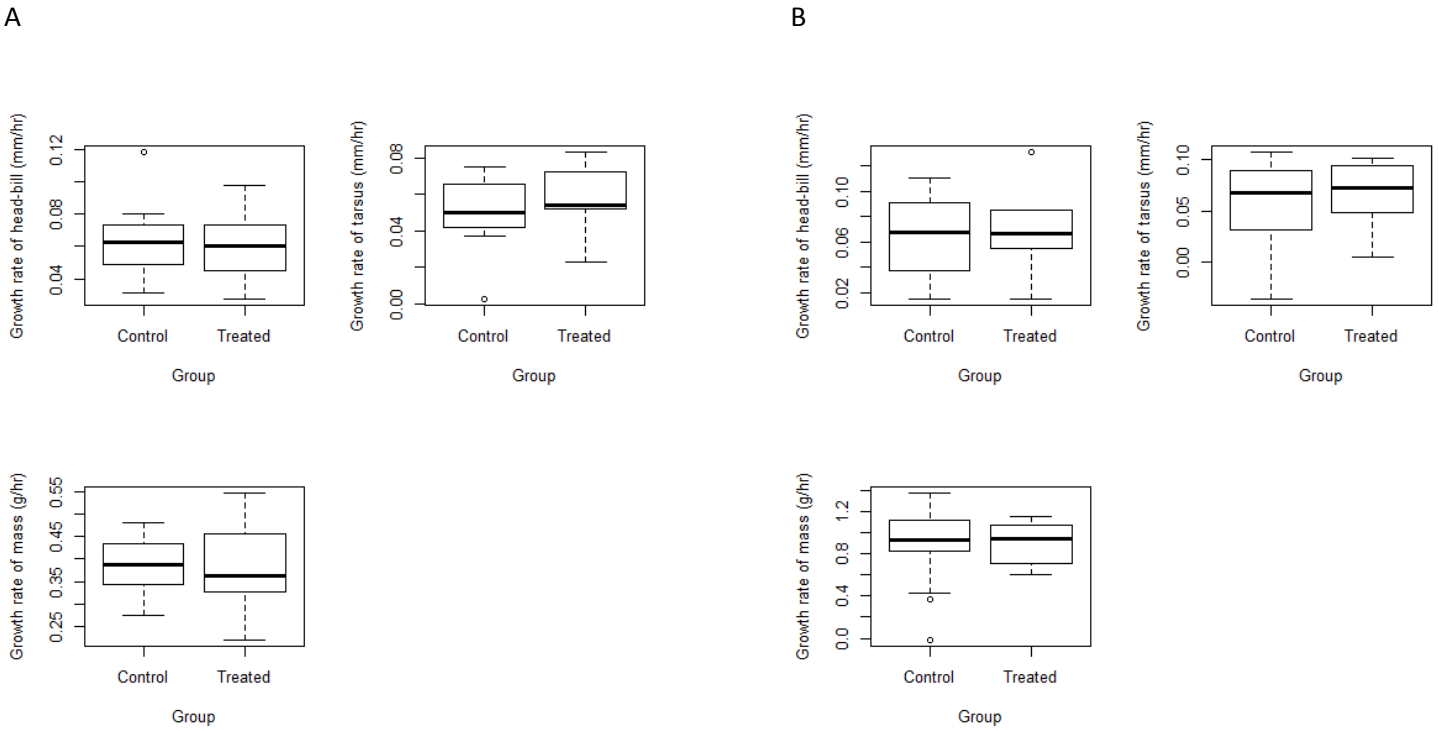


Figure 5.5: Boxplots displaying differences in growth rates of head-bill, tarsus and mass between the medicated and non-medicated (control) nestlings of A) Turtle Doves and B) Woodpigeons.

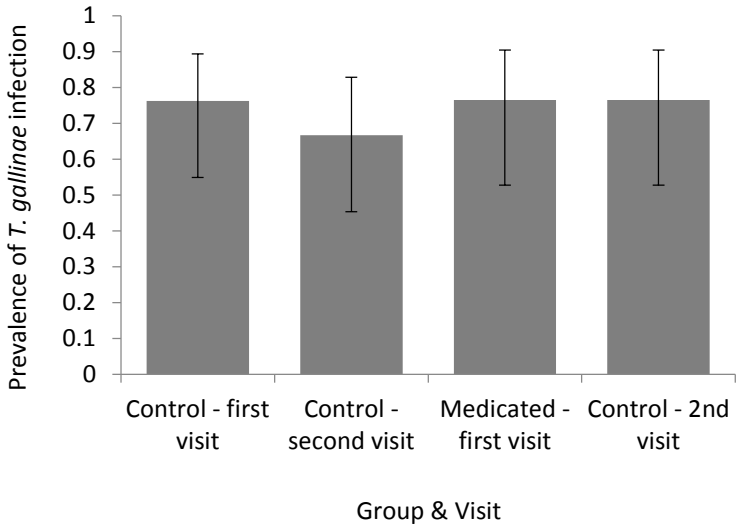
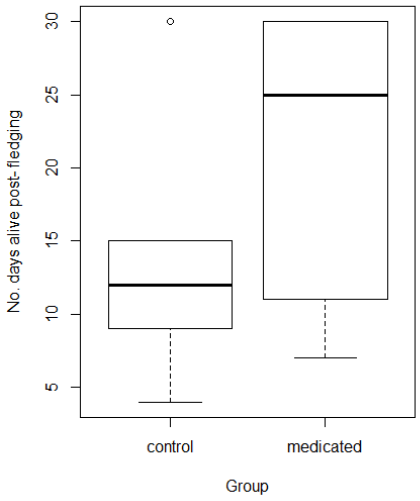


Figure 5.6: Bar chart showing prevalence of *T. gallinae* infection in nestlings on the first and second visit. Standard error bars represent Wilson score intervals. Sample sizes: control – first and visit (N=21), medicated – first and second visit (N=17).

5.3.6 Impact of medication on Turtle Dove post- fledgling survival

The medicated group showed a trend towards surviving for longer and having a greater proportion of survivors (57%, n=12) at the end of the 30 day monitoring period than the control group (29%, n=12) (Figure 5.7). The McNemar's test revealed no significant difference between the two groups in terms of whether they were alive or not at the end of the 30 day period (χ^2=0.5, df=1, p-value =0.48). The power of this study was low (0.18). In order to detect a significant effect ($p<0.05$) with a power of at least 0.8, a sample size of 56 for each group is required.

A



B

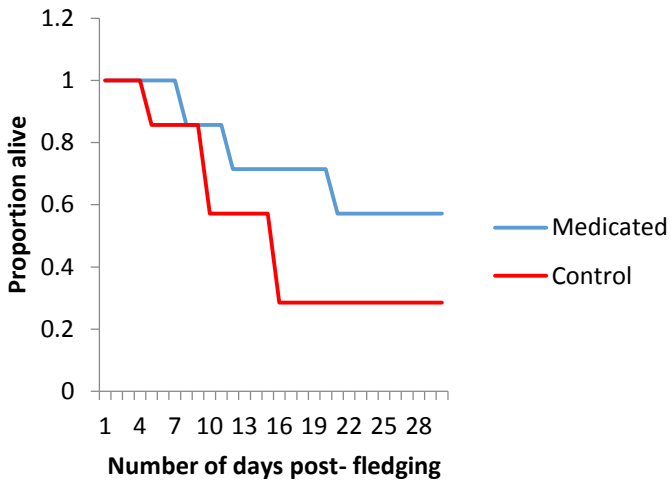


Figure 5.7: A) Boxplot and B) line graph displaying the number of days alive post- fledgling between the control group and the medicated group.

5.3.7 *T. gallinae* strains

Samples from 2013 were identified based on the ITS region whereas samples from 2014/ 2015 were identified based on both the ITS and Fe-hyd region. Two Woodpigeon nestlings sampled in 2013 were infected with the Tcl-1 strain and one was infected with the GEO strain. Of the 20 Woodpigeon nestlings that were sampled in 2014, 95% (n=19) were infected with the Type C strain, one of which was also co-infected with the Type A strain. The last nestling was infected with the Type A strain (0.05%, n=1). Two Type C subtypes were detected: C2 and C4. All the samples bar one were infected with C4 (n=12). The C2 subtype was isolated from

the Woodpigeon nestling with coinfection however the subtype for Type A was not detected. The individual with a single Type A infection carried the A1 subtype. One sample that was identified as Type C based on the ITS region was infected with a new Fe-hyd type (OTU10 in this study) which is a sister taxa to the Type A clade (Figure 2.4 in Chapter 2). This variant differs by 4bp to the A1 subtype and is hereafter referred to as A3-WPNT. Strain information was acquired for one Woodpigeon sample in 2015 which was co-infected with the GEO and Type C strains. The Fe-hyd region was not successfully sequenced for this sample.

The majority of Turtle Dove nestlings sampled from 2013 were infected with the GEO strain (50%, n=15). The Type C strain infected 37% (n=11) of the samples, Tcl-1 infected 10% (n=3) and Type A infected 0.03% (n=1). The slight majority of Turtle Dove nestlings sampled in 2014 (n=15) were infected with the Type A strain (33%, n=5). The remaining nestlings were infected with GEO (27%, n=4), Type C (13%, n=2), Type III (13%, n=2) and a new strain identified during this study, WQR-Env (first mentioned in Chapter 3) (13%, n=2). Four out of the five Type A infected nestlings carry the subtype A1 but the fifth nestling is infected with the new subtype from this study, A3-WPNT. Fe-hyd subtype information was only acquired for one Type C sample, which was infected with C8-TD (first mentioned in Chapter 2). The Fe-hyd region was not successfully sequenced for the GEO strains, Type III strains or WQR-Env strains. No coinfections were detected in Turtle Dove nestlings.

5.4 Discussion

These findings show that there is a high prevalence of coinfection with haemosporidian parasites in adult Turtle Doves. There is no evidence however, of a detrimental effect on measures of reproductive output or body condition. Coinfection with three parasites appears to be correlated with the probability of having fledglings when compared to a double coinfection but there was only moderate power to this study. Additional samples are needed to more rigorously test this trend. There was no evidence of any of the *T. gallinae* strains having a negative impact on nestling mass at 7 days old. The medication trials did not reveal any differences in nestling growth between the medicated and control group nor was there a significant difference in Turtle Dove post- fledgling survival. The majority of these analyses are limited by sample size therefore certain findings are still discussed where there is an indication of a possible trend.

Reproductive output was measured by whether the bird nested during the breeding season and whether it had fledglings. Neither coinfection nor the type of *T. gallinae* strain was associated with nesting attempts suggesting there was no impact on the chance of breeding. There was an indication, however, that infection with a strain associated with virulence (Type A) resulted in an individual being less likely to nest than one infected with a strain not typically associated with virulence (Type C or GEO). This may be a result of the trade-off between immunity and reproduction with regards to fitness. The level of virulence of

parasites could affect investment in immune function, as less virulent ones may be tolerated as long as they don't exceed a certain threshold so that resources can be better invested in reproduction (Zylberberg et al., 2015). The presence of virulent parasites may justify the expenses incurred with activating an immune response (Zylberberg et al., 2015). In this case, infection with the Type A strain may cause resources previously allocated to finding a mate and nesting to be diverted to immune defence, resulting in the individual being less successful with the first stage of breeding (Sheldon and Verhulst, 1996, Schmid-Hempel, 2003).

Previous research on Mauritian Pink Pigeons revealed a negative effect of double parasite infection on fledging success, (Bunbury, 2006) and although not significant, infection with two parasites in this study did give the lowest probability of producing offspring. Birds with a triple parasite infection were more likely to produce fledglings than birds with a double parasite infection. This was an unexpected finding, given the costly impact of parasitic infection on hosts (Sheldon and Verhulst, 1996), and requires further investigation before firm conclusions are made but it is not unprecedented. House Martins *Delichon urbicum* with double blood parasite infections invested more in current reproduction than those with single infections, despite being in poorer physical condition (Marzal et al., 2008). The authors hypothesized that hosts with single infection were more likely to control or clear the infection and therefore invest in immune response and maintenance, despite the trade-offs with other life history traits (Boots and Bowers, 1999, Sandland and Minchella, 2003, Marzal et al., 2007, Marzal et al., 2008). Hosts with double infection, where the effect is more likely to be lethal in this case, invest in 'terminal investment' by maximising current reproduction (Minchella and Loverde, 1981, Sandland and Minchella, 2004, Marzal et al., 2008). This hypothesis could explain our finding in Turtle Doves, should it hold with a larger sample size, except double infections can potentially be cleared whereas triple infections may be more likely to be lethal. The analysis did not control for coinfection between specific blood parasite genera or the *T. gallinae* strain due to limitations in sample size. Comparisons of single infections of each parasite on the host's reproductive output would allow the effect of each parasite to be determined before considering coinfections between them. It may be possible, for example, that an infection between three parasites with low levels of virulence is tolerated more than a double infection where one parasite is highly virulent.

The impact of *T. gallinae* strains on producing fledglings was investigated and although no significant associations were found, some possible patterns began to emerge. Birds infected with a strain not typically associated with virulence (Type C or GEO) appeared more likely to have fledglings than birds infected with a strain associated with virulence (Type A). The Type C strain is generally considered to be non- pathogenic (Sansano-Maestre et al., 2009), whereas there are no published reports of clinical infection with the GEO strain. Tolerating this type of infection and investing resources in reproduction is most likely to maximise fitness of the host. Birds infected with the Type A strain during this study did not exhibit clinical

signs however the immune system may be controlling infection but not completely clearing it. In this instance, morbidity and mortality associated with clinical infection is being avoided in favour of lifetime reproduction but at a cost of current reproductive output (Sheldon and Verhulst, 1996). During the analysis that considered each strain separately, there was an indication that infection with the GEO strain could be detrimental to reproductive output when comparing it to the Type C strain. This is a relatively novel strain to the UK bird population, first detected as part of this study and previously shown to be associated with Africa when comparing it's prevalence to France and the UK (Chapter 2). Further investigation into the impact of this strain is warranted as it may have the potential to cause significant sub- lethal effects. It's possible the impact of the GEO strain is more apparent in a vulnerable species which are experiencing other stressors contributing to the population decline (Lafferty and Holt, 2003, Echaubard et al., 2010).

There was no association with the number of parasite infections or *T. gallinae* strain and adult mass. Infection with an increasing number of parasites or infection with the *T. gallinae* strain that is associated with virulence (Type A) was expected to be linked with a decrease in adult mass due to the increased utilization of body reserves in combating infection. House Martins with double blood parasite infections were found to be in poorer physical condition than those with single infections (Marzal et al., 2008). The same is true for coinfection with different parasite species such as helminths, whereby multiple infections in Willow Ptarmigans *Lagopus lagopus* were negatively associated with host body mass (Holmstad et al., 2005). A lack of relationship is not unusual as a study on over 3,000 Passerines varying in infection with *Haemoproteus* sp. and *Leucocytozoon* sp. did not reveal any effect on host body mass (Bennett et al., 1988). Furthermore, different blood parasite genera can have different effects as shown in Cirl Buntings *Emberiza cirlus*, whereby a lower body condition was detected in individuals infected by *Leucocytozoon cambournaci*, but no such differences were associated with *Plasmodium relictum* infections (Figuerola et al., 1999). Coinfections between the parasites could mask the detrimental effects of single infections, which is a possibility in this sample of Turtle Doves. Again, distinguishing the effects of single parasite infections would increase our understanding of the complex interactions occurring. Although *T. gallinae* strains were evaluated, the sample sizes were not sufficient to investigate each genera of blood parasite. If effects on body condition are not directly visible, it does not rule out detrimental effects of infection on the body. In a study on *Haemoproteus* sp. and *Plasmodium* sp. infection in Seychelles Warblers *Acrocephalus sechellensis* there was no association between infection and body condition however during the energetically demanding provisioning stage of breeding, infected birds had significantly higher oxidative imbalance than non-infected birds (van de Crommenacker et al., 2011). This increased susceptibility to oxidative stress could have longer term detrimental effects by accelerating the degeneration of body functions over time (Finkel and Holbrook, 2000). Indeed, Seychelles Warblers that

were infected earlier in life had lower survival rates than uninfected birds (van Oers et al., 2010). It highlights the need to consider other physiological consequences of infection.

There was no association between *T. gallinae* strain infection and mass of a nestling at 7 days old. It is worth noting that nestlings infected with the GEO strain tended to be at the lower end of the range of measurements observed, considering the previous results suggesting that the GEO strain could be linked with a lower probability of producing fledglings. The proportion of available seed-rich habitat was found to be a strong predictor of Turtle Dove mass at 7 days old therefore food availability, rather than infection status, appears to be a more important determinant of body condition (Dunn et al., 2016a). There was no difference between medicated and non-medicated nestlings in terms of growth. Reduced growth in non-medicated nestlings was expected as resources allocated to growth are re-directed towards fighting infection. This was shown to be the case with ectoparasites infecting nestling house martins where parasite loads increased daily metabolic rate and negatively affected body mass at 16 days old (Moller et al., 1994). Screening the nestlings on the first and second visit revealed no difference in *T. gallinae* prevalence in the medicated group i.e. prevalence remained high. This resulted in the medicated group being similar to the control group in terms of *T. gallinae* prevalence. Re-infection rates of *T. gallinae* would have been high, considering the transmission route of parent to offspring via regurgitated crop milk (Stabler, 1947), therefore nestlings are likely to be re-infected in their subsequent feed. It is unlikely the medication provides protection against re-infection, as repeated medication is recommended when the possibility of re-infection is high and all Pigeons should be treated simultaneously (Swinnerton et al., 2005, Spartrix, n.d.). This would be difficult to control for in the field however medication trials with captive birds, if possible, would confirm the impact of infection on nestling growth. This is important, considering that a reduced size at the stage of fledging has been shown to negatively impact post- fledging survival and recruitment success (Ringsby et al., 1998, Both et al., 1999, Naef-Daenzer et al., 2001, Becker and Bradley, 2007), including in Turtle Doves (Dunn et al 2016). Other stressful rearing conditions such as food and nutrient limitation, adverse weather and sibling competition can also depress growth rates (Ricklefs, 1968, Dijkstra et al., 1990, Cooch et al., 1991, Dawson and Bidwell, 2005). Food availability has been shown to compromise fledging success in Turtle Doves therefore exploring how this interacts with infection would increase our understanding of the drivers of Turtle Dove population decline.

Despite the lack of an association of *T. gallinae* strain infection on nestling mass and high re-infection rates in the nest leading to a persistent prevalence of *T. gallinae* infection, an effect of medication on post- fledgling survival was hinted at by the results, although it was not significant. Medicated nestlings survived for longer with a larger proportion being alive at the end of the 30 day monitoring period. These results need further support with larger sample sizes to confirm

whether this trend is significant. If this is indeed the case, it suggests that the medication did confer an advantage not reflected by growth indicators and concurs with medication experiments carried out in Pink Pigeons where survival in nestlings with and without signs of clinical disease was higher following medication (Swinnerton et al 2005). It may have reduced infection intensity or oxidative stress susceptibility. Future studies could measure a wider range of physiological parameters indicative of health.

In conclusion, this study has provided an insight into the potential impact of coinfection and infection by *T. gallinae* strains on Turtle Doves in terms of reproductive output, body condition, nestling body condition and post- fledging survival. Larger sample sizes to further examine the effect on reproductive effort, particularly the effect of triple infection on the probability of producing fledglings, is warranted. The potential effect of medication on post- fledging survival is also intriguing and measuring other indicators of health, such as oxidative parameters in the blood plasma, may reveal other associations that could explain the observed variation. Theories concerning the trade-off between immunity and reproductive effort have explained the variation in reproductive effort however taking measures of the immune system function would provide extra support. Lastly, parasite strains, species and genera may all vary in their effects on the host which could potentially confound results. Ideally, the effects of single parasite infection would be established in addition to the effects of coinfection between them. Although strains of *T. gallinae* and the most common blood parasites are considered here, it does not consider the full extent of the potential pathogen community within a host. A full evaluation, screening for bacteria, viruses and fungal pathogens would reveal the true extent of coinfection which the application of Next Generation Sequencing could render more practical for future surveys. Furthermore, the continuation of this longitudinal data set would allow the interaction of coinfection with environmental variables to be explored.

Chapter 6

Discussion

6.1 Summary

This thesis investigates the molecular epidemiology of *T. gallinae* with respect to the declining UK Turtle Dove population, but the dynamics of this multi-host pathogen have implications for a wide range of other bird populations and species. Next Generation Sequencing (NGS) technology was applied to *T. gallinae* infection surveillance for the first time, to evaluate infection status in samples from Turtle Dove populations from the UK, France, Senegal, Burkina Faso, as well as other species of wild UK birds. Using this approach I also provided systematic evidence for a recently identified *T. gallinae* transmission pathway via shared environmental resources (supplementary food and water) in the UK. Coinfection with haemosporidian parasites was also found to be common in UK Turtle Doves. NGS not only allowed the simultaneous detection of multiple strains within one sample, but also eased the practicality of analysing a large number of samples by pooling them for sequencing runs (Long et al., 2011, Kessner et al., 2013, Schlotterer et al., 2014, Cao and Sun, 2015). Avian blood parasites are frequently used as a classic model system for investigating the ecological and evolutionary dynamics of host-parasite associations because they are widespread, abundant and easily assayed (Valkiunas, 2004). Whether the findings are generalizable to other systems ought to be explored and *T. gallinae* shares these characteristics, which makes it another useful model system for host-parasite ecology. Investigating the genetic diversity and infection patterns of parasites provides an insight into their evolutionary histories and aids the identification of causal factors contributing to disease dynamics, allowing the prediction of wildlife disease outbreaks (Barrett et al., 2008). This is important for conservation management , especially in light of the relatively recent finch trichomonosis epidemics in the UK and central/ western Europe (Robinson et al., 2010, Neimanis et al., 2010, Lawson et al., 2011a, Ganas et al., 2014).

This chapter will begin by discussing the application of metabarcoding to disease surveillance and then summarizing the main findings from the thesis. The findings are then examined in the context of five main areas: parasite population structure, transmission pathways, multi-host dynamics, multi-parasite dynamics and impacts on host fitness. The limitations of the study and ideas for future work are then discussed.

6.2 Application of metabarcoding & main findings

Metabarcoding is an emerging discipline which to date has largely been applied to diet analysis from faecal samples and characterising microbial communities in soil and aquatic systems (Pompanon et al., 2012, Tedersoo et al., 2014). Only a few

studies have used it to detect and identify parasites but these already reflect a diversity of applications such as: the intestinal nematode communities of Rufous Mouse Lemurs *Microcebus rufus*; the causative agent and vector involved in a Sindbis virus (SINV) outbreak in Sweden; the characterisation of Black Band Disease on Coral *Porites lutea*; and the simultaneous assessment of gut parasites in addition to diet in the Banded Leaf Monkey *Presbytis femoralis* (Aivelo et al., 2015, Bergqvist et al., 2015, Sere et al., 2016, Srivathsan et al., 2016). This thesis demonstrates the applicability of metabarcoding to a disease surveillance program on a much wider scale, both geographically and temporally. The recognized genetic diversity of *T. gallinae* has increased as a result of this study, with the detection of seven new ITS types (also referred to as strains) and nine new Fe-hyd sub- types. The sub-types have increased the known diversity of the Type C strain and represent first identification of diversity within the Tcl-1 strain. The A1 subtype, responsible for European Finch trichomonosis epidemics, remains the dominant variant of the Type A strain found in free-ranging European birds. The Fe-hyd region could not be amplified for three ITS types found during this study: GEO, Type III and WQR-Env. This hinders its application as a marker for detecting fine-scale genetic variation that has thus far provided useful insights into the evolutionary history of *T. gallinae* (Chi et al., 2013, Sansano-Maestre et al., 2016). Geographical variation in *T. gallinae* strain prevalence was revealed when comparing the strain composition present in populations of Columbidae from different countries. The Type A strain was most prevalent in the UK, the Type C strain was most likely to be found in France, the GEO strain was more common in Senegal and the Tcl – 1 strain was linked with Burkina Faso. Furthermore, there was significant temporal variation in *T. gallinae* strain prevalence over a five year period, suggesting turnover of lineages between years.

Shared resources pose a significant risk as a transmission pathway, with the detection of *T. gallinae* in 20%-57% of food and 40%-67% of water resources. Furthermore, repeated detection of *T. gallinae* in the same resource over a season indicates that re-infection rates of resources were high in some cases. Although *T. gallinae* infection was more likely in species of Columbidae than gamebirds or Passerines, there was no association between infection by the Type A strain and bird order. Infection by the Type A strain was, however, more likely in birds sampled on sites with supplementary feeding.

There was a high prevalence of coinfection between *T. gallinae* and at least one blood parasite. There was no evidence of a detrimental effect of coinfection or a particular *T. gallinae* strain infection on reproductive output, adult body condition, nestling body condition or post- fledgling survival. However, these analyses were likely hindered by small sample sizes and there were suggestions of trends that warrant further investigation. The increase in reproductive output between double parasite infection and triple parasite infection could be explained by the 'terminal investment' hypothesis but further research is required to support this theory.

6.3 Population structure of *T. gallinae*

Geographical variation in the genetic structure of parasite populations is likely to be driven by host specificity, host mobility and environmental conditions (Huyse et al., 2005). Low host specificity, highly mobile hosts and/ or an absence of physical barriers will facilitate the exchange of strains between populations (Barrett et al., 2008, Li et al., 2011, Shi et al., 2014). *T. gallinae* is considered a generalist parasite as it can infect a wide range of avian taxa (Stabler, 1947), There is still geographical variation in *T. gallinae* strain composition when comparisons are made within years and it remains relatively stable when comparing populations of Columbidae or just Turtle Dove populations, indicating that the composition of host species is not driving the observed variation. A meta-analysis investigated whether the type of life cycle was a driver of parasite dispersal in trematodes whereby some species only use aquatic hosts to complete their life cycle whereas others use birds or mammals as final hosts (Blasco-Costa and Poulin, 2013). Species of trematode limited to completing their life cycle within water showed the highest genetic structure than those who dispersed among separate aquatic habitats through a bird or mammal host (Blasco-Costa and Poulin, 2013). *T. gallinae* is also reliant upon its host for indirect dispersal. Host dispersal between the countries studied here is relatively limited as Turtle Doves are the only long-distant migrant, although some species of Columbidae, such as Woodpigeons, are short distance migrants and travel between the UK and the continent. This could explain the associations of some strains with specific countries however the shared presence of a number of strains indicates a level of pathogen dispersal. The migration route of the Turtle Dove across the western Palaearctic flyway could be the mechanism behind this. The UK and French populations may share wintering grounds where transmission is possible. Furthermore, stopover points along the western palearctic flyway could provide further opportunities for transmission (Eraud et al., 2013). The geographical populations could be viewed as metapopulations which are linked by migration, in the same way that breeding colonies of seabirds represent metapopulations. Seabirds are site faithful to their breeding grounds, forming dense colonies which favour the local maintenance of parasites (Rothschild and Clay, 1961, Furness and Monaghan, 1987). Additionally, they undertake long-distance movements during migration and foraging which facilitates large-scale dissemination of parasites (Egevang et al., 2010, Fuller et al., 2012, Altizer et al., 2013). A heterogeneous distribution of parasites among the islands making up the Iles Eparses, and seabird species suggests that there is a level of independence between the metacommunities (McCoy et al., 2016). Investigating what types of host movements are responsible for dissemination and how these movements change with infection status would contribute to understanding parasite population structure within a metapopulation framework (McCoy et al., 2016).

Studies revealing temporal dynamics in the genetic structure of a parasite population infecting wildlife populations are limited. Temporal variation in the strain composition of *T. gallinae* was indicated over a five year period in the UK

but the study period was too short to reveal whether these fluctuations in strain prevalence were a regular cyclical pattern. Cyclical dynamics have previously been demonstrated in the prevalence of three blood parasite lineages (two *Plasmodium* and one *Haemoproteus*) infecting Great Reed Warblers *Acrocephalus arundinaceus* which appeared to fluctuate in parallel with a periodicity of about three to four years (Bensch et al., 2007). It is currently unknown what factors may be driving this pattern but knowledge on extrinsic parameters such as climatic variation affecting vector distribution and alternative hosts was highlighted (Bensch et al., 2007). Research on how parasite communities in host populations change over time has revealed different dynamics at different timescales. Short-term stability (two years) of macro-parasite communities was shown in Three-spined Sticklebacks *Gasterosteus aculeatus* in freshwater lochs of Scotland (de Roij and MacColl, 2012). Seasonal variation in infection dynamics was apparent in helminth parasite communities of the Pacific Fat Sleeper *Dormitator iatifrons* from Tres Palos Lagoon, Mexico which was associated with environmental changes during the dry and rainy seasons (Violante-Gonzalez et al., 2008). Long-term stability (10 years) of cyclical dynamics was shown in the prevalence of common helminths in Bank Voles *Clethrionomys glareolus* and Red Voles *Clethrionomys rutilus* as it closely followed the changes in host density (Haukisalmi et al., 1988). A high turnover in gastrointestinal parasite prevalence and composition was revealed in troops of Guinea Baboons *Papio papio* when examined during two surveys 20 years apart (Ebbert et al., 2013). One study assessed the variation both within hosts and between hosts over three years, whereby there was a high turnover of species within hosts but the overall species composition in the Rufous Mouse Lemur *Microcebus rufus* population remained similar (Aivelo et al., 2015).

Environmental conditions which differentially affect the transmission of the parasite ought to be considered as transmission success has been shown to be lineage specific and partly shaped by locality-specific effects (Szollosi et al., 2011). Temporal dynamics of lineage diversity can also reflect interspecific interactions between parasites. Mixed strain infections of *Plasmodium chabaudi* were examined in laboratory mice which revealed that the more virulent strains had a competitive advantage (de Roode et al., 2005). Transmission experiments showed that competitive suppression of a strain within hosts also suppresses that strain's transmission to mosquitoes (de Roode et al., 2005). The processes occurring in natural populations may be far more complex, as shown by the non- parallel annual variation in the prevalence of two *Plasmodium* parasites reported in a population of Blue Tits which highlighted that different biological processes were underpinning variation in this system (Knowles et al., 2011). A longitudinal study of *T. gallinae* in wild bird populations would allow the teasing apart of the contributing factors behind temporal variation and provide an insight into the evolutionary dynamics of this parasite.

6.4 Transmission pathway

Environmental transmission of *T. gallinae* through shared resources, particularly bird feeding stations, was long suspected and accepted based on circumstantial evidence (Forzan et al., 2010, Neimanis et al., 2010, Robinson et al., 2010). This is the first study to provide direct systematic evidence of *T. gallinae* occurrence and persistence in the environment, and suggests this transmission route could be a significant risk for the spread of *T. gallinae*. It has led to the development of supplementary feeding trials which are testing the delivery and level of seed provisioning whilst minimizing the risk of parasite transmission to wild birds. If successful, the supplementary feeding option will be incorporated into an agri-environment scheme. Wider sampling, both geographically and temporally, would increase our understanding of the extent to which it is a transmission route. The increased likelihood of detecting *T. gallinae* in full water trays, compared to those which are less than half full, and during warmer daily temperatures increases our understanding of the conditions that facilitate *T. gallinae* survival in the environment and potential mechanisms behind its persistence. The predicted changes in the UK brought on by climate change (IPCC, 2013), namely warmer temperatures and increased rainfall may increase the rate of *T. gallinae* environmental transmission. Flooding can drive water-borne epidemics and although *T. gallinae* is not a water-borne pathogen, increased rainfall would increase the number of water resources in the environment which may increase rates of transmission (Cann et al., 2013). On the other hand, increased rainfall would render water resources common and potentially discourage birds from drinking at higher densities, resulting in a decreased rate of transmission (Baylis et al., 1999, Linthicum et al., 1999). Previous attempts to evaluate the relative impact of environmental parameters on the transmission of parasites have found them difficult to measure (Koelle et al., 2005, Wearing and Rohani, 2006). The interactions among different factors may be additive, multiplicative or antagonistic, with a non- linear effect on transmission (Koelle et al., 2005, Wearing and Rohani, 2006). Furthermore, these interactions and effects could vary at different temporal or spatial scales (Koelle et al., 2005, Wearing and Rohani, 2006). Overall, further sampling of *T. gallinae* from the environment at varying temporal and spatial scales is needed to understand the role of environmental transmission in the epidemiology of *T. gallinae*.

The increased probability of detecting *T. gallinae* in high intensity resources (such as grain piles) rather than low intensity resources (representing more natural foraging areas) has important implications considering farming intensification and the consequential reduction in available foraging habitat (Stoate et al., 2001, Robinson and Sutherland, 2002, Newton, 2004). It may encourage birds to crowd whilst feeding on the limited resources that are left or result in an increasing dependence on unnatural food sources such as spilt grain on farms which also encourage high bird feeding densities. The availability of more hosts for transmission is expected to drive the evolution of increased virulence (Mennerat et al., 2010, Pulkkinen et al., 2010). The current view regarding virulence is that

there should be an intermediate level adopted by the parasite in order to balance host exploitation as a means to develop propagules and maintain host survival to maximise long term survival (Alizon et al., 2009). Increasing virulence would increase host mortality although if there is higher host availability, this reduces the associated fitness cost of virulence (Mennerat et al., 2010). The emergence of the bacterial fish disease *Flavobacterium columnare* in salmon fish farms in northern Finland was due to an increase in bacterial virulence (Pulkkinen et al., 2010). The co-occurrence of parasite strains and high stocking density of the host enhanced transmission opportunities and promoted competition between strains which provided an environment that promoted the evolution of virulence (Pulkkinen et al., 2010). Being aware of similar conditions in other ecosystems will facilitate early detection of virulent strains and could allow preventative measures to be put in place in order to control the risk of disease outbreaks.

6.5 Multi-host dynamics

Prevalence of *T. gallinae* infection in species of columbidae is persistently high whereas prevalence of infection is more variable and tends to be lower in Passerines. This is unsurprising given that infection is likely to be due to environmental transmission, which is less effective than the transmission route in species of Columbidae and more liable to be affected by abiotic factors. Host species may vary in quality as well as quantity, which has implications for the transmission of the parasite. Columbids may be high quality hosts, given they are the natural host of the parasite whereas Passerines could be lower quality hosts which may be associated with higher host mortality and also support the observed patterns in prevalence in the two bird families (Woolhouse et al., 2001, Power and Mitchell, 2004, Rigaud et al., 2010, Fenton et al., 2015). The high prevalence of infection in species of Columbidae and an effective environmental transmission route would elevate the exposure of other bird hosts to the parasite, resulting in spillover (Daszak, 2000, Power and Mitchell, 2004). If the parasite infects a host species that transmits poorly to subsequent hosts then this can drive a decline in environmental transmission of the parasite, resulting in a lower prevalence of infection in the host species which is known as the dilution effect (Keesing et al., 2006). It is likely that both of these factors are at play in the *T. gallinae* system. When factors that promote environmental transmission of the parasite, such as condition of the shared resource, co-occur with those that encourage high host density, transmission from species of Columbidae to Passerines is effective and results in a high prevalence of infection in Passerines. When shared resource conditions are not in favour of enhancing *T. gallinae* survival and host density is less, transmission from species of Columbidae to Passerines is ineffective and because Passerines are a lower quality host in terms of being less able to transmit to subsequent hosts, prevalence of infection in Passerine populations declines.

Although a pathogenic impact was rarely observed in species of Columbidae and Passerines sampled during this study i.e. the majority of infections were sub-

clinical, the Type A strain has caused epidemics in Passerines and species of Columbidae in the past (Villanua et al., 2006, Neimanis et al., 2010, Robinson et al., 2010, Ganas et al., 2014). Parasites may evolve higher virulence in their host depending on community context. A trichomonosis epidemic in Spanish Woodpigeons was associated with the provision of supplementary feeding where high host density may have relaxed the fitness constraints of parasite virulence and although the presence of multiple strains was not determined, these conditions explain the initiation of a disease outbreak. Virulence in low quality hosts is more likely if spillover is rare, as the low quality host contributes little to parasite fitness hence there is no selective constraint on parasite virulence in that host (Woolhouse et al., 2001). On the other hand, if the quality of the host is not an issue, the high virulence may reflect recent infection in the new host where optimal virulence has not yet been achieved (Woolhouse et al., 2001). The latter theory explains *T. gallinae* infection in Passerines in the UK, where initial reports were of trichomonosis epidemics but recently, the Type A strain is being detected in Passerines but with no clinical signs in the infected birds (Robinson et al., 2010, Lawson et al., 2011a, Chi et al., 2013, Ermgassen et al., 2016). Parasite virulence and transmission involve trade-offs among virulence in different host species, variation in host species quality and patterns of transmission so all of these factors should be considered when exploring relationships between a parasite and multiple hosts (Rigaud et al., 2010).

6.6 Multi-parasite dynamics

The high prevalence of coinfection between *T. gallinae* and haemosporidian parasites reveals that *T. gallinae* is more likely to coexist with distantly related parasites than those of the same species. Parasite coinfection within a host may lead to exclusion by the most effective competitor or ongoing competitive interactions. These interactions may be through direct competition for host resources, which is likely for strains of the same species, or indirectly through the host immune system, which is more likely for more distantly related parasites (Mideo, 2009). Direct competition may favour the evolution of increased virulence if it is positively correlated with competitive ability, as shown for the malaria parasite strains in laboratory mice and *Pasteuria ramosa* strains in Daphnia (de Roode et al., 2005, Ben-Ami et al., 2008). A similar pattern has also been observed for different parasites where *Echinostoma caproni* increases in virulence when present in its snail host alongside another trematode *Schistosoma mansoni* (Sandland et al., 2007). If different species are not competing for the same resources, increased virulence is still the best strategy if higher densities of parasites are more likely to overcome the non-specific response of the immune system (Mideo, 2009). Coinfections between *T. gallinae* strains were rarely observed in the avian hosts sampled during this study, despite using NGS specifically to detect coinfections. This could be because coinfection between strains resulted in direct competition and was resolved rapidly by the exclusion of one strain over the other. It does not appear that this was achieved through

increasing virulence as there was an absence of clinical signs in the birds sampled. *T. gallinae* strains are likely to be co-transmitted in species of Columbidae, during the regurgitation of crop milk, and an increased probability of co-transmission with strains of the same species has been shown theoretically to favour less virulent strains (Alizon, 2013). Competitive exclusion could have been achieved by a different mechanism, known as interference competition whereby the growth, reproduction or transmission of competitors is inhibited, either chemically or mechanically (Mideo, 2009). Coinfection between different blood parasite species and between blood parasites and *T. gallinae* in Turtle Doves was common. Evidence from other systems has shown that virulence appears to be lineage specific in haemosporidia (van Rooyen et al., 2013) but measures of blood parasite virulence in Turtle Doves were not considered during this study. Blood parasites are likely to be co-transmitted and whether this selects for higher or lower levels of virulence in the competing parasites of different species, depends on the relative virulence of the species involved (Alizon, 2013). If the species have co-evolved in the host for a long period of time and co-transmission rates are the same, this selects for lower levels of virulence (Alizon, 2013). The type of competitive interaction that these species employ i.e. exploitation competition, immune-mediated apparent competition or inference competition will also impact the evolution of virulence (Mideo, 2009, Alizon, 2013). The dynamics of competition in addition to mediation by the host immune system need to be taken into account when assessing the outcome.

6.7 Impacts on host fitness

The selection for virulence has been considered in the context of multiple hosts and coinfection between multiple strains and parasites within the host. The strategy of increased virulence to increase the probability of transmission under these conditions is worrying, particularly if the host is a species of conservation concern and their survival as a species is not required for that of the parasite. Consideration has been given to how the prevalence of infection is driven by host specificity, host dispersal, environmental conditions, transmission pathways, multiple hosts and multiple parasites. However, there are also traits relating to host fitness that must be considered – the immunological capacity to either prevent parasitic infection or to clear the infection after it has established (Atkinson and van Riper III, 1991), which in turn can be suppressed by stressors (Appleby et al., 1999, Lochmiller and Deerenberg, 2000, Navarro, 2004, Christe et al., 2012). Turtle Doves differ from other species of Columbidae in that they perform long-distance migration and therefore are exposed to a host of other stressors not experienced by resident species. Indeed, their population decline has been attributed to some of them. These additional stressors could increase their susceptibility to infection relative to other species of Columbidae. Multiple stressors could also act simultaneously on both parasites and their hosts. These stressors could vary both temporally, as with seasonal food availability, and spatially, such as nesting habitat availability and hence co-occur with each other

or occur sequentially (Cable et al., 2017). When they co-occur, their effects could range from being additive, synergistic or antagonistic (Folt et al., 1999, Anthony et al., 2007, Noges et al., 2016, Prado et al., 2016). No associations between *T. gallinae* strain infection or coinfection with haemosporidians and measures of reproduction or body condition were discovered during this study however further work is needed to increase the sample sizes before conclusions are made. It is still possible that infection is having a negative impact on Turtle Doves as we know one strain is capable of causing mortality (Stockdale et al., 2015) and not all potential sub-lethal impacts were considered during this analysis. Teasing apart the relative impact of different stressors on Turtle Doves, particularly if they are interacting, will be difficult. However, interacting stressors are beginning to be explored, with a couple of interesting studies highlighted here. Northern Leopard Frog *Rana pipiens* tadpoles were exposed to stressors both singly and in combination, which included: infection by a trematode parasite, exposure to predator chemical cues and exposure to a herbicide (Koprivnikar, 2010). Parasite infection and predator pressure or the presence of herbicide proved to be particularly deleterious for the tadpoles (Koprivnikar, 2010). Increasing seawater temperatures were found to increase European Flat Oyster *Ostrea edulis* larval mortalities, although increasing seawater acidification lowered bacterial growth which may help prevent bacterial pathogenicity in larvae (Prado et al., 2016). The net effect of these two stressors could result in little change on European Flat Oyster populations despite the underlying mechanisms. These examples highlight that the type of stressor in an interaction is important as they can have different effects and that the stability of a population does not necessarily mean they are not experiencing stressors.

6.8 Limitations & future work

The main limitation of this study was the sample size of Turtle Doves due to their rarity in the UK and difficulty in catching them, which resulted in low power of the analyses concerning the impact of infection on measures of reproductive output, mass, nestling mass and post- fledgling survival. The study was also limited geographically to sites in East Anglia. An attempt to broaden the geographical scope to include a site in Hampshire was unsuccessful as only one Turtle Dove was caught and radio-tracking the individual was unsuccessful. Further sampling of Turtle Doves over future years and on a wider geographical scale has the potential to examine some of the trends hinted upon by preliminary analysis, such as the impact of the strain associated with virulence on reproductive output. In addition to small sample sizes, the likelihood of recapturing Turtle Doves is extremely low and only happened once over a four year period of fieldwork. The turnover of parasite strains within hosts therefore could not be assessed, nor could the potential for the host to clear *T. gallinae* or blood parasite infection naturally. Whether the temporal variation in strain composition is due to a turnover within the host could not be established. Examining within host parasite dynamics in another species of Columbidae is possible, but the additional environmental

stressors experienced by Turtle Doves compared to the common species of Columbidae may hinder generalizations being made between species. However, it would still be worthwhile to examine *T. gallinae* strain dynamics in the general avian population as epidemics do occur in other species.

Increased certainty in the results of this study would be achieved with further measures to prevent contamination between samples in place, as discussed in Chapter two. An increase in the types of replicates would also be beneficial. There were no biological replicates i.e. duplicate samples taken from the bird but taking multiple oral swabs or blood samples would have to be considered from an ethical view point. Technical replicates in this study only involved running 10% sample duplicates in the NGS runs. Repeated DNA extractions were not possible for *T. gallinae* isolates as the entirety of the sample was used for the first extraction. This was to ensure maximum DNA yield however if cultures were set up and maintained under laboratory conditions, this would increase the yield of parasite from one sample and allow multiple DNA extractions to take place. Future studies ought to involve replicates from the PCR through to the sequencing stage, and sequence the same sample on different runs to produce technical replicates of every stage. Robust error rates could be calculated and taken into account when considering parasite identification.

When considering the potential impact of *T. gallinae* infection on nestling growth and Turtle Dove post-fledging survival with medication trials, one result suggested that the medication did not actually have an effect in terms of clearing infection. There was no difference in *T. gallinae* prevalence in medicated nestlings between the first and second visits (Figure 5.6). Although a trend of medicated Turtle Dove nestlings being more likely to be alive at the end of the 30 day monitoring period was hinted at, the effect of the medication needs to be established before further trials investigating this trend takes place. At the moment, this potential trend appears more likely to be due to chance.

This study has highlighted some interesting patterns regarding the Type A *T. gallinae* strain, which is associated with virulence, which would be worth exploring in future studies. Firstly, it appears to exhibit temporal variation in prevalence in the UK bird community and the continued annual screening of these populations, in addition to wider geographic sampling, will establish whether this trend is cyclical and allow the investigation of potential drivers. This would have important implications for managing future trichomonosis outbreaks and shed light on the selective pressures being experienced by this strain. Infected birds sampled on farms that provided supplementary feeding were more likely to be infected by this Type A strain, which suggests that supplementary feeding is playing an important role in the transmission of this strain. This raises the question of whether the Type A strain has a competitive advantage over other strains in environmental transmission. Although other strains were also detected in shared food resources, they are not necessarily effectively transmitted to subsequent hosts. Conducting *T. gallinae* detection trials on grain under

laboratory conditions whereby the survival of different strains is investigated would clarify whether the Type A strain is more suited to survival in the environment. Transmission experiments with birds fed single strain infected grain piles and mixed strain infected grain piles would allow the strains' ability to be transmitted from a grain pile to be compared, in addition to confirming whether the Type A strain outcompetes the other strains. It is also important to establish the role that the pseudocyst plays in the life cycle of *T. gallinae* and the implications this has for environmental transmission. Conducting environmental sampling nationwide in addition to sampling the bird populations local to the environmental resource would reveal the extent to which strains in the environment are correlated with strain detected in birds feeding at those sites, whether contamination of the food source is more likely from the wild birds or gamebirds and if there is an association between prevalence/diversity of *T. gallinae* in relation to bird diversity at a site. This information would help develop epidemiological models and further understand the dynamics of *T. gallinae*.

Lastly, although the Type A strain is associated with virulence, there were limited observations of the Type A strain causing trichomonosis during the period of fieldwork conducted for this study, although there was a high mortality rate in 2012 (Stockdale et al., 2015). Assessing what factors drive the virulence of this strain would improve our understanding of the dynamics of disease and evaluate the risk of future epidemics. This would be quite a challenge as numerous factors need to be accounted for, including: previous history of *T. gallinae* infection, within host community of parasites, potential environmental stressors such as stage of the life cycle i.e. breeding, habitat availability, food/ foraging habitat availability and predation pressure. Furthermore, there could be genetic changes driving virulence which would be uncovered by comparative analysis of *T. gallinae* genomes.

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Appendix

Table 7.1: Abbreviations used in Table 7.4. Locations of farms detailed in methods of Chapter 2 and Chapter 3 along with maps (Figure 2.1 & Figure 3.1)

Farm		Source	
Abbreviations	Name	Abbreviations	Name
AH	Abbotts Hall	BP	Bait piles
CF	Cobbs Farm	FP	Farm plots
F	Flambirds	H20 tray	Water tray
GC	Golf Course	H20C	Control water tray
HL	Hobbs Lot	TP	Trial plot
LI	Limesbrook	H20	Water source (natural)
MF	Manor Farm	GF	Gamebird feeder
OB	Ouse Bridge		
OP	Orwell Pit		
PG	Perry Green		
SM	Sunnymead		
UH	Upp Hall		

Table 7.2: Table listing all of *T. gallinae* samples analysed for thesis. Locations: Fr.=France,Sen=Senegal, B.Faso=Burkina Faso, Host: NT=nestling, DNA extraction: ext.=extraction, AA=ammonium acetate method, PCR=result from up to three repeats (see Chapter 2 methods for details), Fe-hyd: un-ID=unidentified as no match to reference database (see Chapter 2 methods for details). Sequences not yet submitted to GenBank.

Date	Location	Host	Host ID	Sample ID	Culture quality	DNA ext.	PCR	ITS	Fe-hyd
07/05/2013	UK	Woodpigeon NT	FH44752	A01	Swollen pouch, liquid cloudy	Qiagen	-	NA	NA
07/05/2013	UK	Woodpigeon NT	FH44753	A02	Pouch not swollen, liquid clear	Qiagen	-	NA	NA
17/05/2013	UK	Carrion Crow	FH78601	A03	Pouch not swollen, liquid clear	Qiagen	-	NA	NA
21/05/2013	UK	Turtle Dove	EG59102	A04	Swollen pouch, liquid slightly cloudy	Qiagen	+	Type C	NA
21/05/2013	UK	Turtle Dove	EG59103	A05	Slightly swollen pouch, liquid clear	Qiagen	+	Type C	NA
21/05/2013	UK	Woodpigeon	EH55310	A06	Slightly swollen pouch, liquid clear	Qiagen	-	NA	NA
22/05/2013	UK	Woodpigeon	FH44761	A07	Slightly swollen pouch, liquid clear	Qiagen	+	Type C	NA
22/05/2013	UK	Woodpigeon	FH44762	A08	Swollen pouch, liquid clear	Qiagen	-	NA	NA
22/05/2013	UK	Woodpigeon	FH44763	A09	Slightly swollen pouch, liquid clear	Qiagen	+	Type C	NA
22/05/2013	UK	Jackdaw	EG59104	A10	Swollen pouch, liquid clear	Qiagen	-	NA	NA
22/05/2013	UK	Turtle Dove	EG59105	A11	Swollen pouch, liquid clear	Qiagen	+	NA	NA
22/05/2013	UK	Turtle Dove	EG59106	A12	Slightly swollen pouch, liquid clear	Qiagen	+	GEO	NA
23/05/2013	UK	Woodpigeon	FH44754	A13	Slightly swollen pouch, liquid clear	Qiagen	+	NA	NA
23/05/2013	UK	Turtle Dove	EG59107	A14	Swollen pouch, liquid slightly cloudy	Qiagen	+	GEO	NA
23/05/2013	UK	Turtle Dove	EG59108	A15	Swollen pouch, liquid cloudy	Qiagen	+	Type	NA

								C	
23/05/2013	UK	Jackdaw	EG59109	A16	Slightly swollen pouch, liquid clear	Qiagen	+	NA	NA
								Type	
23/06/2013	UK	Turtle Dove	EG59110	A17	Swollen pouch, liquid slightly cloudy	Qiagen	+	C	NA
					Slightly swollen pouch, liquid slightly				
28/05/2013	UK	Turtle Dove	EG59051	A18	cloudy	Qiagen	+	GEO	NA
29/05/2013	UK	Collared Dove	EG59052	A19	Swollen pouch, liquid slightly cloudy	Qiagen	+	NA	NA
					Slightly swollen pouch, liquid slightly			Type	
29/05/2013	UK	Turtle Dove	EG59111	A20	cloudy	Qiagen	+	C	NA
					Slightly swollen pouch, liquid slightly				
29/05/2013	UK	Woodpigeon	FH44755	A21	cloudy	Qiagen	-	NA	NA
30/05/2013	UK	Woodpigeon	FH44756	A22	Slightly swollen pouch, liquid clear	Qiagen	-	NA	NA
								Type	
03/06/2013	UK	Turtle Dove NT	EG82565	A23	Swollen pouch, liquid slightly cloudy	Qiagen	+	C	NA
					Slightly swollen pouch, liquid slightly				
03/06/2013	UK	Stock Dove	EG82567	A24	cloudy	Qiagen	+	Tcl-1	NA
		Red Legged			Slightly swollen pouch, liquid slightly				
03/06/2013	UK	Partridge	RLP	A25	cloudy	Qiagen	+	Tcl-1	NA
								Type	
03/06/2013	UK	Turtle Dove NT	EG82566	A26	Swollen pouch, liquid slightly cloudy	Qiagen	+	C	NA
								Type	
04/06/2013	UK	Woodpigeon	FH44757	A27	Swollen pouch, liquid slightly cloudy	Qiagen	+	C	NA
					Pouch not swollen, liquid slightly				
05/06/2013	UK	Woodpigeon	FH44758	A28	cloudy	Qiagen	+	Tcl-1	NA
					Pouch slightly swollen, liquid slightly				
05/06/2013	UK	Woodpigeon	FH44759	A29	cloudy	Qiagen	+	Tcl-1	NA

05/06/2013	UK	Stock Dove	EG59112	A30	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
05/06/2013	UK	UH/ PS 1		A32	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
05/06/2013	UK	UH/ H20 1		A33	Pouch not swollen, liquid not cloudy	Qiagen	-	NA	NA
05/06/2013	UK	LI/ GF 1		A34	Pouch slightly swollen, liquid not cloudy	Qiagen	+	NA	NA
05/06/2013	UK	LI/ BP 1		A35	Pouch slightly swollen, liquid clear	Qiagen	+	Tcl-1	NA
05/06/2013	UK	LI/ TP 1		A36	Pouch slightly swollen, liquid clear	Qiagen	-	NA	NA
05/06/2013	UK	LI/ GGF 1		A37	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
05/06/2013	UK	LI/ GGF 2		A38	Pouch swollen, liquid slightly cloudy	Qiagen	-	NA	NA
05/06/2013	UK	LI/ GF 2		A39	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
05/06/2013	UK	LI/ GF 3		A40	Pouch not swollen, liquid clear	Qiagen	+	Tcl-1	NA
05/06/2013	UK	LI/ GGF 3		A41	Pouch not swollen, liquid clear	Qiagen	-	NA	NA
05/06/2013	UK	LI/ H20 1		A42	Pouch not swollen, liquid clear	Qiagen	+	NA	NA
06/06/2013	UK	UH/ BP 1		A43	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
06/06/2013	UK	UH/ FP 1		A44	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
06/06/2013	UK	UH/ H20 1		A45	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
06/06/2013	UK	F/ BP 1		A46	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
06/06/2013	UK	F/ BP 2		A47	Pouch slightly swollen, liquid slightly	Qiagen	+	Tcl-1	NA

				cloudy				
06/06/2013	UK	F/ TP 1	A48	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
06/06/2013	UK	F/ H20 1	A49	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
06/06/2013	UK	AH/ BP 1	A50	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
06/06/2013	UK	AH/ FP 1	A51	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
08/06/2013	UK	Collared Dove	DEAD	A52	Pouch swollen, liquid cloudy	Qiagen	+	Type C	NA
10/06/2013	UK	Woodpigeon NT	FH48244	A53	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
10/06/2013	UK	Woodpigeon NT	FH48245	A54	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
11/06/2013	UK	Rook	Rook 1	A55	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
11/06/2013	UK	Rook	Rook 2	A56	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
11/06/2013	UK	Rook	Rook 3	A57	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
11/06/2013	UK	Rook	Rook 4	A58	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
11/06/2013	UK	Carrion Crow		A59	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
11/06/2013	UK	Carrion Crow		A60	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
11/06/2013	UK	Magpie		A61	Pouch slightly swollen, liquid clear	Qiagen	+	Tcl-1	NA
13/06/2013	UK	Turtle Dove	EG59113	A62	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
17/06/2013	UK	MF/ BP 2		A63	Pouch swollen, liquid cloudy	Qiagen	-	NA	NA
17/06/2013	UK	BP (N)		A64	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
17/06/2013	UK	MF/ BP 1		A65	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA

17/06/2013	UK	MF/ H20 1		A66	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
17/06/2013	UK	OP/ H20 2		A67	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
17/06/2013	UK	HL/ BP 1		A68	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
17/06/2013	UK	OP/ H20 1		A69	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
17/06/2013	UK	MF/ TP 1		A70	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
18/06/2013	UK	OB/ BP 1		A71	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
18/06/2013	UK	OB/ BP 2		A72	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
18/06/2013	UK	Turtle Dove	EG59114	A73	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Type C	NA
19/06/2013	UK	Turtle Dove	EG59115	A74	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
20/06/2013	UK	Turtle Dove	EG59119	A75	Pouch very swollen, liquid very cloudy	Qiagen	+	GEO	NA
20/06/2013	UK	Stock Dove	EG59117	A76	Pouch swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
20/06/2013	UK	Turtle Dove	EG59118	A77	Pouch very swollen, liquid very cloudy	Qiagen	+	GEO	NA
20/06/2013	UK	Turtle Dove	EG59116	A78	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
24/06/2013	UK	Turtle Dove NT	EG82569	A79	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
24/06/2013	UK	Turtle Dove NT	EG82570	A80	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
24/06/2013	UK	Turtle Dove NT	EG82568	A81	Pouch very swollen, liquid cloudy	Qiagen	+	GEO	NA
25/06/2013	UK	Feral Pigeon	FP1303	A82	Pouch swollen, liquid cloudy	Qiagen	+	Type A	NA

25/06/2013	UK	Blackbird	LB70806	A91	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Feral Pigeon	FP1304	A84	Pouch swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Turtle Dove	EG82571	A85	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
25/06/2013	UK	Feral Pigeon	FP1301	A86	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Pied Wagtail	X112922	A87	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	LI/ BP 1		A88	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
25/06/2013	UK	LI/ GF 3		A89	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	LI/ TP 1		A90	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
25/06/2013	UK	Blackbird	LB70806	A83	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
25/06/2013	UK	Chiffchaff	X112917	A92	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Feral Pigeon	FP1302	A93	Pocuh swollen, liquid slightly cloudy	Qiagen	-	NA	NA
25/06/2013	UK	Blackbird	LB70808	A94	Pouch slightly swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Chaffinch	X112918	A95	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	Robin	X112920	A96	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
25/06/2013	UK	LI/ GGF 3	N/A	A97	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA

25/06/2013	UK	Turtle Dove	EG59120	A98	Pouch slightly swollen,liquid slightly cloudy	Qiagen	+	GEO	NA
25/06/2013	UK	Robin	X112919	A99	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
26/06/2013	UK	AH/ FP 1		A100	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
26/06/2013	UK	AH/ BP 1		B01	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
26/06/2013	UK	Collared Dove	EG82572	B02	Pouch not swollen, liquid not cloudy	Qiagen	+	Tcl-1	NA
26/06/2013	UK	UH/ GF 1		B03	Pouch swollen, liquid cloudy	Qiagen	-	NA	NA
26/06/2013	UK	Dunnock	X112924	B04	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
26/06/2013	UK	UH/ PS 1		B05	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
26/06/2013	UK	UH/ BP 1		B06	Pouch swollen, liquid cloudy	Qiagen	+	NA	NA
26/06/2013	UK	Starling	LB70819	B07	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
27/06/2013	UK	Stock Dove	EG59122	B08	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
27/06/2013	UK	Stock Dove	EG59125	B09	Pouch slighlt swollen, liquid slightly cloudy	Qiagen	+	NA	NA
27/06/2013	UK	Stock Dove	EG59123	B10	Pouch slightly swollen liquid slighlt cloudy	Qiagen	-	NA	NA
27/06/2013	UK	Stock Dove	EG59121	B11	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
27/06/2013	UK	Stock Dove	EG59124	B12	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
28/06/2013	UK	Stock Dove	EG59126	B13	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
01/07/2013	UK	Turtle Dove	EG82573	B14	Pouch not swollen, liquid slightly	Qiagen	+	GEO	NA

					cloudy				
01/07/2013	UK	Turtle Dove	EG82574	B15	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
03/07/2013	UK	GC/ BP 1		B16	Pouch very swollen, liquid very cloudy	Qiagen	-	NA	NA
04/07/2013	UK	AH/ FP 1		B17	Pouch very swollen, liquid cloudy	Qiagen	-	NA	NA
04/07/2013	UK	LI/ TP 1		B18	Pouch very swollen, liquid cloudy	Qiagen	-	NA	NA
04/07/2013	UK	Turtle Dove NT	EG82575	B19	Pouch very swollen, liquid cloudy	Qiagen	+	Type C	NA
04/07/2013	UK	Turtle Dove NT	EG82576	B20	Pouch very swollen, liquid cloudy	Qiagen	+	Type C	NA
03/07/2013	UK	MF/ BP 1		B21	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
03/07/2013	UK	OB/ BP 2		B22	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
03/07/2013	UK	MF/ BP 2		B23	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
03/07/2013	UK	MF/ TP 1		B24	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
03/07/2013	UK	HL/ BP 1		B25	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
03/07/2013	UK	OB/ BP 1		B26	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
04/07/2013	UK	F/ BP 2		B28	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
04/07/2013	UK	F/ TP 1		B30	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
04/07/2013	UK	LI/ BP 1		B31	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA

04/07/2013	UK	AH/ BP 1		B32	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
04/07/2013	UK	UH/ GF 1		B33	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
04/07/2013	UK	LI/ GF 3		B34	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
04/07/2013	UK	UH/ BP 1		B35	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
08/07/2013	UK	Turtle Dove	EG82577	B36	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
09/07/2013	UK	OB/ BP 2		B37	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
09/07/2013	UK	OB/ BP 1		B38	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
10/07/2013	UK	Stock Dove	EG59133	B39	Pouch v.v. swollen, liquid v. cloudy	Qiagen	+	Type A	NA
10/07/2013	UK	HL/ BP 1		B40	Pouch v.v. swollen, liquid v. cloudy	Qiagen	-	NA	NA
10/07/2013	UK	MF/ TP 1		B41	Pouch v.v. swollen, liquid v. cloudy	Qiagen	-	NA	NA
10/07/2013	UK	Stock Dove	EG59129	B42	Pouch v. swollen, liquid v. cloudy	Qiagen	+	Type A	NA
15/07/2013	UK	Turtle Dove NT	EG59134	B43	Pouch v. swollen, liquid v. cloudy	Qiagen	+	Type C	NA
15/07/2013	UK	Turtle Dove NT	DEAD	B44	Pouch v. swollen, liquid v. cloudy	Qiagen	+	Type C	NA
10/07/2013	UK	Turtle Dove	EG59131	B45	Pouch v. swollen, liquid v. cloudy	Qiagen	+	Type A	NA
10/07/2013	UK	OP/ H20 2		B46	Pouch not swollen, liquid cloudy	Qiagen	-	NA	NA
10/07/2013	UK	MF/ BP 1		B47	Pouch not swollen, liquid slightly	Qiagen	-	NA	NA

					cloudy				
10/07/2013	UK	GC/ BP 1		B48	Pouch swollen, liquid slightly cloudy	Qiagen	+	NA	NA
10/07/2013	UK	Stock Dove	EG59128	B49	Pouch swollen, liquid cloudy	Qiagen	+	NA	NA
10/07/2013	UK	Stock Dove	EG59132	B50	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
10/07/2013	UK	Turtle Dove	EG59127	B51	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
10/07/2013	UK	Stock Dove	EG59130	B52	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
10/07/2013	UK	MF/ BP 2		B53	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
11/07/2013	UK	UH/ GF 1		B54	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
11/07/2013	UK	Woodpigeon NT		B55	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
11/07/2013	UK	UH/ PS 1		B56	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
11/07/2013	UK	LI/ GF 3		B57	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
11/07/2013	UK	UH/ BP 1		B58	Pouch not swollen, liquid not cloudy	Qiagen	+	NA	NA
11/07/2013	UK	LI/ BP 1		B59	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
11/07/2013	UK	Woodpigeon NT	FH44760	B60	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
11/07/2013	UK	F/ BP 2		B63	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA

12/07/2013	UK	AH/ FP 1		B64	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
12/07/2013	UK	Woodpigeon NT	FH44760	B65	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
12/07/2013	UK	AH/ BP 1		B66	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
12/07/2013	UK	Woodpigeon NT		B67	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
16/07/2013	UK	Turtle Dove NT	EG59136	B68	Pouch swollen, liquid very cloudy	Qiagen	+	GEO	NA
16/07/2013	UK	Turtle Dove NT	EG59135	B69	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
17/07/2013	UK	HL/ BP 1		B70	Pouch v.v. swollen, liquid cloudy	Qiagen	-	NA	NA
17/07/2013	UK	MF/ BP 2		B71	Pouch swollen, liquid cloudy	Qiagen	-	NA	NA
17/07/2013	UK	OB/ BP 1		B72	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
17/07/2013	UK	MF/ TP 1		B73	Pouch v.v. swollen, liquid cloudy	Qiagen	-	NA	NA
17/07/2013	UK	MF/ BP 1		B74	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
17/07/2013	UK	HL/ FP 1		B76	Pouch v.v. swollen, liquid v. cloudy	Qiagen	-	NA	NA
18/07/2013	UK	LI/ BP 1		B77	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
18/07/2013	UK	LI/ GF 3		B78	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
18/07/2013	UK	AH/ BP 1		B79	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
18/07/2013	UK	AH/ FP 1		B80	Pouch slightly swollen, liquid slightly	Qiagen	-	NA	NA

					cloudy				
19/07/2013	UK	UH/ BP 1		B83	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
19/07/2013	UK	UH/ PS 1		B84	Pouch not swollen, liquid not cloudy	Qiagen	+	NA	NA
19/07/2013	UK	UH/ GGF 1		B85	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
19/07/2013	UK	UH/ H20 2		B86	Pouch v.v. swollen, liquid cloudy	Qiagen	-	NA	NA
19/07/2013	UK	UH/ GF 1		B87	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
19/07/2013	UK	F/ BP 2		B88	Pouch not swollen, liquid slightly cloudy	Qiagen	+	GEO	NA
23/07/2013	UK	HL/ BP 1		B89	Pouch not swollen, liquid slightly	Qiagen	-	NA	NA
23/07/2013	UK	Turtle Dove	EG82581	B90	Pouch swollen, liquid cloudy	Qiagen	+	Tcl-1	NA
21/07/2013	UK	Turtle Dove NT	EG59137	B91	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
21/07/2013	UK	Turtle Dove NT	EG59138	B92	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Tcl-1	NA
24/07/2013	UK	OB/ BP 2		B96	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
24/07/2013	UK	MF/ BP 2		B97	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
24/07/2013	UK	OB/ BP 1		B98	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
24/07/2013	UK	MF/ BP 1		B99	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
24/07/2013	UK	MF/ TP 1		B100	Pouch slightly swollen, liquid cloudy	Qiagen	-	NA	NA
25/07/2013	UK	UH/ PS 1		C1	Pouch v v swollen, liquid cloudy	Qiagen	-	NA	NA
25/07/2013	UK	UH/ BP 1		C2	Pouch slightly swollen, liquid very	Qiagen	+	NA	NA

					cloudy				
25/07/2013	UK	F/ BP 2		C3	Pouch not swollen, liquid cloudy	Qiagen	+	NA	NA
25/07/2013	UK	UH/ GF 1		C4	Pouch not swollen, liquid cloudy	Qiagen	-	NA	NA
25/07/2013	UK	UH/ GGF 1		C6	Pouch v v swollen, liquid black	Qiagen	-	NA	NA
25/07/2013	UK	Collared Dove NT	EG59140	C9	Pouch slightly swollen, liquid cloudy	Qiagen	+	NA	NA
25/07/2013	UK	Collared Dove NT	EG59139	C10	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
25/07/2013	UK	AH/ BP 1		C11	Pouch not swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
25/07/2013	UK	AH/ FP 1		C12	Pouch slightly swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
26/07/2013	UK	Turtle Dove NT	EG59141	C14	Pouch swollen, liquid v cloudy	Qiagen	-	NA	NA
26/07/2013	UK	Turtle Dove NT	EG59142	C15	Pouch swollen, liquid v cloudy	Qiagen	+	Type C	NA
26/07/2013	UK	LI/ BP 1		C16	Pouch not swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
26/07/2013	UK	LI/ GF 3		C17	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
29/07/2013	UK	Turtle Dove NT	DEAD	C18	Pouch v swollen, liquid v cloudy	Qiagen	+	Type C	NA
10/07/2013	UK	MF/ H20 1		C19	N/A	Qiagen	inconclusive	NA	NA
18/07/2013	UK	LI/ H20 3		C20	N/A	Qiagen	inconclusive	NA	NA
18/07/2013	UK	LI/ H20 4		C21	N/A	Qiagen	inconclusive	NA	NA
29/07/2013	UK	Turtle Dove NT	EG82583	C22	Pouch not swollen, liquid not cloudy	Qiagen	+	Tcl-1	NA
29/07/2013	UK	Turtle Dove NT	DEAD	C23	Pouch not swollen, liquid very slightly cloudy	Qiagen	+	Type C	NA

29/07/2013	UK	Turtle Dove NT	EG82582	C24	Pouch not swollen, liquid not cloudy	Qiagen	+	Type C	NA
30/07/2013	UK	Turtle Dove NT		C25	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
05/08/2013	UK	Turtle Dove NT	EG59143	C26	Pouch swollen, liquid cloudy	Qiagen	+	Type A	NA
05/08/2013	UK	Turtle Dove NT	DEAD	C27	Pouch not swollen, liquid slightly cloudy	Qiagen	+	Type C	NA
08/08/2013	UK	Turtle Dove NT	EG82584	C32	Pouch not swollen, liquid not cloudy	Qiagen	+	NA	NA
24/07/2013??	UK	OP/ H20 2		C34	N/A	Qiagen	inconclusive	NA	NA
24/07/2013	UK	MF/ H20 1		C35	N/A	Qiagen	inconclusive	NA	NA
16/08/2013	UK	Woodpigeon NT	DEAD	C37	Pouch not swollen, liquid slightly cloudy	Qiagen	-	NA	NA
19/08/2013	UK	Turtle Dove NT		C39	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
19/08/2013	UK	Turtle Dove NT		C40	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
20/08/2013	UK	Turtle Dove NT	EG59145	C41	Pouch not swollen, liquid not cloudy	Qiagen	+	GEO	NA
20/08/2013	UK	Turtle Dove NT	EG59144	C42	Pouch not swollen, liquid not cloudy	Qiagen	+	GEO	NA
21/08/2013	UK	Turtle Dove NT	DEAD	C43	Pouch not swollen, liquid not cloudy	Qiagen	+	GEO	NA
21/08/2013	UK	Turtle Dove NT	EG59146	C44	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
21/08/2013	UK	Turtle Dove NT	EG59147	C45	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
22/08/2013	UK	Bluetit	DO98427	C49	Pouch not swollen, liquid not cloudy	Qiagen	-	NA	NA
22/08/2013	UK	Great Tit	DO98425	C50	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
22/08/2013	UK	Great Tit	DO98426	C51	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
22/08/2013	UK	Great Tit	DO98424	C52	Pouch not swollen, liquid slightly	Qiagen	-	NA	NA

					cloudy				
26/08/2013	UK	Great Tit	DO98428	C55	Pouch not swollen, liquid not cloudy	Qiagen	-	NA	NA
28/08/2013	UK	Turtle Dove NT	EG59148	C56	Pouch slightly swollen, liquid not cloudy	Qiagen	+	GEO	NA
28/08/2013	UK	Turtle Dove NT	EG59149	C57	Pouch slightly swollen, liquid not cloudy	Qiagen	+	GEO	NA
04/09/2013	UK	Turtle Dove NT	EG82585	C58	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
04/09/2013	UK	Turtle Dove NT	EG59150	C59	Pouch swollen, liquid cloudy	Qiagen	+	GEO	NA
25/07/2013	UK	F/ H20 1		C60	N/A	Qiagen	inconclusive	NA	NA
25/07/2013	UK	UH/ H20 2		C61	N/A	Qiagen	inconclusive	NA	NA
25/07/2013	UK	UH/ H20 1		C62	N/A	Qiagen	inconclusive	NA	NA
26/07/2013	UK	LI/ H20 4		C65	N/A	Qiagen	inconclusive	NA	NA
26/07/2013	UK	LI/ H20 3		C66	N/A	Qiagen	inconclusive	NA	NA
26/07/2013	UK	LI/ H20 2		C67	N/A	Qiagen	inconclusive	NA	NA
03/07/2013	UK	MF/ H20 1		C69	N/A	Qiagen	inconclusive	NA	NA
03/07/2013	UK	OP/ H20 2		C70	N/A	Qiagen	inconclusive	NA	NA
04/07/2013	UK	UH/ H20 1		C71	N/A	Qiagen	inconclusive	NA	NA
04/07/2013	UK	F/ H20 1		C72	N/A	Qiagen	inconclusive	NA	NA
11/07/2013	UK	F/ H20 1		C73	N/A	Qiagen	inconclusive	NA	NA
11/07/2013	UK	LI/ H20 2		C74	N/A	Qiagen	inconclusive	NA	NA
11/07/2013	UK	UH/ H20 2		C75	N/A	Qiagen	inconclusive	NA	NA
17/07/2013	UK	HL/ H20 1		C76	N/A	Qiagen	inconclusive	NA	NA
17/07/2013	UK	MF/ H20 1		C77	N/A	Qiagen	inconclusive	NA	NA
18/07/2013	UK	LI/ H20 2		C79	N/A	Qiagen	-	NA	NA

19/07/2013	UK	F/ H20 1		C80	N/A	Qiagen	inconclusive	NA	NA
02/09/2013	UK	Greenfinch	TL08129	C90	Pouch slightly swollen, liquid slightly cloudy	Qiagen	-	NA	NA
02/09/2013	UK	Robin	DO98435	C91	Pouch not swollen, liquid not cloudy	Qiagen	+	NA	NA
03/09/2013	UK	Goldfinch	DO98469	C92	Pouch very swollen, liquid very cloudy	Qiagen	+	NA	NA
03/09/2013	UK	Bluetit	DO98447	C93	Pouch slightly swollen, liquid slightly cloudy	Qiagen	+	NA	NA
03/09/2013	UK	Great Tit	DO98456	C94	Pouch not swollen, liquid not cloudy	Qiagen	+	NA	NA
03/09/2013	UK	Great Tit	TL08124	C95	Pouch not swollen, liquid slightly cloudy	Qiagen	+	NA	NA
03/09/2013	UK	Bluetit	DO98463	C96	Pouch slightly swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Chaffinch	DO98453	C97	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Dunnock	DO98451	C98	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Goldfinch	DO98436	C99	Pouch not swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98458	C100	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98465	D1	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Dunnock	DO98446	D2	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	LO60695	D3	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Dunnock	DO98449	D4	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98468	D5	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Woodpigeon	FH78602	D6	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	DO98464	D7	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98467	D8	Pouch slightly swollen, liquid slightly	Qiagen	inconclusive	NA	NA

					cloudy				
03/09/2013	UK	Dunnock	DO98450	D9	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	DO98445	D10	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	TL08130	D11	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Chaffinch		D12	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	DO98437	D13	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Dunnock	DO98452	D14	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98466	D15	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	D098443	D16	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	DO98455	D17	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Great Tit	TL08126	D18	Pouch not swollen, liquid not cloudy	Qiagen	inconclusive	NA	NA
03/09/2013	UK	Bluetit	DO98448	D19	Pouch slightly swollen, liquid slightly cloudy	Qiagen	inconclusive	NA	NA
28/02/2014	Sen.	Namaqua Dove	?	E01 *	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Namaqua Dove	RL63604	E02*	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
24/02/2014	Sen.	Namaqua Dove	RL63601	E03	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
24/02/2014	Sen.	Namaqua Dove	?	E04	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
24/02/2014	Sen.	Namaqua Dove	?	E05	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Laughing Dove	DE24971	E06	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Namaqua Dove	?	E07	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA

25/02/2014	Sen.	Namaqua Dove	RL63604	E08	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Namaqua Dove	RL63605	E09	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Laughing Dove	DE84972	E10	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Laughing Dove	DE84973	E11	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C9
25/02/2014	Sen.	Laughing Dove	DE84974	E12	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
25/02/2014	Sen.	Turtle Dove	EY79803	E13	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
25/02/2014	Sen.	Turtle Dove	EY79801	E14	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
26/02/2014	Sen.	Namaqua Dove	RL63606	E15	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
26/02/2014	Sen.	Black Billed Wood Dove	RL63608	E16	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
26/02/2014	Sen.	Namaqua Dove	RL63607	E17	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Laughing Dove	DE84975	E18	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	NA
27/02/2014	Sen.	Black Billed Wood Dove	LH80101	E19	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-BBWD	NA
27/02/2014	Sen.	Laughing Dove	DE84975	E20	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO-TD	un-ID
27/02/2014	Sen.	Turtle Dove	EY79803	E21	Pouch swollen, liquid cloudy	AA	+	Type C	C8
27/02/2014	Sen.	Turtle Dove	EY79804	E22	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-1	T1-TD
27/02/2014	Sen.	Black Billed Wood Dove	LH80102	E23	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA

27/02/2014	Sen.	Namaqua Dove	RL63609	E24	Pouch swollen, liquid cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove	RL63611	E25	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove	RL63612	E26	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove	RL63613	E27	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Turtle Dove	EY79806	E28	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove	RL63614	E29	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
27/02/2014	Sen.	Turtle Dove	EY79806	E30	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
27/02/2014	Sen.	Black Billed Wood Dove	LH80103	E31	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-BBWD	NA
27/02/2014	Sen.	Laughing Dove	?	E32	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove	RL63610	E33	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
27/02/2014	Sen.	Namaqua Dove?	?	E34	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C10
28/02/2014	Sen.	Black Billed Wood Dove	LH80105	E35	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Black Billed Wood Dove	LH80104	E36	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Laughing Dove	DE84982	E37	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/02/2014	Sen.	Namaqua Dove	RL63619	E38	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA

28/02/2014	Sen.	Laughing Dove	DE84980	E39	Pouch swollen, liquid cloudy	AA	+	Type C	C9
28/02/2014	Sen.	Namaqua Dove	RL63618	E40	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Namaqua Dove	RL63616	E41	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Laughing Dove	DE84979	E42	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Black Billed Wood Dove	LH80106	E43	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-BBWD	OTU8
28/02/2014	Sen.	Black Billed Wood Dove	LH80109	E44	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-BBWD	NA
28/02/2014	Sen.	Black Billed Wood Dove	?	E45	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
28/02/2014	Sen.	Namaqua Dove	RL63617	E46	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
28/02/2014	Sen.	Laughing Dove	DE84981	E47	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C10
28/02/2014	Sen.	Black Billed Wood Dove	LH80110	E48	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Black Billed Wood Dove	LH80108	E49	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
28/02/2014	Sen.	Namaqua Dove	RL63620	E50	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Namaqua Dove	RL63621	E51	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
28/02/2014	Sen.	Namaqua Dove	RL63622	E52	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA

28/02/2014	Sen.	Namaqua Dove	RL63623	E53	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
28/02/2014	Sen.	Turtle Dove	EY79807	E54	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/02/2014	Sen.	Vinaceous Dove	DE84983	E55	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C9
01/03/2014	Sen.	Black Billed Wood Dove	LH80111	E56	Pouch swollen, liquid cloudy	AA	+	Tcl-BBWD	NA
01/03/2014	Sen.	Namaqua Dove	RL63626	E57	Pouch swollen, liquid cloudy	AA	+	GEO	NA
01/03/2014	Sen.	Namaqua Dove	RL63625	E58	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
01/03/2014	Sen.	Namaqua Dove	RL63624	E59	Pouch slightly swollen, liquid slightly cloudy	AA	+	Sen-NQD	un-ID
01/03/2014	Sen.	Turtle Dove	EY79808	E60	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
01/03/2014	Sen.	Laughing Dove	DE84984	E61	Pouch swollen, liquid cloudy	AA	+	Type c	C9
01/03/2014	Sen.	Namaqua Dove	RL63627	E62	Pouch swollen, liquid cloudy	AA	+	GEO	un-ID
01/03/2014	Sen.	Black Billed Wood Dove	LH80113	E63	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-BBWD	OTU8
01/03/2014	Sen.	Namaqua Dove	RL63628	E64	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO-NQD	un-ID
01/03/2014	Sen.	Black Billed Wood Dove	LH80112	E65	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type A	OTU12
01/03/2014	Sen.	Namaqua Dove	RL63629	E66	Pouch swollen, liquid cloudy	AA	+	GEO	NA
01/03/2014	Sen.	Namaqua Dove	RL63630	E67	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
01/03/2014	Sen.	Namaqua Dove	RL63631	E68	Pouch swollen, liquid cloudy	AA	+	Type	NA

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01/03/2014	Sen.	Laughing Dove	DE84985	E69	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
01/03/2014	Sen.	Black Billed Wood Dove	LH80114	E70	Pouch swollen, liquid cloudy	AA	+	Tcl-BBWD	NA
01/03/2014	Sen.	Laughing Dove	DE84983	E71	Pouch not swollen, liquid not cloudy	AA	+	GEO-LD & Tcl-LD	NA
01/03/2014	Sen.	Laughing Dove	DE84988	E72	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/03/2014	Sen.	Laughing Dove	DE84991	E73	Pouch not swollen, liquid not cloudy	AA	+	GEO-TD	un-ID
02/03/2014	Sen.	Turtle Dove	EY79809	E74	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/03/2014	Sen.	Namaqua Dove	RL63632	E75	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	NA
02/03/2014	Sen.	Laughing Dove	DE84989	E76	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/03/2014	Sen.	Laughing Dove	DE84992	E77	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	OTU13
02/03/2014	Sen.	Laughing Dove	DE84993	E78	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO	un-ID
02/03/2014	Sen.	Laughing Dove	DE84994	E79	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/03/2014	Sen.	Laughing Dove	DE84995	E80	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/03/2014	Sen.	Laughing Dove	DE84990	E81	Pouch not swollen, liquid not cloudy	AA	+	Type C	C9
02/03/2014	Sen.	Laughing Dove	DE84996	E82	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
03/03/2014	Sen.	Laughing Dove	DE84986	E83	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
03/03/2014	Sen.	Laughing Dove	Unringed	E84	Pouch slightly swollen, liquid not cloudy	AA	+	Type C	C6

03/03/2014	Sen.	Laughing Dove	DE84997	E85	Pouch not swollen, liquid not cloudy	AA	+	Type C	C6
03/03/2014	Sen.	Laughing Dove	DE84998	E86	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
03/03/2014	Sen.	Laughing Dove	DE84999	E87	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C9
03/03/2014	Sen.	Turtle Dove	EY79811	E88	Pouch not swollen, liquid not cloudy	AA	+	Tcl-1	T2-TD
03/03/2014	Sen.	Turtle Dove	EY79810	E89	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
03/03/2014	Sen.	Laughing Dove	DE85000	E90	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	un-ID
27/05/2014	Fr.	Turtle Dove	GY103608	Fr 01	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
27/05/2014	Fr.	Woodpigeon	EA701885	Fr 02	Pouch swollen, liquid slightly cloudy	AA	+	Type C	C4
27/05/2014	Fr.	Turtle Dove	GY103636	Fr 03	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY69429	Fr 04	Pouch swollen, liquid cloudy	AA	+	Type C	C8
27/05/2014	Fr.	Turtle Dove	GY103643	Fr 05	Pouch not swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
27/05/2014	Fr.	Turtle Dove	GY69594	Fr 06	Trapped air when sealed, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Collared Dove	FA48474	Fr 07	Pouch swollen, liquid cloudy	AA	+	Type C	C6
26/05/2014	Fr.	Turtle Dove	Captive	Fr 08	Pouch swollen, liquid cloudy	AA	+	Type C	NA
27/05/2014	Fr.	Turtle Dove	GY120904	Fr 09	N/A	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY69820	Fr 10	Pouch not swollen, liquid not cloudy	AA	+	NA	NA

27/05/2014	Fr.	Turtle Dove	GY103641	Fr 11	Pouch slightly swollen, liquid not cloudy	AA	+	Type A	A1
27/05/2014	Fr.	Turtle Dove	GY103640	Fr 12	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY103637	Fr 13	Pouch not swollen, liquid slightly cloudy	AA	+	Type III	un-ID
27/05/2014	Fr.	Turtle Dove	GY103638	Fr 14	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY103642	Fr 15	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY103639	Fr 16	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
27/05/2014	Fr.	Woodpigeon	EA701884	Fr 17	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
27/05/2014	Fr.	Turtle Dove	GY69316	Fr 18	Pouch swollen, liquid cloudy	AA	+	Type C	C8
28/05/2014	Fr.	Turtle Dove	GY89757	Fr 19	Pouch slightly swollen, liquid not cloudy	AA	+	GEO	un-ID
28/05/2014	Fr.	Turtle Dove	GY69313	Fr 20	Pouch slightly swollen, liquid not cloudy	AA	+	Tcl-1	un-ID
28/05/2014	Fr.	Turtle Dove	GY68895	Fr 21	Pouch swollen, liquid slightly cloudy	AA	+	Type C	C8
28/05/2014	Fr.	Turtle Dove	GY68891	Fr 22	Pouch swollen, liquid slightly cloudy	AA	+	Type C	un-ID
28/05/2014	Fr.	Turtle Dove	GY130067	Fr 23	Pouch swollen, liquid cloudy	AA	+	Type C	C8
28/05/2014	Fr.	Turtle Dove	GY130065	Fr 24	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130064	Fr 25	Pouch not swollen, liquid not cloudy	AA	+	NA	NA

28/05/2014	Fr.	Turtle Dove	GY130070	Fr 26	Pouch not swollen, liquid not cloudy	AA	+	GEO	NA
28/05/2014	Fr.	Turtle Dove	GY130063	Fr 27	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY69998	Fr 28	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130066	Fr 29	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130069	Fr 30	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130068	Fr 31	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130061	Fr 32	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY69462	Fr 33	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
28/05/2014	Fr.	Turtle Dove	GY130062	Fr 34	Pouch not swollen, liquid not cloudy	AA	+	GEO	un-ID
27/05/2014	Fr.	Turtle Dove	GY69439	Fr 35	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY103603	Fr 36	Pouch swollen, liquid cloudy	AA	+	Type C	C8
30/05/2014	Fr.	Turtle Dove	GY120905	Fr 37	Pouch slightly swollen	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY103644	Fr 38	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY69414	Fr 39	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY103645	Fr 40	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY103646	Fr 41	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY103628	Fr 42	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY130306	Fr 43	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA

30/05/2014	Fr.	Turtle Dove	GY130305	Fr 44	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY130304	Fr 45	Pouch not swollen, liquid slightly cloudy	AA	+	GEO	un-ID
30/05/2014	Fr.	Turtle Dove	GY130303	Fr 46	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY130302	Fr 47	Pouch slightly swollen, liquid cloudy	AA	+	GEO	NA
30/05/2014	Fr.	Turtle Dove	GY103647	Fr 48	Pouch swollen, liquid cloudy	AA	+	Type C	C7
30/05/2014	Fr.	Turtle Dove	GY103627	Fr 49	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY130301	Fr 50	Pouch slightly swollen, liquid cloudy	AA	+	Tcl-1	un-ID
30/05/2014	Fr.	Turtle Dove	GY103650	Fr 51	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
30/05/2014	Fr.	Turtle Dove	GY103648	Fr 52	Pouch not swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
30/05/2014	Fr.	Turtle Dove	GY103649	Fr 53	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type III	NA
30/05/2014	Fr.	Turtle Dove	GY120906?	Fr 54	Pouch swollen, liquid cloudy	AA	+	Type C	C8
30/05/2014	Fr.	Turtle Dove	GY120584	Fr 55	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
30/05/2014	Fr.	Turtle Dove	GY120908	Fr 56	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C8
30/05/2014	Fr.	Turtle Dove	GY120907	Fr 57	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C8
30/05/2014	Fr.	Turtle Dove	GY120909	Fr 58	Pouch slightly swollen, liquid slightly	AA	+	NA	NA

					cloudy				
02/06/2014	Fr.	Turtle Dove	GY130073	Fr 59	Pouch slightly swollen, liquid slightly cloudy	AA	+	GEO & Type III	NA
02/06/2014	Fr.	Turtle Dove	GY130074	Fr 60	Pouch swollen, liquid cloudy	AA	+	Type C	C11
02/06/2014	Fr.	Turtle Dove	?	Fr 61	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-1	un-ID
02/06/2014	Fr.	Turtle Dove	GY103881	Fr 62	Pouch not swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
02/06/2014	Fr.	Turtle Dove	GY120669	Fr 63	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	GY120732	Fr 64	Pouch swollen, liquid cloudy	AA	+	Tcl-1	NA
02/06/2014	Fr.	Turtle Dove	GY119787	Fr 65	Pouch not swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
02/06/2014	Fr.	Turtle Dove	GY130078	Fr 66	Pouch swollen, liquid cloudy	AA	+	Type C	C8
02/06/2014	Fr.	Turtle Dove	GY130051	Fr 67	Pouch not swollen, liquid slightly cloudy	AA	+	Tcl-1	NA
02/06/2014	Fr.	Turtle Dove	GY130077	Fr 68	Pouch slightly swollen, liquid cloudy	AA	+	GEO	un-ID
02/06/2014	Fr.	Turtle Dove	GY130075	Fr 69	Pouch swollen, liquid cloudy	AA	+	Type C	C8
02/06/2014	Fr.	Turtle Dove	GY130072	Fr 70	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	?	Fr 71	Pouch swollen, liquid cloudy	AA	+	Tcl-1	un-ID

02/06/2014	Fr.	Turtle Dove	?	Fr 72	Pouch swollen, liquid cloudy	AA	+	Type C	NA
02/06/2014	Fr.	Turtle Dove	GY120683	Fr 73	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	GY130076	Fr 74	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	GY69349	Fr 75	Pouch slightly swollen, liquid cloudy	AA	+	Tcl-1	T1-TD
02/06/2014	Fr.	Turtle Dove	GY130079	Fr 76	Pouch not swollen, liquid not cloudy	AA	+	Type III	NA
02/06/2014	Fr.	Turtle Dove	GY130080	Fr 77	Pouch not swollen, liquid not cloudy	AA	+	Type III	NA
02/06/2014	Fr.	Turtle Dove	GY130082	Fr 78	Pouch swollen, liquid cloudy	AA	+	Type C	C8
02/06/2014	Fr.	Turtle Dove	GY130081	Fr 79	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	GY120912	Fr 80	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
02/06/2014	Fr.	Turtle Dove	GY120911	Fr 81	Pouch swollen, liquid slightly cloudy	AA	+	Tcl-1	T2-TD
07/05/2014	UK	MF/ BP 1		F01	Liquid not cloudy	AA	+	NA	NA
07/05/2014	UK	HL/ BP 1		F02	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	F/ BP 3		F03	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	LI/ BP 1		F04	Liquid not cloudy	AA	+	NA	NA
08/05/2014	UK	AH/ BP 1		F05	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	SM/ BP 1		F06	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	PG/ BP 1		F07	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	UH/ BP 1		F08	Liquid not cloudy	AA	-	NA	NA
08/05/2014	UK	CF/ BP 1		F09	Liquid not cloudy	AA	-	NA	NA
14/05/2014	UK	MF/ TP 2		F10	Liquid cloudy	AA	-	NA	NA
14/05/2014	UK	OP/ FP 1		F11	Liquid cloudy	AA	-	NA	NA

14/05/2014	UK	HL/ FP 1	F12	Liquid cloudy	AA	-	NA	NA
14/05/2014	UK	OB/ TP 1	F13	Liquid cloudy	AA	-	NA	NA
14/05/2014	UK	MF/ H20 tray	F14	Liquid cloudy	AA	+	NA	NA
14/05/2014	UK	HL/ BP 1	F15	Liquid cloudy	AA	-	NA	NA
14/05/2014	UK	MF/ BP 1	F16	Liquid cloudy	AA	-	NA	NA
14/05/2014	UK	HL/ H20 tray	F17	Liquid cloudy	AA	-	NA	NA
15/05/2014	UK	SM/ BP 1	F18	Liquid cloudy	AA	+	NA	NA
15/05/2014	UK	LI/ H20 tray	F19	Liquid cloudy	AA	-	NA	NA
15/05/2014	UK	UH/ FP 1	F20	Liquid cloudy	AA	+	NA	NA
15/05/2014	UK	AH/ BP 1	F21	Liquid cloudy	AA	+	NA	NA
15/05/2014	UK	PG/ FP 1	F22	Liquid cloudy	AA	+	NA	NA
15/05/2014	UK	UH/ BP 1	F23	Liquid cloudy	AA	-	NA	NA
15/05/2014	UK	LI/ H20C	F24	Liquid cloudy	AA	-	NA	NA
15/05/2014	UK	LI/ BP 1	F25	Liquid slightly cloudy	AA	-	NA	NA
15/05/2014	UK	SM/ FP 1	F26	Liquid slightly cloudy	AA	+	NA	NA
15/05/2014	UK	LI/ TP 1	F27	Liquid slightly cloudy	AA	+	NA	NA
15/05/2014	UK	PG/ H20 tray	F28	Liquid not cloudy	AA	-	NA	NA
19/05/2014	UK	F/ H20 tray	F40	Liquid cloudy	AA	-	NA	NA
19/05/2014	UK	F/ BP 3	F42	Liquid cloudy	AA	?	NA	NA
12/05/2014	UK	OB/ BP 2	F44	Liquid cloudy	AA	?	NA	NA
12/05/2014	UK	OP/ BP 1	F45	Liquid cloudy	AA	-	NA	NA
20/05/2014	UK	UH/ BP 1	F49	Liquid cloudy	AA	+	Type A	NA
20/05/2014	UK	PG/ BP 1	F50	Liquid cloudy	AA	+	Type	NA

20/05/2014	UK	AH/ H20 tray		F51	Liquid cloudy	AA	+	A & Type C Type A	NA
20/05/2014	UK	UH/ FP 1		F52	Liquid cloudy	AA	-	NA	NA
20/05/2014	UK	AH/ FP 2		F53	Liquid cloudy	AA	-	NA	NA
20/05/2014	UK	PG/ FP 1		F54	Liquid cloudy	AA	?	NA	NA
20/05/2014	UK	AH/ BP 1		F55	Liquid cloudy	AA	+	Type A	NA
20/05/2014	UK	SM/ H20C		F56	Liquid not cloudy	AA	+	Type A	NA
20/05/2014	UK	PG/ H20 tray		F57	Liquid not cloudy	AA	?	NA	NA
20/05/2014	UK	SM/ BP 1		F58	Liquid cloudy	AA	+	Type A	NA
19/05/2014	UK	Turtle Dove	EG82586	F59	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
20/05/2014	UK	Woodpigeon	FH44764	F60	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
19/05/2014	UK	Red Legged Partridge	RLP/ PG	F61	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
04/06/2014	UK	Stock Dove	EG59154	F62	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
04/06/2014	UK	Stock Dove	EG59152	F63	Pouch swollen, liquid cloudy	AA	+	Type C	C4
06/06/2014	UK	Red Legged Partridge	RLP/ UH	F64	Pouch not swollen, liquid not cloudy	AA	+	NA	NA

03/06/2014	UK	Turtle Dove	EG59151	F65	Pouch swollen, liquid cloudy	AA	+	Type C	C8
04/06/2014	UK	Chaffinch	X112930	F66	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
04/06/2014	UK	Woodpigeon	FH44766	F67	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
04/06/2014	UK	Stock Dove	EX75298	F68	Pouch not swollen, liquid slightly cloudy	AA	+	Type A	NA
04/06/2014	UK	Stock Dove	EG82594	F69	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
02/06/2014	UK	Goldfinch	X112957	F70	Pouch swollen, liquid cloudy	AA	+	Type A	NA
03/06/2014	UK	HL/ H20C		F71	Liquid cloudy	AA	-	NA	NA
03/06/2014	UK	MF/ H20C		F72	Liquid not cloudy	AA	-	NA	NA
03/06/2014	UK	HL/ H20 tray		F73	Liquid not cloudy	AA	-	NA	NA
03/06/2014	UK	MF/ BP 1		F74	Liquid slightly cloudy	AA	-	NA	NA
03/06/2014	UK	MF/ TP 2		F75	Liquid not cloudy	AA	-	NA	NA
03/06/2014?	UK	HL/ FP 1?		F76	Liquid not cloudy	AA	-	NA	NA
03/06/2014	UK	HL/ BP 1		F77	Liquid not cloudy	AA	?	NA	NA
03/06/2014	UK	MF/ H20 tray		F78	Liquid not cloudy	AA	+	Tcl-1	NA
05/06/2014	UK	LI/ H20 tray		F79	Liquid cloudy	AA	-	NA	NA
05/06/2014	UK	LI/ BP 1		F80	Liquid cloudy	AA	+	Type A	NA
05/06/2014	UK	AH/ BP 1		F81	Liquid cloudy	AA	+	Type A	NA
05/06/2014	UK	SM/ FP 1		F82	Liquid slightly cloudy	AA	+	Type	NA

05/06/2014	UK	AH/ H20 tray		F83	Liquid not cloudy	AA	+	A Type A	NA
05/06/2014	UK	SM/ BP 1		F84	Liquid not cloudy	AA	+	A Type A	NA
05/06/2014	UK	SM/ H20 tray		F85	Liquid not cloudy	AA	+	A Type A	NA
05/06/2014	UK	AH/ H20C		F86	Liquid slightly cloudy	AA	-	NA	NA
05/06/2014	UK	AH/ FP 2		F87	Liquid slightly cloudy	AA	+	A Type A	NA
05/06/2014	UK	LI/ TP 1		F88	Liquid cloudy	AA	?	NA	NA
05/06/2014	UK	SM/ H20C		F89	Liquid not cloudy	AA	-	NA	NA
05/06/2014	UK	LI/ H20C		F90	Liquid cloudy	AA	?	NA	NA
06/06/2014	UK	PG/ H20C		F91	Liquid not cloudy	AA	?	NA	NA
06/06/2014	UK	PG/ BP 1		F92	Liquid slightly cloudy	AA	?	NA	NA
06/06/2014	UK	PG/ FP 1		F93	Liquid not cloudy	AA	-	NA	NA
06/06/2014	UK	PG/ H20 tray		F94	Liquid not cloudy	AA	-	NA	NA
06/06/2014	UK	UH/ H20C		F95	Liquid cloudy	AA	-	NA	NA
06/06/2014	UK	UH/ BP 1		F96	Liquid cloudy	AA	-	NA	NA
06/06/2014	UK	UH/ H20 tray		F97	Liquid cloudy	AA	-	NA	NA
06/06/2014	UK	UH/ FP 1		F98	Liquid cloudy	AA	-	NA	NA
10/06/2014	UK	Stock Dove	EG82593	F99	Pouch swollen, liquid cloudy	AA	+	Type C	C4
10/06/2014	UK	Woodpigeon	FH44767	F100	Pouch not swollen, liquid cloudy	AA	-	NA	NA
11/06/2014	UK	Stock Dove	EG59157	G01	Pouch swollen, liquid cloudy	AA	-	NA	NA

11/06/2014	UK	Turtle Dove	EG59155	G02	Pouch swollen, liquid cloudy	AA	+	Type A	A1
11/06/2014	UK	Stock Dove	EG59156	G03	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
12/06/2014	UK	Jackdaw	EG59158	G04	Pouch swollen, liquid not cloudy	AA	-	NA	NA
12/06/2014	UK	Turtle Dove	EG79960	G05	Pouch swollen, liquid cloudy	AA	+	Type A	A1
12/06/2014	UK	Woodpigeon	FH55351	G06	Pouch slightly swollen, liquid cloudy	AA	-	NA	NA
12/06/2014	UK	Woodpigeon	FH55352	G07	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
12/06/2014	UK	Turtle Dove	EG59010	G08	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
12/06/2014	UK	Magpie	EG59159	G09	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
12/06/2014	UK	Magpie	EG82596	G10	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type A	NA
13/06/2014	UK	Stock Dove	EY79812	G11	Pouch not swollen, liquid slightly cloudy	AA	+	Type A	NA
13/06/2014	UK	Woodpigeon	FH44769	G12	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	Woodpigeon	FH44768	G13	Pouch swollen, liquid cloudy	AA	+	WQR-Env	NA
13/06/2014	UK	Stock Dove	EG82600	G14	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	Stock Dove	EG82599	G15	Pouch swollen, liquid cloudy	AA	+	Type A	A1
13/06/2014	UK	Stock Dove	EG82598	G16	Pouch not swollen, liquid cloudy	AA	+	NA	NA
13/06/2014	UK	Stock Dove	EG82597	G17	Pouch swollen, liquid cloudy	AA	+	Type	un-ID

								A	
13/06/2014	UK	Chaffinch	X112931	G18	Pouch slightly swollen, liquid cloudy	AA	-	NA	NA
13/06/2014	UK	Turtle Dove NT	DEAD	G19	Pouch swollen, liquid cloudy	AA	+	Type III	A1
17/06/2014	UK	Woodpigeon NT	FH85602	G20	Pouch swollen, liquid cloudy	AA	+	Type C	C4
17/06/2014	UK	Stock Dove	EG59162	G21	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
17/06/2014	UK	Stock Dove	EG59161	G22	Pouch swollen, liquid slightly cloudy	AA	+	NA	NA
17/06/2014	UK	Turtle Dove	EG59160	G23	Pouch swollen, liquid cloudy	AA	+	Type A	NA
16/06/2014	UK	Woodpigeon	FH85601	G24	Pouch not swollen, liquid slightly cloudy	AA	+	WQR-Env	NA
17/06/2014	UK	Woodpigeon NT	FH85603	G25	Pouch swollen, liquid cloudy	AA	+	Type C	C4
18/06/2014	UK	Collared Dove	EG59163	G27	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
18/06/2014	UK	Collared Dove	DEAD	G28	Pouch swollen, liquid cloudy	AA	+	Type C	C11
11/06/2014	UK	CF/ BP 1		G29	Liquid not cloudy	AA	+	NA	NA
11/06/2014	UK	CF/ TP 1		G30	Liquid slightly cloudy	AA	-	NA	NA
11/06/2014	UK	SM/ H20 tray		G31	Liquid cloudy	AA	-	NA	NA
11/06/2014	UK	SM/ BP 1		G32	Liquid cloudy	AA	-	NA	NA
11/06/2014	UK	SM/ FP 1		G33	Liquid cloudy	AA	-	NA	NA
11/06/2014	UK	SM/ H20C		G34	Liquid slightly cloudy	AA	-	NA	NA
11/06/2014	UK	AH/ H20 tray		G35	Liquid slightly cloudy	AA	-	NA	NA

11/06/2014	UK	AH/ H20C	G36	Liquid cloudy	AA	-	NA	NA
11/06/2014	UK	AH/ BP 1	G37	Liquid slightly cloudy	AA	-	NA	NA
11/06/2014	UK	AH/ FP 2	G38	Liquid slightly cloudy	AA	-	NA	NA
11/06/2014	UK	LI/ H20C	G39	Liquid slightly cloudy	AA	-	NA	NA
11/06/2014	UK	LI/ BP 1	G40	Liquid cloudy	AA	-	NA	NA
11/06/2014	UK	LI/ TP 1	G41	Liquid not cloudy	AA	-	NA	NA
11/06/2014	UK	LI/ H20 tray	G42	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	PG/ BP 1	G43	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	PG/ FP 1	G44	Liquid not cloudy	AA	-	NA	NA
12/06/2014	UK	PG/ H20C	G45	Liquid not cloudy	AA	+	Type C	NA
12/06/2014	UK	PG/ H20 tray	G46	Liquid cloudy	AA	+	NA	NA
12/06/2014	UK	UH/ FP 1	G47	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	UH/ H20C	G48	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	UH/ BP 1	G49	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	UH/ H20 tray	G50	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	F/ BP 3	G51	Liquid slightly cloudy	AA	-	NA	NA
12/06/2014	UK	F/ TP 1	G52	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	F/ H20 tray	G53	Liquid cloudy	AA	-	NA	NA
12/06/2014	UK	OB/ TP 1	G54	Liquid slightly cloudy	AA	-	NA	NA
12/06/2014	UK	OB/ BP 2	G55	Liquid slightly cloudy	AA	-	NA	NA
12/06/2014	UK	OB/ H20C	G56	Liquid not cloudy	AA	-	NA	NA
12/06/2014	UK	OB/ H20 tray	G57	Liquid cloudy	AA	?	NA	NA
18/06/2014	UK	LI/ H20C	G58	Liquid cloudy	AA	-	NA	NA

18/06/2014	UK	LI/ TP 1	G59	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	LI/ BP 1	G60	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	LI/ H20 tray	G61	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	UH/ H20 tray	G62	Liquid cloudy	AA	?	NA	NA
18/06/2014	UK	UH/ H20C	G63	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	UH/ FP 1	G64	Liquid not cloudy	AA	-	NA	NA
18/06/2014	UK	UH/ BP 1	G65	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	AH/ H20 tray	G66	Liquid cloudy	AA	?	NA	NA
18/06/2014	UK	AH/ FP 2	G67	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	AH/ BP 1	G68	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	AH/ BP 1	G69	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	CF/ BP 2	G70	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	CF/ TP 1	G71	Liquid slightly cloudy	AA	+	NA	NA
18/06/2014	UK	SM/ FP 1	G72	Liquid slightly cloudy	AA	+	Tcl-1	NA
18/06/2014	UK	SM/ H20C	G73	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	SM/ H20 tray	G74	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	SM/ BP 1	G75	Liquid cloudy	AA	-	NA	NA
17/06/2014	UK	OB/ H20 tray	G76	Liquid cloudy	AA	-	NA	NA
17/06/2014	UK	OB/ TP 1	G77	Liquid cloudy	AA	-	NA	NA
17/06/2014	UK	OB/ BP 2	G78	Liquid cloudy	AA	-	NA	NA
17/06/2014	UK	OB/ H20C	G79	Liquid cloudy	AA	-	NA	NA
13/06/2014	UK	HL/ H20 tray	G80	Liquid cloudy	AA	-	NA	NA
13/06/2014	UK	OP/ BP 2	G81	Liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	HL/ H20C	G82	Liquid slightly cloudy	AA	-	NA	NA

13/06/2014	UK	OP/ FP 2		G83	Liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	OP/ H20C 2		G84	Liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	MF/ TP 2		G85	Liquid slightly cloudy	AA	-	NA	NA
13/06/2014	UK	MF/ H20 tray		G86	Liquid cloudy	AA	-	NA	NA
13/06/2014	UK	MF/ H20C		G87	Liquid cloudy	AA	+	GEO	NA
13/06/2014	UK	MF/ BP 1		G88	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	OP/ FP 2		G89	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	HL/ BP 1		G90	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	MF/ BP 1		G91	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	OP/ BP 2		G92	Liquid cloudy	AA	+	Type C	NA
18/06/2014	UK	MF/ TP 2		G93	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	HL/ FP 1		G94	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	HL/ H20C		G95	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	MF/ H20 tray		G96	Liquid cloudy	AA	+	NA	NA
18/06/2014	UK	OP/ H20 tray 2		G97	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	HL/ H20 tray		G98	Liquid cloudy	AA	-	NA	NA
18/06/2014	UK	OP/ H20C 2		G99	Liquid slightly cloudy	AA	-	NA	NA
18/06/2014	UK	MF/ H20C		G100	Liquid slightly cloudy	AA	-	NA	NA
19/06/2014	UK	Woodpigeon NT	FH85603	H01	Pouch swollen, liquid cloudy	AA	+	Type C	C4
19/06/2014	UK	Jay	DE32902	H02	Pouch swollen, liquid not cloudy	AA	+	NA	NA
19/06/2014	UK	Woodpigeon NT	FH85602	H03	Pouch swollen, liquid cloudy	AA	+	Type C	C4
23/06/2014	UK	Collared Dove	EY79953	H04	Pouch slightly swollen, liquid not	AA	+	NA	NA

					cloudy				
23/06/2014	UK	Moorhen	FH76701	H05	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	Stock Dove	EG59167	H06	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	Stock Dove	EG59164	H07	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	Turtle Dove NT		H08	Pouch slightly swollen, liquid not cloudy	AA	+	Type III	un-ID
25/06/2014	UK	Turtle Dove NT		H09	Pouch swollen, liquid cloudy	AA	+	GEO	un-ID
25/06/2014	UK	Stock Dove	EG59166	H10	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	Stock Dove	EG59165	H11	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
25/06/2014	UK	Woodpigeon	FH85605	H12	Pouch not swollen, liquid slightly cloudy	AA	+	GEO	NA
25/06/2014	UK	Woodpigeon	FH85604	H13	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/06/2014	UK	Turtle Dove	EG59168	H14	Pouch swollen, liquid cloudy	AA	+	Type A	A1
25/06/2014	UK	AH/ FP 2		H15	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	HL/ BP 1		H16	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	AH/ BP 1		H17	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	OP/ H20C 2		H18	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	MF/ BP 1		H19	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	HL/ FP 1		H20	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	AH/ H20C		H21	Liquid cloudy	AA	-	NA	NA

25/06/2014	UK	HL/ H20 tray		H22	Liquid dark green from sampling	AA	?	NA	NA
25/06/2014	UK	MF/ TP 2		H23	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	MF/ H20 tray		H24	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	OP/ H20 tray 2		H25	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	SM/ FP 1		H26	Liquid slightly cloudy	AA	-	NA	NA
24/06/2014	UK	OB/ BP 2		H27	Liquid cloudy	AA	-	NA	NA
24/06/2014	UK	OB/ H20 tray		H28	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	SM/ BP 1		H29	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	OP/ FP 2		H30	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	AH/ H20 tray		H31	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	MF/ H20C		H32	Liquid cloudy	AA	+	Type A & Type C	NA
25/06/2014	UK	OP/ BP 2		H33	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	SM/ H20 tray		H34	Liquid cloudy	AA	+	Type A	NA
24/06/2014	UK	OB/ TP 1		H35	Liquid slightly cloudy	AA	-	NA	NA
24/06/2014	UK	OB/ H20C		H36	Liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	SM/ H20C		H37	Liquid cloudy	AA	?	NA	NA
25/06/2014	UK	HL/ H20C		H38	Liquid cloudy	AA	-	NA	NA
19/06/2014	UK	PG/ BP 1		H39	Liquid cloudy	AA	-	NA	NA
19/06/2014	UK	Woodpigeon NT	FH85603	H40	Liquid cloudy	AA	+	Type C	C4
19/06/2014	UK	Woodpigeon NT	FH85602	H41	Liquid cloudy	AA	+	Type	C4

								C	
19/06/2014	UK	PG/ FP 1		H42	Liquid slightly cloudy	AA	-	NA	NA
19/06/2014	UK	PG/ H20 tray		H43	Liquid slightly cloudy	AA	-	NA	NA
19/06/2014	UK	PG/ H20C		H44	Liquid not cloudy	AA	+	Type A	NA
25/06/2014	UK	UH/ H20 tray		H45	Liquid slightly cloudy	AA	+	Type A	NA
25/06/2014	UK	PG/ H20 tray		H46	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	PG/ BP 1		H47	Liquid not cloudy	AA	+	Type A	NA
25/06/2014	UK	UH/ FP 1		H48	Liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	PG/ H20C		H49	Liquid slightly cloudy	AA	-	NA	NA
25/06/2014	UK	PG/ FP 1		H50	Liquid not cloudy	AA	-	NA	NA
25/06/2014	UK	UH/ BP 1		H51	Liquid cloudy	AA	-	NA	NA
25/06/2014	UK	UH/ H20C		H52	Liquid cloudy	AA	?	NA	NA
26/06/2014	UK	LI/ H20 tray		H53	Liquid cloudy	AA	-	NA	NA
26/06/2014	UK	F/ BP 3		H54	Liquid slightly cloudy	AA	-	NA	NA
26/06/2014	UK	F/ H20 tray		H55	Liquid slightly cloudy	AA	-	NA	NA
26/06/2014	UK	CF/ TP 1		H56	Liquid cloudy	AA	?	NA	NA
26/06/2014	UK	F/ H20C		H57	Liquid slightly cloudy	AA	-	NA	NA
26/06/2014	UK	F/ TP 1		H58	Liquid slightly cloudy	AA	-	NA	NA
26/06/2014	UK	LI/ TP 1		H59	Liquid cloudy	AA	?	NA	NA
26/06/2014	UK	LI/ BP 1		H60	Liquid cloudy	AA	?	NA	NA
26/06/2014	UK	CF/ BP 2		H61	Liquid very cloudy	AA	?	NA	NA
27/06/2014	UK	Greenfinch	TP21417	H62	Pouch not swollen, liquid sllightly	AA	-	NA	NA

					cloudy				
27/06/2014	UK	Greenfinch	TP21418	H63	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
27/06/2014	UK	Dunnock	X112958	H64	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
02/07/2014	UK	Turtle Dove NT		H65	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/07/2014	UK	Turtle Dove NT		H66	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
27/06/2014	UK	Greenfinch	TP21414	H67	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
01/07/2014	UK	Woodpigeon NT	FH55353	H68	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
27/06/2014	UK	Greenfinch	TP21416	H69	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
01/07/2014	UK	Collared Dove	EY79956	H70	Pouch very swollen, liquid very cloudy	AA	+	NA	NA
27/06/2014	UK	Woodpigeon	FH44837	H71	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
01/07/2014	UK	Woodpigeon NT	FH55354	H72	Pouch swollen, liquid cloudy	AA	-	NA	NA
27/06/2014	UK	Greenfinch	TP21415	H73	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/07/2014	UK	AH/ H20C		H74	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	AH/ FP 2		H75	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	AH/ H20 tray		H76	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	LI/ H20 tray		H77	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	LI/ BP 1		H78	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	LI/ H20C		H79	Liquid not cloudy	AA	-	NA	NA
02/07/2014	UK	LI/ TP 1		H80	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	UH/ H20C		H81	Liquid cloudy	AA	-	NA	NA

02/07/2014	UK	UH/ BP 1		H82	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	UH/ FP 1		H83	Liquid cloudy	AA	?	NA	NA
02/07/2014	UK	UH/ H20 tray		H84	Liquid cloudy	AA	?	NA	NA
02/07/2014	UK	PG/ FP 1		H85	Liquid cloudy	AA	?	NA	NA
02/07/2014	UK	PG/ BP 1		H86	Liquid cloudy	AA	?	NA	NA
02/07/2014	UK	PG/ H20 tray		H87	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	PG/ H20C		H88	Liquid cloudy	AA	-	NA	NA
02/07/2014	UK	AH/ BP 1		H89	Liquid cloudy	AA	-	NA	NA
04/07/2014	UK	Turtle Dove NT	EY79823	H90	Pouch not swollen, liquid cloudy	AA	+	NA	NA
04/07/2014	UK	Turtle Dove NT	EY79824	H91	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
04/07/2014	UK	Woodpigeon NT		H92	Pouch slightly swollen, liquid cloudy	AA	+	NA	NA
04/07/2014	UK	Woodpigeon NT		H93	Pouch slightly swollen, liquid cloudy	AA	+	NA	NA
04/07/2014	UK	Woodpigeon NT	FH55354	H94	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
04/07/2014	UK	Woodpigeon NT	FH55356	H95	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
07/07/2014	UK	Woodpigeon	FH85611	H96	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
07/07/2014	UK	Woodpigeon NT		H97	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
07/07/2014	UK	Woodpigeon NT		H98	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
07/07/2014	UK	Blackbird	LB70741	H99	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
07/07/2014	UK	Whitethroat	X648350	H100	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
07/07/2014	UK	Whitethroat	X648351	I01	Pouch not swollen, liquid not cloudy	AA	+	NA	NA

09/07/2014	UK	Woodpigeon	FH76703	I02	Pouch slightly swollen, liquid slightly cloudy	AA	+	Type C	C4
09/07/2014	UK	Turtle Dove NT		I03	Pouch very swollen, liquid very cloudy	AA	+	Type A	A1
09/07/2014	UK	Turtle Dove NT		I04	Pouch very swollen, liquid very cloudy	AA	+	Type A	A1
09/07/2014	UK	Turtle Dove NT		I05	Pouch slightly swollen, liquid not cloudy	AA	+	NA	NA
09/07/2014	UK	Turtle Dove NT		I06	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
09/07/2014	UK	Woodpigeon NT	FH76706	I07	Pocuh swollen, liquid cloudy	AA	+	Type C	C4
09/07/2014	UK	Woodpigeon NT	FH76702	I08	Pouch slightly swollen, liquid not cloudy	AA	+	Type C	C4
09/07/2014	UK	Woodpigeon NT	FH76705	I09	Pouch not swollen, liquid slightly cloudy	AA	+	Type C	OTU10
08/07/2014	UK	AH/ FP 2		I10	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	AH/ H20C		I11	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	AH/ BP 1		I12	Liquid not cloudy	AA	-	NA	NA
08/07/2014	UK	AH/ H20 tray		I13	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	PG/ H20 tray		I14	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	PG/ FP 1		I15	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	PG/ H20C		I16	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	PG/ BP 1		I17	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	UH/ FP 1		I18	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	UH/ BP 1		I19	Liquid cloudy	AA	-	NA	NA

08/07/2014	UK	UH/ H20C	I20	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	UH/ H20 tray	I21	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	LI/ H20C	I22	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	LI/ BP 1	I23	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	LI/ H20 tray	I24	Liquid cloudy	AA	-	NA	NA
10/07/2014	UK	CF/ TP 1	I25	Liquid cloudy	AA	-	NA	NA
08/07/2014	UK	LI/ TP 1	I26	Liquid cloudy	AA	-	NA	NA
10/07/2014	UK	CF/ BP 2	I27	Liquid cloudy	AA	+	NA	NA
09/07/2014	UK	SM/ H20 tray	I28	Liquid cloudy	AA	-	NA	NA
09/07/2014	UK	SM/ BP 1	I29	Liquid cloudy	AA	-	NA	NA
09/07/2014	UK	SM/ H20C	I30	Liquid cloudy	AA	-	NA	NA
09/07/2014	UK	SM/ FP 1	I31	Liquid cloudy	AA	-	NA	NA
09/07/2014	UK	F/ TP 1	I32	Liquid cloudy	AA	-	NA	NA
09/07/2014	UK	F/ H20C	I33	Liquid cloudy	AA	+	NA	NA
09/07/2014	UK	F/ H20 tray	I34	Liquid cloudy	AA	?	NA	NA
09/07/2014	UK	F/ BP 3	I35	Liquid cloudy	AA	-	NA	NA
11/07/2014	UK	HL/ H20C	I36	Liquid cloudy	AA	?	NA	NA
11/07/2014	UK	HL/ FP 1	I37	Liquid cloudy	AA	-	NA	NA
11/07/2014	UK	HL/ BP 1	I38	Liquid cloudy	AA	-	NA	NA
11/07/2014	UK	HL/ H20 tray	I39	Liquid cloudy	AA	?	NA	NA
11/07/2014	UK	MF/ H20C	I40	Liquid not cloudy	AA	-	NA	NA
11/07/2014	UK	MF/ H20 tray	I41	Liquid cloudy	AA	-	NA	NA
11/07/2014	UK	MF/ BP 1	I42	Liquid cloudy	AA	?	NA	NA
11/07/2014	UK	MF/ TP 2	I43	Liquid cloudy	AA	-	NA	NA

11/07/2014	UK	OB/ BP 2		I44	Liquid cloudy	AA	-	NA	NA
11/07/2014	UK	OB/ H20C		I45	Liquid slightly cloudy	AA	+	NA	NA
11/07/2014	UK	OB/ TP 1		I46	Liquid not cloudy	AA	-	NA	NA
11/07/2014	UK	OB/ H20 tray		I47	Liquid cloudy	AA	-	NA	NA
10/07/2014	UK	Woodpigeon	FH85612	I48	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
10/07/2014	UK	Stock Dove	EY79825	I49	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
10/07/2014	UK	Woodpigeon	FH85613	I50	Pouch not swollen, liquid slightly cloudy	AA	+	NA	NA
11/07/2014	UK	Woodpigeon NT	FH76707	I51	Pouch very swollen, liquid very cloudy	AA	-	NA	NA
11/07/2014	UK	Woodpigeon NT	FH70708	I52	Pouch very swollen, liquid very cloudy	AA	+	Type C	C4
11/07/2014	UK	Turtle Dove NT		I53	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
11/07/2014	UK	Turtle Dove NT		I54	Pouch swollen, liquid cloudy	AA	+	Type A	A1
11/07/2014	UK	Woodpigeon NT		I55	Pouch swollen, liquid not cloudy	AA	-	NA	NA
11/07/2014	UK	Woodpigeon NT		I56	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
11/07/2014	UK	Turtle Dove NT		I57	Pouch swollen, liquid cloudy	AA	+	Type A	OTU10
11/07/2014	UK	Turtle Dove NT		I58	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
11/07/2014	UK	Woodpigeon NT	FH76704	I59	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
11/07/2014	UK	Woodpigeon NT	FH76702	I60	Pouch not swollen, liquid slightly cloudy	AA	+	Type C	NA
11/07/2014	UK	Rook	FH76706	I61	Pouch swollen, liquid not cloudy	AA	-	NA	NA

14/07/2014	UK	Woodpigeon NT		I62	Pouch swollen, liquid slightly cloudy	AA	+	NA	NA
14/07/2014	UK	Woodpigeon NT		I63	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
14/07/2014	UK	Woodpigeon NT		I64	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
14/07/2014	UK	Woodpigeon NT		I65	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
14/07/2014	UK	Woodpigeon NT		I66	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
14/07/2014	UK	Woodpigeon NT		I67	Pouch slightly swollen, liquid slightly cloudy	AA	+	NA	NA
14/07/2014	UK	Woodpigeon NT		I68	Pouch not swollen, liquid slightly cloudy	AA	-	NA	NA
14/07/2014	UK	Woodpigeon NT		I69	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
16/07/2014	UK	Woodpigeon NT	FH76709	I70	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
16/07/2014	UK	Woodpigeon NT	FH76710	I71	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
16/07/2014	UK	Turtle Dove NT	EG59169	I72	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
16/07/2014	UK	Turtle Dove NT	EG59170	I73	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
14/07/2014	UK	Turtle Dove NT		I74	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
14/07/2014	UK	Turtle Dove NT		I75	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
16/07/2014	UK	SM/ H20 tray		I76	Liquid cloudy	AA	+	NA	NA
16/07/2014	UK	SM/ BP 1		I77	Liquid not cloudy	AA	+	NA	NA
16/07/2014	UK	SM/ H20C		I78	Liquid not cloudy	AA	+	NA	NA
16/07/2014	UK	SM/ FP 1		I79	Liquid slightly cloudy	AA	+	NA	NA
16/07/2014	UK	HL/ BP 1		I80	Liquid cloudy	AA	-	NA	NA

16/07/2014	UK	HL/ FP 1	I81	Liquid cloudy	AA	-	NA	NA
16/07/2014	UK	HL/ H20C	I82	Liquid cloudy	AA	?	NA	NA
16/07/2014	UK	HL/ H20 tray	I83	Liquid cloudy	AA	?	NA	NA
16/07/2014	UK	MF/ H20C	I84	Liquid cloudy	AA	?	NA	NA
16/07/2014	UK	MF/ TP 2	I85	Liquid cloudy	AA	?	NA	NA
16/07/2014	UK	MF/ H20 tray	I86	Liquid cloudy	AA	-	NA	NA
16/07/2014	UK	MF/ BP 1	I87	Liquid cloudy	AA	+	NA	NA
16/07/2014	UK	OB/ H20C	I88	Liquid not cloudy	AA	+	NA	NA
16/07/2014	UK	OB/ BP 2	I89	Liquid slightly cloudy	AA	-	NA	NA
16/07/2014	UK	OB/ TP 1	I90	Liquid cloudy	AA	-	NA	NA
16/07/2014	UK	OB/ H20 tray	I91	Liquid cloudy	AA	-	NA	NA
17/07/2014	UK	AH/ H20 tray	I92	Liquid cloudy	AA	-	NA	NA
17/07/2014	UK	AH/ BP 1	I93	Liquid not cloudy	AA	+	NA	NA
17/07/2014	UK	AH/ FP 2	I94	Liquid cloudy	AA	+	NA	NA
17/07/2014	UK	AH/ H20C	I95	Liquid cloudy	AA	?	NA	NA
18/07/2014	UK	UH/ H20 tray	I96	Liquid slightly cloudy	AA	-	NA	NA
18/07/2014	UK	UH/ H20C	I97	Liquid cloudy	AA	+	NA	NA
18/07/2014	UK	UH/ FP 1	I98	Liquid cloudy	AA	-	NA	NA
18/07/2014	UK	UH/ BP 1	I99	Liquid cloudy	AA	+	NA	NA
18/07/2014	UK	PG/ FP 1	I100	Liquid cloudy	AA	-	NA	NA
18/07/2014	UK	F/ H20C	J01	Liquid cloudy	AA	+	NA	NA
18/07/2014	UK	F/ TP 1	J02	Liquid slightly cloudy	AA	+	NA	NA
18/07/2014	UK	F/ BP 3	J03	Liquid slightly cloudy	AA	+	NA	NA
18/07/2014	UK	F/ H20 tray	J04	Liquid not cloudy	AA	+	Type	NA

18/07/2014	UK	PG/ BP 1		J05	Liquid not cloudy	AA	+	C Type C	NA
18/07/2014	UK	LI/ BP 1		J06	Liquid slightly cloudy	AA	+	C Type C	NA
18/07/2014	UK	LI/ TP 1		J07	Liquid cloudy	AA	-	NA	NA
18/07/2014	UK	PG/ H20C		J08	Liquid cloudy	AA	+	C Type C	NA
18/07/2014	UK	LI/ H20 tray		J09	Liquid cloudy	AA	-	NA	NA
18/07/2014	UK	LI/ H20C		J10	Liquid cloudy	AA	+	A Type A	NA
18/07/2014	UK	PG/ H20 tray		J11	Liquid cloudy	AA	+	C Type C	NA
17/07/2014	UK	Woodpigeon NT		J12	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
17/07/2014	UK	Woodpigeon NT		J13	Pouch swollen, liquid cloudy	AA	?	NA	NA
17/07/2014	UK	Woodpigeon NT	FH55359	J14	Pouch swollen, liquid slightly cloudy	AA	?	NA	NA
17/07/2014	UK	Woodpigeon NT		J15	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
22/07/2014	UK	Woodpigeon NT	FH76711	J16	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
22/07/2014	UK	Woodpigeon NT	FH76712	J17	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
22/07/2014	UK	Turtle Dove NT		J18	Pouch swollen, liquid cloudy	AA	+	NA	NA
22/07/2014	UK	Woodpigeon NT		J19	Pouch swollen, liquid slightly cloudy	AA	?	NA	NA
22/07/2014	UK	Woodpigeon NT		J20	Pouch swollen, liquid slightly cloudy	AA	?	NA	NA

31/07/2014	UK	Turtle Dove NT	EG59175	J21	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
31/07/2014	UK	Turtle Dove NT	EG59174	J22	Pouch swollen, liquid slightly cloudy	AA	?	NA	NA
02/08/2014	UK	Turtle Dove	EG59174	J23	Pouch swollen, liquid slightly cloudy	AA	+	NA	NA
02/08/2014	UK	Turtle Dove	EG59175	J24	Pouch slightly swollen, liquid not cloudy	AA	+	Type C	NA
06/08/2014	UK	Woodpigeon NT		J25	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
06/08/2014	UK	Woodpigeon NT		J26	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
06/08/2014	UK	Woodpigeon NT	FH76717	J27	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
06/08/2014	UK	Woodpigeon NT	FH76716	J28	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
08/08/2014	UK	Woodpigeon NT	FH76716	J29	Pouch swollen, liquid slightly cloudy	AA	?	NA	NA
08/08/2014	UK	Woodpigeon NT	FH76717	J30	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
08/08/2014	UK	Woodpigeon NT		J31	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
08/08/2014	UK	Woodpigeon NT		J32	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
11/08/2014	UK	Woodpigeon NT		J33	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
11/08/2014	UK	Woodpigeon NT		J34	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
11/08/2014	UK	Woodpigeon NT		J35	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
13/08/2014	UK	Woodpigeon NT	FH76718	J36	Pouch not swollen, liquid not cloudy	AA	-	NA	NA

13/08/2014	UK	Woodpigeon NT		J37	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT	FH55370	J38	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT	FH55371	J39	Pouch very slightly swollen, liquid not cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT	FH55368	J40	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT		J41	Pouch very slightly swollen, liquid slightly cloudy	AA	-	NA	NA
13/08/2014	UK	Collared Dove NT	EY79962	J42	Pouch swollen, liquid cloudy	AA	+	NA	NA
13/08/2014	UK	Collared Dove NT	EY79961	J43	Pouch swollen, liquid cloudy	AA	+	NA	NA
13/08/2014	UK	Woodpigeon NT		J44	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT	FH55369	J45	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
13/08/2014	UK	Woodpigeon NT		J46	Pouch very slightly swollen, liquid not cloudy	AA	?	NA	NA
14/08/2014	UK	Woodpigeon NT	FH85615	J47	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
15/08/2014	UK	Woodpigeon NT		J48	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
15/08/2014	UK	Woodpigeon NT		J49	Pouch swollen, liquid not cloudy	AA	?	NA	NA
15/08/2014	UK	Woodpigeon NT	FH55370	J50	Pouch swollen, liquid not cloudy	AA	-	NA	NA
15/08/2014	UK	Woodpigeon NT	FH55371	J51	Pouch swollen, liquid not cloudy	AA	?	NA	NA
18/08/2014	UK	Woodpigeon NT		J52	Pouch swollen, liquid cloudy	AA	?	NA	NA

18/08/2014	UK	Woodpigeon NT		J53	Pouch swollen, liquid cloudy	AA	?	NA	NA
20/08/2014	UK	Woodpigeon NT	FH85617	J54	Pouch swollen, liquid cloudy	AA	+	Type C	C4
20/08/2014	UK	Woodpigeon NT	FH85616	J55	Pouch swollen, liquid cloudy	AA	+	Type C	C4
02/09/2014	UK	House Sparrow	TT94092	J56	Pouch not swollen, liquid not cloudy	AA	+	Type C	C4
02/09/2014	UK	House Sparrow	TT94094	J57	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
02/09/2014	UK	House Sparrow	TT94078	J58	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Greenfinch	TT94077	J59	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Goldfinch	D883202	J60	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Greenfinch	TT94086	J61	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Greenfinch	TT94090	J62	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Chaffinch	?	J63	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	House Sparrow	?	J64	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
02/09/2014	UK	Greenfinch	TT94089	J65	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
02/09/2014	UK	Greenfinch	TT94093	J66	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
21/05/2014	UK	Blackbird	LE97168	ESJ1	Clear	AA	-	NA	NA
21/05/2014	UK	Starling	LE97170	ESJ2	Clear	AA	-	NA	NA
21/05/2014	UK	Jay	DE32902	ESJ3	Localised cloudiness	AA	-	NA	NA
21/05/2014	UK	Woodpigeon	FH44765	ESJ4	Clear	AA	-	NA	NA
21/05/2014	UK	Jay	DE32901	ESJ5	Clear	AA	-	NA	NA
21/05/2014	UK	Starling	LE97169	ESJ6	Clear	AA	?	NA	NA
21/05/2014	UK	Starling	LE97174	ESJ7	Clear	AA	-	NA	NA
21/05/2014	UK	Starling	LE97173	ESJ8	Some small bubbles, clear	AA	-	NA	NA

21/05/2014	UK	Starling	LE97172	ESJ9	Clear	AA	-	NA	NA
21/05/2014	UK	Starling	LE97171	ESJ10	Clear	AA	-	NA	NA
22/05/2014	UK	Yellowhammer	X648349	ESJ11	Clear	AA	-	NA	NA
22/05/2014	UK	Yellowhammer	TP21409	ESJ12	Clear	AA	-	NA	NA
22/05/2014	UK	Yellowhammer	TP21410	ESJ13	Clear	AA	-	NA	NA
22/05/2014	UK	Yellowhammer	TP21411	ESJ14	Clear	AA	-	NA	NA
29/05/2014	UK	Jackdaw		NJ1	Clear	AA	?	NA	NA
30/05/2014	UK	Turtle Dove	EY79952	NJ2	Clear	AA	+	NA	NA
28/05/2014	UK	Grey Partridge	EG82587	ESJ15	Clear	AA	-	NA	NA
28/05/2014	UK	Grey Partridge	EG82588	ESJ16	Clear	AA	-	NA	NA
28/05/2014	UK	Jay	DE32903	ESJ17	Clear	AA	-	NA	NA
29/05/2014	UK	Turtle Dove	EG82589	ESJ18	Pouch swollen, liquid cloudy	AA	+	GEO	un-ID
29/05/2014	UK	Turtle Dove	EG82590	ESJ19	clear	AA	-	NA	NA
29/05/2014	UK	House Sparrow	TP21413	ESJ20	Clear	AA	-	NA	NA
29/05/2014	UK	Dunnock	TP21412	ESJ21	Clear	AA	-	NA	NA
29/05/2014	UK	Stock Dove	EG82591	ESJ22	Liquid slightly cloudy	AA	?	NA	NA
29/05/2014	UK	Stock Dove	EG82592	ESJ23	Liquid slightly cloudy	AA	?	NA	NA
29/05/2014	UK	Collared Dove	EG82593	ESJ24	Liquid slightly cloudy	AA	-	NA	NA
30/05/2014	UK	Pheasant		ESJ25	clear	AA	-	NA	NA
30/05/2014	UK	Red Legged Partridge		ESJ26	Clear	AA	-	NA	NA
30/05/2014	UK	Red Legged Partridge		ESJ27	Clear	AA	-	NA	NA
30/05/2014	UK	Pheasant		ESJ28	Clear	AA	-	NA	NA
30/05/2014	UK	Pheasant		ESJ29	Pouch swollen, liquid cloudy	AA	+	Type A	A1

30/07/2014	UK	Woodpigeon NT		ESJ30	Pouch swollen, liquid cloudy	AA	?	NA	NA
30/07/2014	UK	Woodpigeon NT		ESJ31	Pouch swollen, liquid cloudy	AA	?	NA	NA
28/07/2014	UK	Turtle Dove NT	EG79826	ESJ32	Pouch swollen, liquid cloudy	AA	+	WQR-Env	NA
28/07/2014	UK	Turtle Dove NT	EG79827	ESJ33	Pouch swollen, liquid cloudy	AA	+	GEO	NA
28/07/2014	UK	Turtle Dove NT	EY79827	ESJ34	Pouch swollen, liquid cloudy	AA	?	NA	NA
28/07/2014	UK	Turtle Dove NT	EY79826	ESJ35	Pouch swollen, liquid cloudy	AA	+	GEO	NA
30/06/2014	UK	Collared Dove NT	DEAD	ESR1	Pouch very swollen, liquid very cloudy	AA	+	WQR-Env	NA
27/06/2014	UK	Turtle Dove NT	?	ESR2	Pouch swollen, liquid cloudy	AA	+	NA	NA
27/06/2014	UK	Turtle Dove NT	?	ESR3	Pouch swollen, liquid cloudy	AA	+	GEO	NA
27/06/2014	UK	Collared Dove NT	EY79954	ESR4	Pouch swollen, liquid cloudy	AA	+	NA	NA
01/07/2014	UK	Stock Dove	EY79957	ESR5	Pouch swollen, liquid cloudy	AA	+	NA	NA
27/06/2014	UK	Collared Dove NT	EY79955	ESR6	Pouch very swollen, liquid very cloudy	AA	+	NA	NA
01/07/2014	UK	HL/ BP 1		ESR7	Liquid cloudy, muddy	AA	-	NA	NA
01/07/2014	UK	HL/ H20 tray		ESR8	Liquid cloudy	AA	-	NA	NA
01/07/2014	UK	HL/ FP 1		ESR9	Liquid cloudy	AA	-	NA	NA
01/07/2014	UK	HL/ H20C		ESR10	Liquid very cloudy	AA	-	NA	NA
31/07/2014	UK	Turtle Dove NT	EG59172	ESR11	Pouch swollen, liquid cloudy	AA	+	Type C	C8
31/07/2014	UK	Turtle Dove NT	EG59173	ESR12	Pouch swollen, liquid cloudy	AA	+	Type C	NA
01/08/2014	UK	Woodpigeon NT	FH76714	ESR13	Pouch swollen, liquid cloudy	AA	+	Type C	C4

01/08/2014	UK	Woodpigeon NT	FH76713	ESR14	Pouch swollen, liquid cloudy	AA	+	NA	C4
02/08/2014	UK	Turtle Dove	EG59172	ESR15	Pouch swollen, liquid cloudy	AA	+	Type C	C8
02/08/2014	UK	Turtle Dove	EG59173	ESR16	Pouch swollen, liquid cloudy	AA	+	NA	NA
24/07/2014	UK	Woodpigeon NT		ESR17	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
24/07/2014	UK	Woodpigeon NT		ESR18	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
04/08/2014	UK	Woodpigeon NT		ESR19	Pouch very swollen, liquid very cloudy	AA	+	Type C & Type A	C2
04/08/2014	UK	Woodpigeon NT		ESR20	Pouch very swollen, liquid very cloudy	AA	+	Type A	A1
04/08/2014	UK	Woodpigeon NT		ESR21	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
04/08/2014	UK	Woodpigeon NT		ESR22	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
04/08/2014	UK	Woodpigeon NT		ESR23	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
04/08/2014	UK	Woodpigeon NT		ESR24	Pouch slightly swollen, liquid not cloudy	AA	?	NA	NA
07/08/2014	UK	Turtle Dove NT		ESR25	Pouch very swollen, liquid very cloudy	AA	+	Type A	NA
06/08/2014	UK	Woodpigeon NT		ESR26	Pouch slightly swollen, liquid slightly cloudy	AA	?	NA	NA
06/08/2014	UK	Woodpigeon NT		ESR27	Pouch slightly swollen, liquid slightly	AA	?	NA	NA

					cloudy				
06/08/2014	UK	Woodpigeon NT		ESR28	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
06/08/2014	UK	Woodpigeon NT		ESR29	Pouch slightly swollen, liquid slightly cloudy	AA	-	NA	NA
13/08/2014	UK	Woodpigeon NT		ESR30	Pouch very swollen, liquid very cloudy	AA	+	Type C	C4
13/08/2014	UK	Woodpigeon NT		ESR31	Pouch very swollen, liquid very cloudy	AA	+	Type C	C4
15/08/2014	UK	Woodpigeon NT		ESR32	Pouch very swollen, liquid very cloudy	AA	?	NA	NA
15/08/2014	UK	Woodpigeon NT		ESR33	Pouch very swollen, liquid very cloudy	AA	+	Type C	C4
27/08/2014	UK	Woodpigeon NT		ESR34	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
27/08/2014	UK	Woodpigeon NT		ESR35	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
29/08/2014	UK	Woodpigeon NT	FH85619	ESR36	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
29/08/2014	UK	Woodpigeon NT		ESR37	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
02/09/2014	UK	Greenfinch	TT94076	ESR38	Pouch swollen, liquid cloudy	AA	+	Type A	A1
02/09/2014	UK	Greenfinch	TT94081	ESR39	Pouch swollen, liquid cloudy	AA	+	Type A	A1
03/09/2014	UK	Collared Dove NT	EG59058	ESR40	Pouch very swollen, liquid cloudy	AA	+	NA	NA
03/09/2014	UK	Collared Dove NT	EG59057	ESR41	Pouch very swollen, liquid cloudy	AA	+	NA	NA
02/09/2014	UK	House Sparrow	TT94091	ESR42	Pouch swollen, liquid cloudy	AA	+	Type	A1

								A	
19/05/2015	UK	PG/ BP 1		CO1	cloudy	AA	?	NA	NA
21/05/2015	UK	LI/ BP 1		CO2	cloudy, white mould	AA	-	NA	NA
21/05/2015	UK	AH/ BP 1		CO3	cloudy, white mould	AA	?	NA	NA
19/05/2015	UK	UH/ BP 1		CO4	slightly cloudy	AA	?	NA	NA
26/05/2015	UK	Stock Dove		CO5	swollen pouch, liquid cloudy	AA	+	Type A	A1
26/05/2015	UK	Stock Dove		CO6	swollen pouch, liquid cloudy	AA	+	Type A	A1
26/05/2015	UK	UH/ BP 1		CO7	cloudy, white mould	AA	?	NA	NA
26/05/2015	UK	PG/ BP 1		CO8	cloudy, white mould	AA	?	NA	NA
29/05/2015	UK	LI/ BP 1		CO9	Cloudy	AA	?	NA	NA
29/05/2015	UK	Sizewell		CO10	Cloudy	AA	?	NA	NA
31/05/2015	UK	Bait pile	Rodgers/ BP 1	CO11	Very cloudy	AA	?	NA	NA
29/05/2015	UK	HL/ BP 1		CO12	Cloudy	AA	?	NA	NA
28/05/2015	UK	AH/ BP 1		CO13	Cloudy	AA	?	NA	NA
30/05/2015	UK	Dereks/ BP 1		CO14	Cloudy	AA	?	NA	NA
29/05/2015	UK	OB/ BP 1		CO15	Cloudy	AA	?	NA	NA
29/05/2015	UK	Feltwell		CO16	Cloudy	AA	?	NA	NA
29/05/2015	UK	Stuston		CO17	Cloudy	AA	?	NA	NA
29/05/2015	UK	Abbey Farm		CO18	Cloudy	AA	?	NA	NA
29/05/2015	UK	Ray's Kelsale		CO19	Cloudy	AA	+	NA	NA
03/06/2015	UK	Woodpigeon	FH78614	CO20	Pouch not swollen, liquid clear	AA	?	NA	NA
03/06/2015	UK	Pheasant		CO21	Pouch not swollen, liquid clear	AA	?	NA	NA

01/06/2015	UK	Woodpigeon	FH78615	CO22	Pouch not swollen, liquid clear	AA	?	NA	NA
01/06/2015	UK	Moorhen	FH78613	CO23	Pouch not swollen, liquid clear	AA	?	NA	NA
01/06/2015	UK	Blackbird	LE97305	CO24	Pouch not swollen, liquid clear	AA	+	NA	NA
01/06/2015	UK	Stock Dove	EY79813	CO25	Pouch not swollen, liquid clear	AA	-	NA	NA
04/06/2015	UK	Yellowhammer	TX56503	CO26	Pouch swollen, liquid cloudy	AA	+	Type A	A1
04/06/2015	UK	Yellowhammer	TX56501	CO27	Pouch swollen, liquid cloudy	AA	+	Type A	A1
04/06/2015	UK	Turtle Dove	EG82505	CO28	Pouch swollen, liquid cloudy	AA	+	Tcl-1	un-ID
11/06/2015	UK	Woodpigeon NT		CO29	Pouch swollen, liquid cloudy	AA	+	Type A	NA
11/06/2015	UK	Woodpigeon NT		CO30	Pouch swollen, liquid cloudy	AA	?	NA	NA
01/06/2015	UK	Frampton		CO31	Cloudy	AA	?	NA	NA
04/06/2015	UK	PG/ BP 1		CO32	Cloudy	AA	+	NA	NA
03/06/2015	UK	LI/ BP 1		CO33	Cloudy	AA	-	NA	NA
04/06/2015	UK	UH/ BP 1		CO34	Cloudy	AA	?	NA	NA
04/06/2015	UK	AH/ BP 1		CO35	Cloudy	AA	?	NA	NA
12/06/2015	UK	Stock Dove	EY79963	CO36	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
12/06/2015	UK	Woodpigeon	FH44837	CO37	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
12/06/2015	UK	Woodpigeon	FH55402	CO38	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
12/06/2015	UK	Stock Dove	EY79964	CO39	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
13/05/2015	UK	Control seed swab		K01	Cloudy	AA	?	NA	NA
13/05/2015	UK	PG/ BP 1		K02	Cloudy	AA	?	NA	NA

13/05/2015	UK	UH/ BP 1		K03	Cloudy and white fungus	AA	?	NA	NA
13/05/2015	UK	LI/ BP 1		K04	Cloudy	AA	?	NA	NA
13/05/2015	UK	AH/ BP 1		K05	Cloudy	AA	?	NA	NA
26/05/2015	UK	Bullfinch	Z405521	K06	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
26/05/2015	UK	Blackbird	LH25589	K09	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
26/05/2015	UK	Dunnock	TXO6427	K10	Pouch not swollen, liquid not cloudy	AA	+	NA	un-ID
26/05/2015	UK	Great Tit	Z405523	K11	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
26/05/2015	UK	Bullfinch	Z146354	K12	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Long Tailed Tit	HJP145	K13	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
26/05/2015	UK	Great Tit	D802683	K16	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Dunnock	TX86779	K17	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Blackcap	Z405575	K18	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
26/05/2015	UK	Dunnock	TV18363	K19	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Dunnock	Y848383	K20	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Yellowhammer	TV18920	K21	Pouch not swollen, liquid not cloudy	AA	+	NA	un-ID
26/05/2015	UK	Whitethroat	Z405062	K22	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
26/05/2015	UK	Blackbird	LH25593	K23	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Long Tailed Tit	HJP183	K25	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
26/05/2015	UK	Chiffchaff	HJP152	K26	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
26/05/2015	UK	Stock Dove	EY36558	K27	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
27/06/2015	UK	Red Legged Partridge	RLP UH	K28	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
27/06/2015	UK	Woodpigeon	FH78611	K29	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
28/05/2015	UK	Red Legged	RLP Dereks	K30	Pouch not swollen, liquid not cloudy	AA	?	NA	NA

		Partridge							
29/05/2015	UK	Greenfinch	TT94226	K31	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
29/05/2015	UK	Greenfinch	TT94227	K32	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
29/05/2015	UK	Woodpigeon	FH78612	K33	Pouch slightly swollen, liquid not cloudy	AA	-	NA	NA
03/06/2015	UK	Woodpigeon	FH78641	K34	Pouch swollen, liquid cloudy	AA	+	Type A	A1
04/06/2015	UK	Red Legged Partridge	RLP LI	K36	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
04/06/2015	UK	Yellowhammer	TX56502	K37	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
04/06/2015	UK	Chaffinch	D563411	K38	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Great Tit	D563408	K39	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Rook	FH55483	K40	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Turtle Dove	EG82506	K41	Pouch slightly swollen, liquid slightly cloudy	AA	+	Tcl-1	T2-TD
04/06/2015	UK	Woodpigeon	FH55482	K42	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
04/06/2015	UK	Blackcap	D563405	K43	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Blackcap	D563407	K44	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Woodpigeon	FH55481	K45	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Chaffinch	D563409	K46	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Great Tit	D563406	K47	Pouch very slightly swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Blackcap	D563402	K48	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Bluetit	D563403	K49	Pouch not swollen, liquid not cloudy	AA	?	NA	NA

04/06/2015	UK	Blackcap	D563404	K50	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Bluetit	D563401	K51	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Chaffinch	D563410	K52	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
04/06/2015	UK	Turtle Dove	EY79814	K53	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
05/06/2015	UK	Chaffinch	unringed	K54	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
05/06/2015	UK	Greenfinch	TT94228	K56	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
05/06/2015	UK	House Sparrow	TT94063	K58	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
05/06/2015	UK	Chaffinch	D883562	K59	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
05/06/2015	UK	House Sparrow	TT94229	K60	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	Goldfinch	D530630	K61	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Goldfinch	D563416	K62	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	Great Tit	D563417	K63	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	Dunnock	TX07204	K64	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	House Sparrow	TX56504	K65	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	Chaffinch	D563421	K66	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
09/06/2015	UK	Great Tit	D901011	K67	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Goldfinch	D563414	K68	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Goldfinch	D563415	K69	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Chaffinch		K70	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Woodpigeon	FH55301	K71	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
09/06/2015	UK	Collared Dove	EG82507	K72	Pouch not swollen, liquid slightly cloudy	AA	?	NA	NA
18/06/2015	UK	Woodpigeon NT		K73	Pouch not swollen, liquid not cloudy	AA	+	GEO & Type	NA

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18/06/2015	UK	Pheasant		K74	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
18/06/2015	UK	Woodpigeon NT		K75	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
11/06/2015	UK	Woodpigeon NT		K76	Pouch not swollen, liquid not cloudy	AA	+	NA	NA
11/06/2015	UK	Woodpigeon	FH55401	K77	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
11/06/2015	UK	Woodpigeon NT		K78	Pouch not swollen, liquid not cloudy	AA	+	NA	C4
17/06/2015	UK	PG/ BP 1		K79	Cloudy	AA	-	NA	NA
18/06/2015	UK	LI/ BP 1		K80	Cloudy	AA	-	NA	NA
14/06/2015	UK	Bait pile	Rodgers/ BP 1	K81	Cloudy	AA	-	NA	NA
17/06/2015	UK	UH/ BP 1		K82	Cloudy	AA	-	NA	NA
14/06/2015	UK	Dereks/ BP 1		K83	Cloudy	AA	-	NA	NA
12/06/2015	UK	LI/ BP 1		K84	Cloudy with white mould	AA	-	NA	NA
11/06/2015	UK	PG/ BP 1		K85	Cloudy	AA	-	NA	NA
11/06/2015	UK	UH/ BP 1		K86	Cloudy	AA	-	NA	NA
14/06/2015	UK	Martin Down barn/ BP 1		K87	Cloudy	AA	-	NA	NA
18/06/2015	UK	AH/ BP 1		K88	Cloudy	AA	-	NA	NA
11/06/2015	UK	AH/ BP 1		K89	Cloudy	AA	-	NA	NA
25/06/2015	UK	UH/ BP 1		K90	Cloudy	AA	-	NA	NA
25/06/2015	UK	AH/ BP 1		K91	Cloudy	AA	-	NA	NA
25/06/2015	UK	LI/ BP 1		K92	Cloudy	AA	-	NA	NA
25/06/2015	UK	PG/ BP 1		K93	Cloudy	AA	-	NA	NA
26/06/2015	UK	Turtle Dove		K94	Pouch not swollen, liquid not cloudy	AA	+	Tcl-1	T2-TD
27/6/615	UK	Martin Down barn/ BP 1		K95	Cloudy	AA	-	NA	NA

27/6/615	UK	Bait pile	Rodgers/ BP 1	K96	Cloudy	AA	-	NA	NA
27/6/615	UK	Dereks/ BP 1		K97	Cloudy	AA	-	NA	NA
30/07/2015	UK	Greenfinch	TT94267	K98	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883599	K99	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Bluetit	D883593	K100	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Bluetit	D883588	L01	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
30/07/2015	UK	Greenfinch	TT94266	L02	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Chaffinch	D883596	L03	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Dunnock	TT94264	L04	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Dunnock	TT94261	L05	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
30/07/2015	UK	Dunnock	TT94257	L06	Pouch not swollen, liquid not cloudy	AA	-	NA	NA
30/07/2015	UK	Goldfinch	D883600	L07	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Chaffinch	D883602	L08	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Chaffinch	D883609	L09	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Chaffinch	D883605	L10	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883604	L11	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Greenfinch	TT94272	L12	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883606	L13	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	House Sparrow	TT94262	L14	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Great Tit	D883589	L15	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Chaffinch	D883601	L16	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883598	L17	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883590	L18	Pouch not swollen, liquid not cloudy	AA	?	NA	NA

30/07/2015	UK	Dunnock	TT94260	L19	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Dunnock	TT94256	L20	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Great Tit	D883594	L21	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	House Sparrow	TT94259	L22	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	House Sparrow	TT94263	L23	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	House Sparrow	TT94258	L24	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Great Tit	D883592	L25	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883591	L26	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Bluetit	D883584	L27	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
30/07/2015	UK	Goldfinch	D883595	L28	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Dunnock	TT94253	L29	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Goldfinch	D883616	L30	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Chaffinch	D883615	L31	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Chaffinch	D883612	L32	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	House Sparrow	TT94275	L33	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Goldfinch	D883619	L34	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	House Sparrow	TT94273	L35	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	House Sparrow	TT94274	L36	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Robin	D883613	L37	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Dunnock	X602943	L38	Pouch not swollen, liquid not cloudy	AA	?	NA	NA
31/07/2015	UK	Greenfinch	TT94277	L39	Cloudy, white mould	AA	?	NA	NA
01/09/2015	UK	Fountains Abbey Bird feeder		L40	Cloudy	AA	?	NA	NA
01/09/2015	UK	Fountains Abbey Bird table		L41	White mould	AA	?	NA	NA
08/09/2015	UK	Fountains Abbey Bird table		L42		AA	-	NA	NA

08/09/2015	UK	Fountains Abbey Bird table	L43		AA	?	NA	NA
15/09/2015	UK	Fountains Abbey Bird table	L44		AA	-	NA	NA
15/09/2015	UK	Fountains Abbey Bird table	L45		AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove		1	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove		2	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove		3	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove		4	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		5	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove		6	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove		7	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		8	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		9	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove		10	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove		11	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove		12	AA	?	NA	NA
2012-2013	B.Faso	African Collared Dove		13	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		14	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove		15	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		16	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove		17	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove		18	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		19	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove		20	AA	?	NA	NA
2012-2013	B.Faso	African Collared Dove		21	AA	?	NA	NA

2012-2013	B.Faso	Turtle Dove	22	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	24	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	25	AA	+	NA	NA
2012-2013	B.Faso	Vinaceous Dove	26	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	27	AA	+	NA	NA
2012-2013	B.Faso	African Collared Dove	28	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	29	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	30	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	31	AA	-	NA	NA
2012-2013	B.Faso	African Mourning Dove	32	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	33	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	34	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	35	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	36	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	37	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	38	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	39	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	40	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	41	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	42	AA	+	Tcl-1	NA
2012-2013	B.Faso	Turtle Dove	43	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	44	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	45	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	46	AA	-	NA	NA

2012-2013	B.Faso	Laughing Dove	47	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	48	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	49	AA	-	NA	NA
2012-2013	B.Faso	African Mourning Dove	50	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	51	AA	-	NA	NA
2012-2013	B.Faso	Turtle Dove	52	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	53	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	54	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	55	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	56	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	57	AA	+	Tcl-1	NA
2012-2013	B.Faso	Turtle Dove	58	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	59	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	60	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	61	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	62	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	63	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	64	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	65	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	66	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	67	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	68	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	69	AA	?	NA	NA
2012-2013	B.Faso	African Mourning Dove	70	AA	?	NA	NA

2012-2013	B.Faso	Laughing Dove	71	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	72	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	73	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	74	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	75	AA	?	NA	NA
2012-2013	B.Faso	Vinaceous Dove	76	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	77	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	78	AA	?	NA	NA
2012-2013	B.Faso	African Mourning Dove	79	AA	-	NA	NA
2012-2013	B.Faso	African Mourning Dove	80	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	81	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	82	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	83	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	84	AA	+	NA	NA
2012-2013	B.Faso	Vinaceous Dove	85	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	86	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	87	AA	+	Tcl-1	NA
2012-2013	B.Faso	Laughing Dove	88	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	89	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	90	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	91	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	92	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	93	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	94	AA	?	NA	NA

2012-2013	B.Faso	Laughing Dove	95	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	96	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	97	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	98	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	99	AA	-	NA	NA
2012-2013	B.Faso	Vinaceous Dove	100	AA	-	NA	NA
2012-2013	B.Faso	Turtle Dove	101	AA	+	NA	NA
2012-2013	B.Faso	Turtle Dove	102	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	103	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	104	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	105	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	106	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	107	AA	+	GEO	NA
2012-2013	B.Faso	Laughing Dove	108	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	109	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	110	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	111	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	112	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	113	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	114	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	115	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	116	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	117	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	118	AA	?	NA	NA

2012-2013	B.Faso	Laughing Dove	119	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	120	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	121	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	122	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	123	AA	-	NA	NA
2012-2013	B.Faso	Laughing Dove	124	AA	-	NA	NA
2012-2013	B.Faso	Turtle Dove	125	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	126	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	127	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	128	AA	+	NA	NA
2012-2013	B.Faso	African Mourning Dove	130	AA	?	NA	NA
2012-2013	B.Faso	African Mourning Dove	131	AA	?	NA	NA
2012-2013	B.Faso	Turtle Dove	132	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	133	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	134	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	135	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	136	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	137	AA	?	NA	NA
2012-2013	B.Faso	Laughing Dove	138	AA	+	NA	NA
2012-2013	B.Faso	Laughing Dove	139	AA	-	NA	NA
Jan-Feb 2015	Sen.	Laughing Dove	LD01	AA	+	NA	NA
Jan-Feb 2015	Sen.	Laughing Dove	LD02	AA	+	NA	NA
Jan-Feb 2015	Sen.	Laughing Dove	LDS03	AA	+	Type C	NA

Jan-Feb 2015	Sen.	Laughing Dove	LD031	AA	+	NA	NA
Jan-Feb 2015	Sen.	Black Billed Wood Dove	BBWD01	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS01	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS02	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS03	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS04	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS05	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQS06	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQRF28501	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQRF28502	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQRF28503	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Namaqua Dove	NQRF28504	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Vinaceous Dove	TV01	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB01	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB02	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB03	AA	+	GEO	un-ID
Jan-Feb 2015	Sen.	Turtle Dove	TDB05	AA	+	GEO-TD	un-ID
Jan-Feb 2015	Sen.	Turtle Dove	TDB06	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB07	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB08	AA	+	Type III	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB09	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB10	AA	+	Type C	NA

Jan-Feb 2015	Sen.	Turtle Dove	TDB11	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB12	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB13	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB14	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB15	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB16	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB17	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB18	AA	+	T. tenax	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB19A	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB19B	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB20	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB21	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB22	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB23	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB24	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB25	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB26	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB27	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB28	AA	+	T. tenax	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB29	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB30	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDB31	AA	+	Tcl-1	NA

Jan-Feb 2015	Sen.	Turtle Dove	TDB32	AA	+	Tcl-1	un-ID
Jan-Feb 2015	Sen.	Turtle Dove	TDB33	AA	+	NA	un-ID
Jan-Feb 2015	Sen.	Turtle Dove	TDB34	AA	+	T. tenax	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA01	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA03	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA04	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA05	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA06	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA07	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA08	AA	+	GEO	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA09	AA	+	T. tenax	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA10	AA	+	Tcl-1	NA
Jan-Feb 2015	Sen.	Turtle Dove	TDA11	AA	+	NA	NA
Jan-Feb 2015	Sen.	Turtle Dove	TD DEAD	AA	+	GEO	un-ID
Jan-Feb 2015	Sen.	Turtle Dove	Dead 01	AA	+	NA	NA

Table 7.3: Raw data for water sources sampled and analysed using a GLMM in Chapter 3.

Sample	year	month	county	source	Rainfall	Temp.	Trich presence	Type A	Type C	Tcl1	GEO	state
A66	2013	June	East Anglia	Water source	0	13.9	1	0	0	1	0	full
A67	2013	June	East Anglia	Water source	0	13.9	1	0	0	1	0	full
A69	2013	June	East Anglia	Water source	0	13.9	0	na	na	na	na	full
B46	2013	July	East Anglia	Water source	0	14.2	0	na	na	na	na	dry
F14	2014	May	East Anglia	Water tray	0	12.4	1	na	na	na	na	full
F17	2014	May	East Anglia	Water tray	0	12.4	0	na	na	na	na	full
F71	2014	June	East Anglia	Control water tray	0	14.2	0	na	na	na	na	full
F72	2014	June	East Anglia	Control water tray	0	14.2	0	na	na	na	na	full
F73	2014	June	East Anglia	Water tray	0	14.2	0	na	na	na	na	full
F78	2014	June	East Anglia	Water tray	0	14.2	1	0	0	1	0	full
G56	2014	June	East Anglia	Control water tray	0	16.6	0	na	na	na	na	dry
G76	2014	June	Anglia	Water tray	0	14.7	0	na	na	na	na	full

G79	2014	June	East Anglia	Control water tray	0	14.7	0	na	na	na	na	full
G80	2014	June	East Anglia	Water tray	0	18.2	0	na	na	na	na	moist
G82	2014	June	East Anglia	Control water tray	0	18.2	0	na	na	na	na	moist
G84	2014	June	East Anglia	Control water tray	0	18.2	0	na	na	na	na	full
G86	2014	June	East Anglia	Water tray	0	18.2	0	na	na	na	na	moist
G87	2014	June	East Anglia	Control water tray	0	18.2	1	0	0	0	1	moist
G95	2014	June	East Anglia	Control water tray	0	12.1	0	na	na	na	na	full
G97	2014	June	East Anglia	Water tray	0	12.1	0	na	na	na	na	full
G98	2014	June	East Anglia	Water tray	0	12.1	0	na	na	na	na	full
G99	2014	June	East Anglia	Control water tray	0	12.1	0	na	na	na	na	full
G100	2014	June	East Anglia	Control water tray	0	12.1	0	na	na	na	na	full
H18	2014	June	East Anglia	Control water tray	0	13.9	0	na	na	na	na	full
H24	2014	June	East Anglia	Water tray	0	13.9	0	na	na	na	na	full
H25	2014	June	East	Water tray	0	13.9	0	na	na	na	na	moist

H28	2014	June	Anglia East	Water tray	0	11.4	0	na	na	na	na	full
H32	2014	June	Anglia East	Control water tray	0	13.9	1	1	1	0	0	full
H36	2014	June	Anglia East	Control water tray	0	11.4	0	na	na	na	na	full
H38	2014	June	Anglia East	Control water tray	0	13.9	0	na	na	na	na	full
I40	2014	July	Anglia East	Control water tray	0	14.8	0	na	na	na	na	full
I41	2014	July	Anglia East	Water tray	0	14.8	0	na	na	na	na	full
I45	2014	July	Anglia East	Control water tray	0	14.8	1	na	na	na	na	full
I47	2014	July	Anglia East	Water tray	0	14.8	0	na	na	na	na	full
I86	2014	July	Anglia East	Water tray	0	19.8	0	na	na	na	na	full
I88	2014	July	Anglia East	Control water tray	0	19.8	1	na	na	na	na	full
I91	2014	July	Anglia East	Water tray	0	19.8	0	na	na	na	na	full
ESR8	2014	July	Anglia East	Water tray	0	13.2	0	na	na	na	na	full
ESR10	2014	July	Anglia	Control water tray	0	13.2	0	na	na	na	na	full

A33	2013	June	Essex	Water source	0	14.1	0	na	na	na	na	full
A42	2013	June	Essex	Water source	0	14.1	1	na	na	na	na	full
A45	2013	June	Essex	Water source	0	13.9	1	0	0	1	0	full
A49	2013	June	Essex	Water source	0	13.9	0	na	na	na	na	full
B86	2013	July	Essex	Water source	0	19.8	0	na	na	na	na	full
F19	2014	May	Essex	Water tray	0	12.4	0	na	na	na	na	full
F24	2014	May	Essex	Control water tray	0	12.4	0	na	na	na	na	full
F28	2014	May	Essex	Water tray	0	12.4	0	na	na	na	na	full
F40	2014	May	Essex	Water tray	0	17.3	0	na	na	na	na	moist
F41	2014	May	Essex	Control water tray	0	17.3	1	1	0	0	0	full
F46	2014	May	Essex	Control water tray	0	18	1	1	0	0	0	full
F47	2014	May	Essex	Control water tray	0	18	1	1	0	0	0	moist
F48	2014	May	Essex	Water tray	0	18	1	1	0	0	0	full
F51	2014	May	Essex	Water tray	0	18	1	1	0	0	0	full
F56	2014	May	Essex	Control water tray	0	18	1	1	0	0	0	full
F79	2014	June	Essex	Water tray	1	13.1	0	na	na	na	na	full
F83	2014	June	Essex	Water tray	1	13.1	1	1	0	0	0	full
F85	2014	June	Essex	Water tray	1	13.1	1	1	0	0	0	full
F86	2014	June	Essex	Control water tray	1	13.1	0	na	na	na	na	full
F89	2014	June	Essex	Control water tray	1	13.1	0	na	na	na	na	full
F94	2014	June	Essex	Water tray	0	14.6	0	na	na	na	na	full
F95	2014	June	Essex	Control water tray	0	14.6	0	na	na	na	na	full
F97	2014	June	Essex	Water tray	0	14.6	0	na	na	na	na	full
G31	2014	June	Essex	Water tray	0	17	0	na	na	na	na	moist

G34	2014	June	Essex	Control water tray	0	17	0	na	na	na	na	moist
G35	2014	June	Essex	Water tray	0	17	0	na	na	na	na	full
G36	2014	June	Essex	Control water tray	0	17	0	na	na	na	na	full
G39	2014	June	Essex	Control water tray	0	17	0	na	na	na	na	moist
G42	2014	June	Essex	Water tray	0	17	0	na	na	na	na	moist
G45	2014	June	Essex	Control water tray	0	18.4	1	0	1	0	0	full
G46	2014	June	Essex	Water tray	0	18.4	1	na	na	na	na	full
G48	2014	June	Essex	Control water tray	0	18.4	0	na	na	na	na	moist
G50	2014	June	Essex	Water tray	0	18.4	0	na	na	na	na	dry
G53	2014	June	Essex	Water tray	0	18.4	0	na	na	na	na	dry
G58	2014	June	Essex	Control water tray	0	14.9	0	na	na	na	na	moist
G61	2014	June	Essex	Water tray	0	14.9	0	na	na	na	na	dry
G63	2014	June	Essex	Control water tray	0	14.9	0	na	na	na	na	moist
G73	2014	June	Essex	Control water tray	0	14.9	0	na	na	na	na	full
G74	2014	June	Essex	Water tray	0	14.9	0	na	na	na	na	moist
H21	2014	June	Essex	Control water tray	0.3	13.9	0	na	na	na	na	full
H31	2014	June	Essex	Water tray	0.3	13.9	0	na	na	na	na	full
H34	2014	June	Essex	Water tray	0.3	13.9	1	1	0	0	0	full
H43	2014	June	Essex	Water tray	0	14.6	0	na	na	na	na	full
H44	2014	June	Essex	Control water tray	0	14.6	1	1	0	0	0	full
H45	2014	June	Essex	Water tray	0.3	13.9	1	1	0	0	0	dry
H46	2014	June	Essex	Water tray	0.3	13.9	0	na	na	na	na	dry
H49	2014	June	Essex	Control water tray	0.3	13.9	0	na	na	na	na	full
H53	2014	June	Essex	Water tray	0.3	14.6	0	na	na	na	na	full

H55	2014	June	Essex	Water tray	0.3	14.6	0	na	na	na	na	dry
H57	2014	June	Essex	Control water tray	0.3	14.6	0	na	na	na	na	full
H74	2014	July	Essex	Control water tray	2	15.6	0	na	na	na	na	full
H76	2014	July	Essex	Water tray	2	15.6	0	na	na	na	na	dry
H77	2014	July	Essex	Water tray	2	15.6	0	na	na	na	na	moist
H79	2014	July	Essex	Control water tray	2	15.6	0	na	na	na	na	full
H81	2014	July	Essex	Control water tray	2	15.6	0	na	na	na	na	full
H87	2014	July	Essex	Water tray	2	15.6	0	na	na	na	na	full
H88	2014	July	Essex	Control water tray	2	15.6	0	na	na	na	na	full
I11	2014	July	Essex	Control water tray	0	16.8	0	na	na	na	na	full
I13	2014	July	Essex	Water tray	0	16.8	0	na	na	na	na	moist
I14	2014	July	Essex	Water tray	0	16.8	0	na	na	na	na	full
I16	2014	July	Essex	Control water tray	0	16.8	0	na	na	na	na	moist
I20	2014	July	Essex	Control water tray	0	16.8	0	na	na	na	na	full
I21	2014	July	Essex	Water tray	0	16.8	0	na	na	na	na	dry
I22	2014	July	Essex	Control water tray	0	16.8	0	na	na	na	na	full
I24	2014	July	Essex	Water tray	0	16.8	0	na	na	na	na	dry
I28	2014	July	Essex	Water tray	1.4	17	0	na	na	na	na	full
I30	2014	July	Essex	Control water tray	1.4	17	0	na	na	na	na	full
I33	2014	July	Essex	Control water tray	1.4	17	1	na	na	na	na	full
I76	2014	July	Essex	Water tray	0	20.9	1	na	na	na	na	full
I78	2014	July	Essex	Control water tray	0	20.9	1	na	na	na	na	full
I92	2014	July	Essex	Water tray	0	21.8	0	na	na	na	na	full
I96	2014	July	Essex	Water tray	3.1	23.7	0	na	na	na	na	full

I97	2014	July	Essex	Control water tray	3.1	23.7	1	na	na	na	na	full
J01	2014	July	Essex	Control water tray	3.1	23.7	1	na	na	na	na	full
J04	2014	July	Essex	Water tray	3.1	23.7	1	0	1	0	0	full
J08	2014	July	Essex	Control water tray	3.1	23.7	1	0	1	0	0	full
J09	2014	July	Essex	Water tray	3.1	23.7	0	na	na	na	na	full
J11	2014	July	Essex	Water tray	3.1	23.7	1	0	1	0	0	full

Table 7.4: Raw data for food sources sampled and analysed using a GLMM in Chapter 3.

Sample	year	month	county	type	Rainfall	Temp.	Trich presence	Type A	Type C	Tcl1	GEO
A32	2013	June	Essex	high intensity	0	14.1	1	0	0	1	0
A34	2013	June	Essex	high intensity	0	14.1	1	na	na	na	na
A35	2013	June	Essex	high intensity	0	14.1	1	0	0	1	0
A36	2013	June	Essex	low intensity	0	14.1	0	na	na	na	na
A39	2013	June	Essex	high intensity	0	14.1	1	0	0	1	0
A40	2013	June	Essex	high intensity	0	14.1	1	0	0	1	0
A43	2013	June	Essex	high intensity	0	13.9	1	0	0	1	0
A44	2013	June	Essex	low intensity	0	13.9	0	na	na	na	na
A46	2013	June	Essex	high intensity	0	13.9	1	na	na	na	na
A47	2013	June	Essex	high intensity	0	13.9	1	0	0	1	0
A48	2013	June	Essex	low intensity	0	13.9	0	na	na	na	na
A50	2013	June	Essex	high intensity	0	13.9	1	0	0	1	0
A51	2013	June	Essex	low intensity	0	13.9	0	na	na	na	na
A63	2013	June	East	high	0	13.9	0	na	na	na	na

			Anglia	intensity							
			East	high							
A65	2013	June	Anglia	intensity	0	13.9	0	na	na	na	na
			East	high							
A68	2013	June	Anglia	intensity	0	13.9	1	na	na	na	na
			East								
A70	2013	June	Anglia	low intensity	0	13.9	0	na	na	na	na
			East	high							
A71	2013	June	Anglia	intensity	0	17.1	1	0	0	1	0
			East	high							
A72	2013	June	Anglia	intensity	0	17.1	0	na	na	na	na
				high							
A88	2013	June	Essex	intensity	0	14.1	0	na	na	na	na
				high							
A89	2013	June	Essex	intensity	0	14.1	1	0	0	1	0
A90	2013	June	Essex	low intensity	0	14.1	0	na	na	na	na
A100	2013	June	Essex	low intensity	0	15.7	0	na	na	na	na
				high							
B01	2013	June	Essex	intensity	0	15.7	0	na	na	na	na
				high							
B03	2013	June	Essex	intensity	0	15.7	0	na	na	na	na
				high							
B05	2013	June	Essex	intensity	0	15.7	1	na	na	na	na
				high							
B06	2013	June	Essex	intensity	0	15.7	1	na	na	na	na
			East	high							
B16	2013	July	Anglia	intensity	0	16.4	0	na	na	na	na

B17	2013	July	Essex	low intensity	0	18.4	0	na	na	na	na
B18	2013	July	Essex	low intensity	0	18.4	0	na	na	na	na
B21	2013	July	East Anglia	high intensity	0	16.4	0	na	na	na	na
B22	2013	July	East Anglia	high intensity	0	16.4	0	na	na	na	na
B23	2013	July	East Anglia	intensity	0	16.4	0	na	na	na	na
B24	2013	July	East Anglia	low intensity	0	16.4	0	na	na	na	na
B25	2013	July	East Anglia	high intensity	0	16.4	0	na	na	na	na
B26	2013	July	East Anglia	high intensity	0	16.4	0	na	na	na	na
B28	2013	July	Essex	intensity	0	18.4	1	na	na	na	na
B30	2013	July	Essex	low intensity	0	18.4	0	na	na	na	na
B31	2013	July	Essex	high intensity	0	18.4	0	na	na	na	na
B32	2013	July	Essex	high intensity	0	18.4	0	na	na	na	na
B33	2013	July	Essex	high intensity	0	18.4	1	na	na	na	na
B34	2013	July	Essex	high intensity	0	18.4	1	0	0	0	1
B35	2013	July	Essex	intensity	0	18.4	0	na	na	na	na

B37	2013	July	East Anglia	high intensity	0	16.6	0	na	na	na	na
B38	2013	July	East Anglia	high intensity	0	16.6	0	na	na	na	na
B40	2013	July	East Anglia	high intensity	0	14.2	0	na	na	na	na
B41	2013	July	East Anglia	low intensity	0	14.2	0	na	na	na	na
B47	2013	July	East Anglia	high intensity	0	14.2	0	na	na	na	na
B48	2013	July	East Anglia	high intensity	0	14.2	1	na	na	na	na
B53	2013	July	East Anglia	high intensity	0	14.2	0	na	na	na	na
B54	2013	July	Essex	high intensity	0	16.3	0	na	na	na	na
B56	2013	July	Essex	high intensity	0	16.3	1	na	na	na	na
B57	2013	July	Essex	high intensity	0	16.3	1	na	na	na	na
B58	2013	July	Essex	high intensity	0	16.3	1	na	na	na	na
B59	2013	July	Essex	high intensity	0	16.3	0	na	na	na	na
B63	2013	July	Essex	high intensity	0	16.3	1	0	0	0	1
B64	2013	July	Essex	low intensity	0	17	0	na	na	na	na

B66	2013	July	Essex	high intensity	0	17	1	0	0	0	1
B70	2013	July	Anglia East	high intensity	0	21	0	na	na	na	na
B71	2013	July	Anglia East	high intensity	0	21	0	na	na	na	na
B72	2013	July	Anglia East	high intensity	0	21	1	0	0	0	1
B73	2013	July	Anglia East	low intensity	0	21	0	na	na	na	na
B74	2013	July	Anglia East	high intensity	0	21	0	na	na	na	na
B76	2013	July	Anglia	low intensity	0	21	0	na	na	na	na
B77	2013	July	Essex	high intensity	0	20	0	na	na	na	na
B78	2013	July	Essex	high intensity	0	20	1	na	na	na	na
B79	2013	July	Essex	high intensity	0	20	1	na	na	na	na
B80	2013	July	Essex	low intensity	0	20	0	na	na	na	na
B83	2013	July	Essex	high intensity	0	19.8	1	na	na	na	na
B84	2013	July	Essex	high intensity	0	19.8	1	na	na	na	na
B87	2013	July	Essex	high intensity	0	19.8	0	na	na	na	na

B88	2013	July	Essex	high intensity	0	19.8	1	0	0	0	1
B89	2013	July	Anglia East	high intensity	0	20.6	0	na	na	na	na
B96	2013	July	Anglia East	high intensity	0	21.1	0	na	na	na	na
B97	2013	July	Anglia East	high intensity	0	21.1	0	na	na	na	na
B98	2013	July	Anglia East	high intensity	0	21.1	0	na	na	na	na
B99	2013	July	Anglia East	high intensity	0	21.1	0	na	na	na	na
B100	2013	July	Anglia	low intensity	0	21.1	0	na	na	na	na
C1	2013	July	Essex	high intensity	9.9	21.8	0	na	na	na	na
C2	2013	July	Essex	high intensity	9.9	21.8	1	na	na	na	na
C3	2013	July	Essex	high intensity	9.9	21.8	1	na	na	na	na
C4	2013	July	Essex	high intensity	9.9	21.8	0	na	na	na	na
F01	2014	May	Anglia East	high intensity	0	11.5	0	na	na	na	na
F02	2014	May	Anglia	high intensity	0	11.5	0	na	na	na	na
F03	2014	May	Essex	high	12.2	12.1	0	na	na	na	na

				intensity							
F04	2014	May	Essex	high intensity	12.2	12.1	0	na	na	na	na
F05	2014	May	Essex	high intensity	12.2	12.1	0	na	na	na	na
F06	2014	May	Essex	high intensity	12.2	12.1	0	na	na	na	na
F07	2014	May	Essex	high intensity	12.2	12.1	0	na	na	na	na
F08	2014	May	Essex	high intensity	12.2	12.1	0	na	na	na	na
F09	2014	May	Essex	intensity	12.2	12.1	0	na	na	na	na
F10	2014	May	East Anglia	low intensity	0	12.4	0	na	na	na	na
F11	2014	May	East Anglia	low intensity	0	12.4	0	na	na	na	na
F12	2014	May	East Anglia	low intensity	0	12.4	0	na	na	na	na
F13	2014	May	East Anglia	low intensity	0	12.4	0	na	na	na	na
F15	2014	May	East Anglia	high intensity	0	12.4	0	na	na	na	na
F16	2014	May	Anglia	high intensity	0	12.4	0	na	na	na	na
F18	2014	May	Essex	intensity	0	12.4	1	na	na	na	na

F20	2014	May	Essex	low intensity	0	12.4	1	na	na	na	na
F21	2014	May	Essex	high intensity	0	12.4	1	na	na	na	na
F22	2014	May	Essex	low intensity	0	12.4	1	na	na	na	na
F23	2014	May	Essex	high intensity	0	12.4	0	na	na	na	na
F25	2014	May	Essex	high intensity	0	12.4	0	na	na	na	na
F26	2014	May	Essex	low intensity	0	12.4	1	na	na	na	na
F27	2014	May	Essex	low intensity	0	12.4	1	na	na	na	na
F45	2014	May	East Anglia	high intensity	0	10	0	na	na	na	na
F49	2014	May	Essex	high intensity	0	18	1	1	0	0	0
F50	2014	May	Essex	high intensity	0	18	1	1	1	0	0
F52	2014	May	Essex	low intensity	0	18	0	na	na	na	na
F53	2014	May	Essex	low intensity	0	18	0	na	na	na	na
F55	2014	May	Essex	high intensity	0	18	1	1	0	0	0
F58	2014	May	Essex	high intensity	0	18	1	1	0	0	0
F74	2014	June	East Anglia	high intensity	0	14.2	0	na	na	na	na
F75	2014	June	East Anglia	low intensity	0	14.2	0	na	na	na	na

F76	2014	June	East Anglia	low intensity	0	14.2	0	na	na	na	na
F80	2014	June	Essex	high intensity	1	13.1	1	1	0	0	0
F81	2014	June	Essex	high intensity	1	13.1	1	1	0	0	0
F82	2014	June	Essex	low intensity	1	13.1	1	1	0	0	0
F84	2014	June	Essex	high intensity	1	13.1	1	1	0	0	0
F87	2014	June	Essex	low intensity	1	13.1	1	1	0	0	0
F93	2014	June	Essex	low intensity	0	14.6	0	na	na	na	na
F96	2014	June	Essex	high intensity	0	14.6	0	na	na	na	na
F98	2014	June	Essex	low intensity	0	14.6	0	na	na	na	na
G29	2014	June	Essex	high intensity	0	17	1	na	na	na	na
G30	2014	June	Essex	low intensity	0	17	0	na	na	na	na
G32	2014	June	Essex	high intensity	0	17	0	na	na	na	na
G33	2014	June	Essex	low intensity	0	17	0	na	na	na	na
G37	2014	June	Essex	high intensity	0	17	0	na	na	na	na
G38	2014	June	Essex	low intensity	0	17	0	na	na	na	na
G40	2014	June	Essex	high intensity	0	17	0	na	na	na	na
G41	2014	June	Essex	low intensity	0	17	0	na	na	na	na

G43	2014	June	Essex	high intensity	0	18.4	0	na	na	na	na
G44	2014	June	Essex	low intensity	0	18.4	0	na	na	na	na
G47	2014	June	Essex	low intensity	0	18.4	0	na	na	na	na
G49	2014	June	Essex	high intensity	0	18.4	0	na	na	na	na
G51	2014	June	Essex	high intensity	0	18.4	0	na	na	na	na
G52	2014	June	Essex	low intensity	0	18.4	0	na	na	na	na
G54	2014	June	East Anglia	low intensity	0	16.6	0	na	na	na	na
G55	2014	June	East Anglia	high intensity	0	16.6	0	na	na	na	na
G59	2014	June	Essex	low intensity	0	14.9	0	na	na	na	na
G60	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na
G64	2014	June	Essex	low intensity	0	14.9	0	na	na	na	na
G65	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na
G67	2014	June	Essex	low intensity	0	14.9	0	na	na	na	na
G68	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na
G69	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na
G70	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na

G71	2014	June	Essex	low intensity	0	14.9	1	na	na	na	na
G72	2014	June	Essex	low intensity	0	14.9	1	0	0	1	0
G75	2014	June	Essex	high intensity	0	14.9	0	na	na	na	na
G77	2014	June	Anglia East	low intensity	0	14.7	0	na	na	na	na
G78	2014	June	Anglia East	high intensity	0	14.7	0	na	na	na	na
G81	2014	June	Anglia East	high intensity	0	18.2	0	na	na	na	na
G83	2014	June	Anglia East	low intensity	0	18.2	0	na	na	na	na
G85	2014	June	Anglia East	low intensity	0	18.2	0	na	na	na	na
G88	2014	June	Anglia East	high intensity	0	18.2	0	na	na	na	na
G89	2014	June	Anglia East	low intensity	0	12.1	0	na	na	na	na
G90	2014	June	Anglia East	high intensity	0	12.1	0	na	na	na	na
G91	2014	June	Anglia East	high intensity	0	12.1	0	na	na	na	na
G92	2014	June	Anglia East	high intensity	0	12.1	1	0	1	0	0
G93	2014	June	Anglia	low intensity	0	12.1	0	na	na	na	na
G94	2014	June	East	low intensity	0	12.1	0	na	na	na	na

			Anglia								
H15	2014	June	Essex	low intensity	0.3	13.9	0	na	na	na	na
			East	high							
H16	2014	June	Anglia	intensity	0	13.9	0	na	na	na	na
				high							
H17	2014	June	Essex	intensity	0.3	13.9	0	na	na	na	na
			East	high							
H19	2014	June	Anglia	intensity	0	13.9	0	na	na	na	na
			East								
H20	2014	June	Anglia	low intensity	0	13.9	0	na	na	na	na
			East								
H23	2014	June	Anglia	low intensity	0	13.9	0	na	na	na	na
H26	2014	June	Essex	low intensity	0.3	13.9	0	na	na	na	na
			East	high							
H27	2014	June	Anglia	intensity	0	11.4	0	na	na	na	na
				high							
H29	2014	June	Essex	intensity	0.3	13.9	0	na	na	na	na
			East								
H30	2014	June	Anglia	low intensity	0	13.9	0	na	na	na	na
			East	high							
H33	2014	June	Anglia	intensity	0	13.9	0	na	na	na	na
			East								
H35	2014	June	Anglia	low intensity	0	11.4	0	na	na	na	na
				high							
H39	2014	June	Essex	intensity	0	14.6	0	na	na	na	na
H42	2014	June	Essex	low intensity	0	14.6	0	na	na	na	na

H47	2014	June	Essex	high intensity	0.3	13.9	1	1	0	0	0
H48	2014	June	Essex	low intensity	0.3	13.9	0	na	na	na	na
H50	2014	June	Essex	low intensity	0.3	13.9	0	na	na	na	na
H51	2014	June	Essex	high intensity	0.3	13.9	0	na	na	na	na
H54	2014	June	Essex	high intensity	0.3	14.6	0	na	na	na	na
H58	2014	June	Essex	low intensity	0.3	14.6	0	na	na	na	na
H75	2014	July	Essex	low intensity	2	15.6	0	na	na	na	na
H78	2014	July	Essex	high intensity	2	15.6	0	na	na	na	na
H80	2014	July	Essex	low intensity	2	15.6	0	na	na	na	na
H82	2014	July	Essex	high intensity	2	15.6	0	na	na	na	na
H89	2014	July	Essex	high intensity	2	15.6	0	na	na	na	na
I10	2014	July	Essex	low intensity	0	16.8	0	na	na	na	na
I12	2014	July	Essex	high intensity	0	16.8	0	na	na	na	na
I15	2014	July	Essex	low intensity	0	16.8	0	na	na	na	na
I17	2014	July	Essex	high intensity	0	16.8	0	na	na	na	na
I18	2014	July	Essex	low intensity	0	16.8	0	na	na	na	na
I19	2014	July	Essex	high intensity	0	16.8	0	na	na	na	na

I23	2014	July	Essex	high intensity	0	16.8	0	na	na	na	na
I25	2014	July	Essex	low intensity	6.5	14.6	0	na	na	na	na
I26	2014	July	Essex	low intensity	0	16.8	0	na	na	na	na
I27	2014	July	Essex	high intensity	6.5	14.6	1	na	na	na	na
I29	2014	July	Essex	high intensity	1.4	17	0	na	na	na	na
I31	2014	July	Essex	low intensity	1.4	17	0	na	na	na	na
I32	2014	July	Essex	low intensity	1.4	17	0	na	na	na	na
I35	2014	July	Essex	high intensity	1.4	17	0	na	na	na	na
I37	2014	July	East Anglia	low intensity	0	14.8	0	na	na	na	na
I38	2014	July	East Anglia	high intensity	0	14.8	0	na	na	na	na
I43	2014	July	East Anglia	low intensity	0	14.8	0	na	na	na	na
I44	2014	July	East Anglia	high intensity	0	14.8	0	na	na	na	na
I46	2014	July	East Anglia	low intensity	0	14.8	0	na	na	na	na
I77	2014	July	Essex	high intensity	0	20.9	1	na	na	na	na
I79	2014	July	Essex	low intensity	0	20.9	1	na	na	na	na
I80	2014	July	East	high	0	19.8	0	na	na	na	na

			Anglia	intensity							
I81	2014	July	East Anglia	low intensity	0	19.8	0	na	na	na	na
I87	2014	July	East Anglia	high intensity	0	19.8	1	na	na	na	na
I89	2014	July	East Anglia	high intensity	0	19.8	0	na	na	na	na
I90	2014	July	East Anglia	low intensity	0	19.8	0	na	na	na	na
I93	2014	July	Essex	high intensity	0	21.8	1	na	na	na	na
I94	2014	July	Essex	low intensity	0	21.8	1	na	na	na	na
I98	2014	July	Essex	low intensity	3.1	23.7	0	na	na	na	na
I99	2014	July	Essex	high intensity	3.1	23.7	1	na	na	na	na
I100	2014	July	Essex	low intensity	3.1	23.7	0	na	na	na	na
J02	2014	July	Essex	low intensity	3.1	23.7	1	na	na	na	na
J03	2014	July	Essex	high intensity	3.1	23.7	1	na	na	na	na
J05	2014	July	Essex	high intensity	3.1	23.7	1	0	1	0	0
J06	2014	July	Essex	high intensity	3.1	23.7	1	0	1	0	0
J07	2014	July	Essex	low intensity	3.1	23.7	0	na	na	na	na
ESR7	2014	July	East Anglia	high intensity	0	13.2	0	na	na	na	na

ESR9	2014	July	East Anglia	low intensity	0	13.2	0	na	na	na	na

Table 7.5: Species classified by diet for Model 1 in Chapter 4 – prevalence of *T. gallinae* strains in Columbid populations

Species	Diet
Black Billed Wood Dove	Granivorous
Collared Dove	Omnivorous
Feral Pigeon	Omnivorous
Laughing Dove	Omnivorous
Namaqua Dove	Granivorous
Stock Dove	Omnivorous
Turtle Dove	Granivorous
Vinaceous Dove	Herbivorous
Woodpigeon	Herbivorous

Table 7.6: Species classified by diet for Model 2.1 in Chapter 4– prevalence of *T. gallinae* strains in British bird population

Blackbird	Omnivorous
Chaffinch	Omnivorous
Collared Dove	Omnivorous
Dunnock	Omnivorous
Feral Pigeon	Omnivorous
Goldfinch	Omnivorous
Greenfinch	Omnivorous
House Sparrow	Omnivorous
Magpie	Omnivorous
Pheasant	Omnivorous
Red-Legged Partridge	Herbivorous
Robin	Omnivorous
Stock Dove	Omnivorous
Turtle Dove	Granvirous
Woodpigeon	Herbivorous
Yellowhammer	Omnivorous